Study of the establishment of epithelial polarity: Search for new proteins that interact with aPKC

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Introduction
A key issue in developmental biology is the relationship between cell polarity and signal transduction pathways. Most eukaryotic cells are polarized with an asymmetric distribution of molecules and organelles resulting in different functional regions required for cell physiology. The control of this polarity in space and time is essential to coordinate changes in cell morphology with proliferation and morphogenetic movements required for the development of the organism. This control is carried out by signalling pathways, which in many cases are regulated by the subcellular localization of their components. In fact, there is a close relationship between polarity and the control of cell proliferation, since many receptors of intercellular communication pathways that regulate proliferation are located and activated in specific domains of the plasma membrane. Therefore, the understanding of the signalling pathways-cell polarity relationship is crucial for the knowledge of how signals are integrated to induce morphogenesis but also how are modified in aberrant processes as those occurring in cancer. The atypical protein kinase C (aPKC) is a crucial protein in the cell polarity establishment or maintenance and also can participate in many other processes in the cell. aPKC has an enzymatic activity and can regulate different signaling pathways in the cell. In all these processes aPKC interact, depending on the process, with different regulators and modifies different substrates. In addition, aPKC is an oncogene. To understand how cell polarity is established, maintained and modified and also how this polarity can regulate signalling processes we have focused on to find out new proteins that interact with aPKC.

Genetic screen

For the genetic screen, We are using a hypomorphic background for aPKC in a tissue, specifically in the wing (not required for survival and easily detected under a microscope) expressing an RNAi line specific for aPKC under Sal-G4 driver (Fig. 1, 2). This overexpression produce a phenotype that consists in a reduction in the region of vein 2 and 3 that is more severe in males than in females (Fig. 3).

To the expression domain of sal-G4 in the imaginal wing disc appears surrounded in red.

This system is sensitive to changes in genes related with aPKC. So, if you combine this sensitized genetic background with mutations in polarity genes, you can rescue or enhance the phenotype (Fig. 4):

Biochemical screen

For the biochemical screen, we are doing an affinity purification using the protein of interest clone with a protein A domain and a restriction sequence for the TEV protease linked to others different TAGs that can be useful for protein detection. We express this construct in Drosophila embryos and cells culture. To purify my protein from the lysate, we use Dynabeads® cover with IgG that binds to the protein A tag. The elution is with TEV protease, so with only one purification step we can purify my protein with its interacting protein.

Then, trough an electrophoresis gel, we can select the bands that interact specifically with our protein and trough a mass spectrometry analysis we can find out which are these proteins.

Because of aPKC present a regulatory domain that acts inhibiting the kinase domain, we are doing this process with the full length protein and both domains separately. In addition, we have all this constructs with a CAAX sequence in order to generate a constitutively active form of aPKC (Fig. 6).

How to do it?

1. Preliminary results: affinity purification overexpressing aPKC in ectoderm tissues in Drosophila embryos using 69B-Gal4 driver.