Regulation of the apico-basal distribution of the G protein-coupled receptor Smoothened by Hedgehog signalling in drosophila

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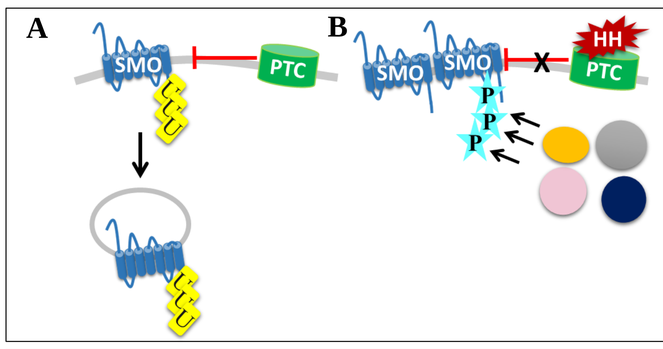
Summary

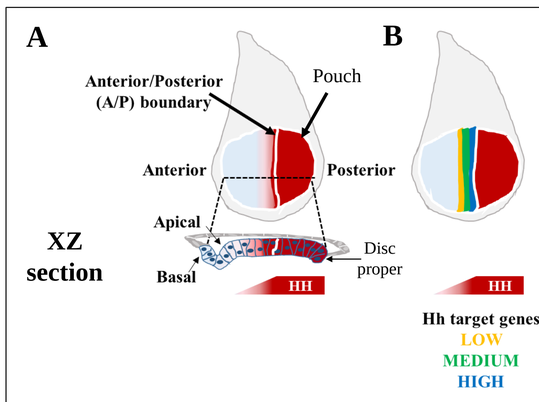
During development, the fate of epithelial cells is controlled by signalling molecules. Epithelial cells display an apico-basal polarity that results in an asymmetric distribution of proteins and lipids. The aim of my project is to understand how apico-basal polarity can modulate cell signalling in the context of the seven-pass transmembrane protein Smoothened (SMO), an oncogene which is required for the transduction of the Hedgehog (HH) morphogenetic signal.

Thanks to a novel method, I could label and follow the SMO molecules present at the surface of epithelial cells from the fly wing primordium, a structure whose development is under HH control.

I could thereby observe a basal enrichment of SMO at the surface of the cells that receive the highest levels of HH. My data support a model in which SMO is initially targeted to the apical region of epithelial cells, before being endocytosed and redistributed to the basal region, where it is stabilized in presence of HH. Finally, I showed that this basal accumulation of SMO requires its phosphorylation by a Protein Kinase A (PKA), Casein Kinase I (CKI) and the kinase called Fused (Fu), a known positive regulator of HH signalling.

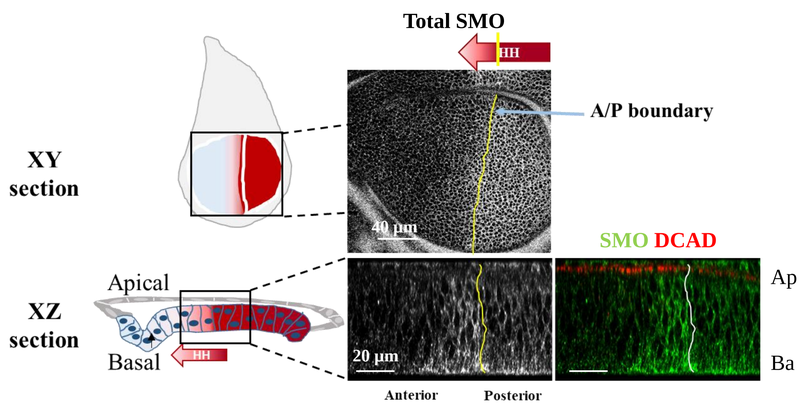


HH controls SMO subcellular location. A) In absence of HH, SMO is inactive, ubiquitinated, internalized and degraded **B)** In its presence, SMO accumulates at the plasma membrane and is hyperphosphorylated. Both events are necessary and sufficient for its activation.



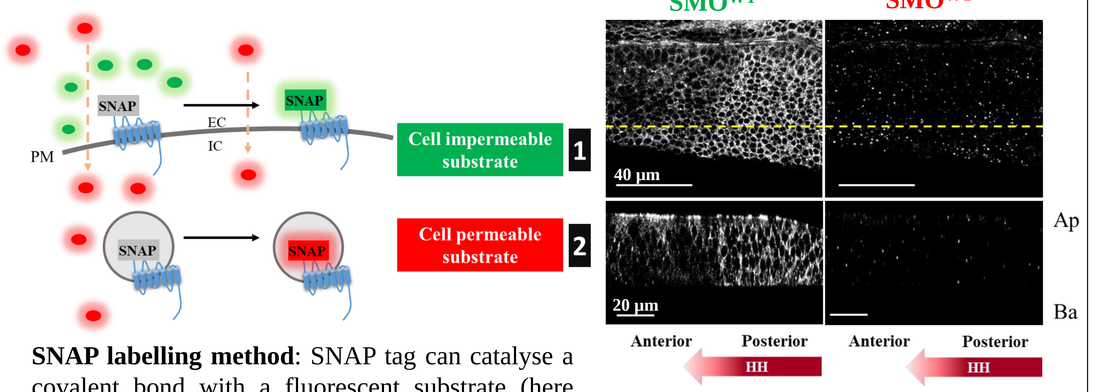
The wing imaginal disc (WID) is a larval epithelium that develops into the adult wing.
A) Representation of a WID (top) with the XZ section of the pouch (bellow) showing apico-basal polarity of its proper epithelium. HH (red) is produced in Posterior (P) compartment cells and received by Anterior (A) compartment cells closest to the A/P boundary (white vertical line), which separates de two compartments.
B) In A cells, a HH concentration gradient is established leading to different target gene transcription.

High levels of HH correlate with SMO basolateral accumulation



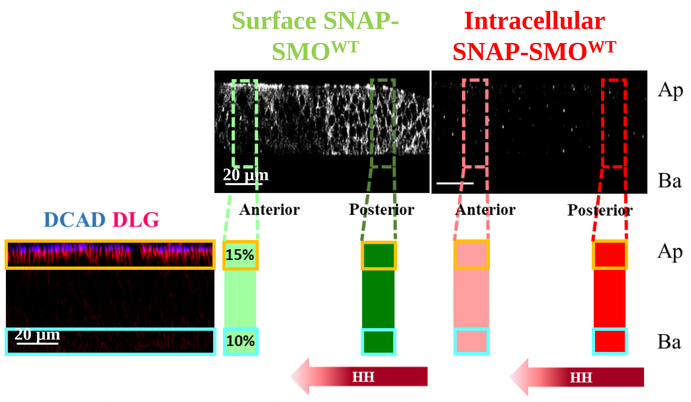
High levels of HH correlate with the basolateral accumulation of SMO.
Antibody labelling of SMO: confocal XY (upper image) and XZ sections (lower images) of the pouch of the WID.
D-Cadherin (DCAD) marks the adherens junctions.
Ap- Apical, Ba- Basal

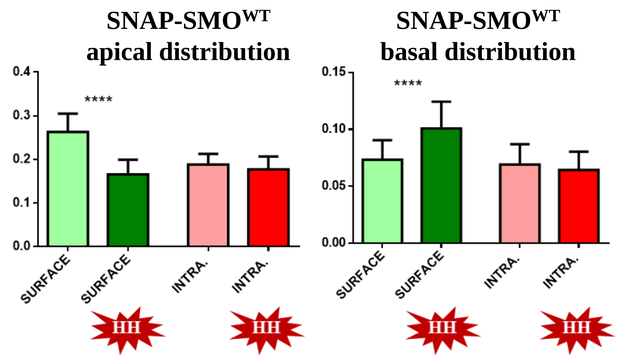
Which population of SMO is affected by HH?



SNAP labelling method: SNAP tag can catalyse a covalent bond with a fluorescent substrate (here green and red circles)

High HH levels correlate with surface SNAP-SMO apical depletion and basal accumulation

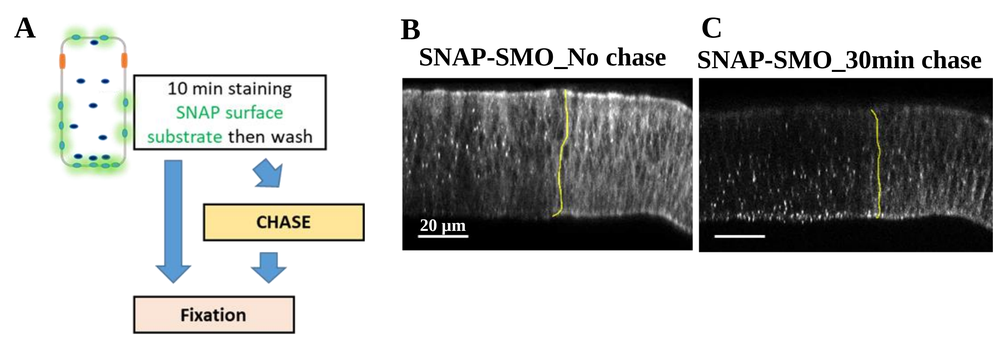




Quantification method: regions where selected in far-anterior (no HH) and posterior compartments (high HH). Apical/subapical and basal domains were considered to be 15% and 10%, respectively of the disc's thickness.

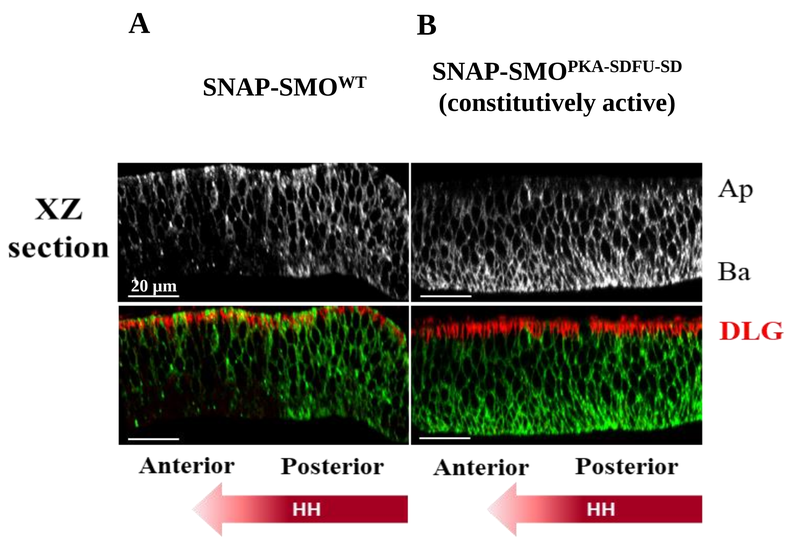
Quantification confirms the correlations between high HH levels and a relative apical depletion (left) and basal enrichment of surface SNAP-SMO (right). The intracellular levels are not affected by HH. T-test: p values of <0.0001 (****), 12 discs.

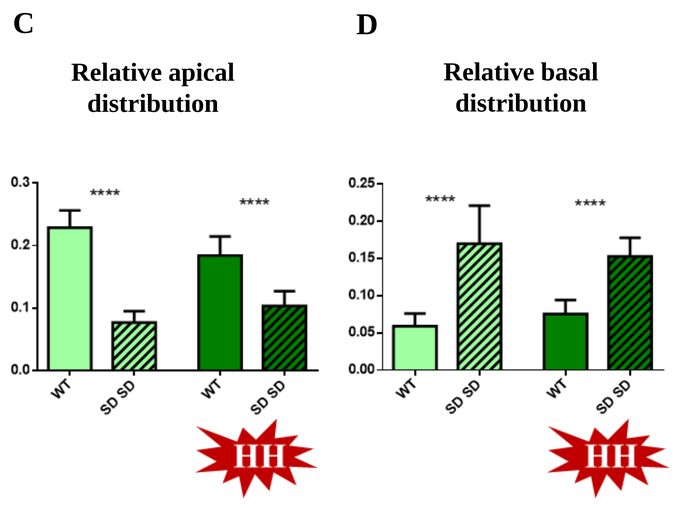
Pulse-chase experiments suggest that surface SNAP-SMO is apically depleted and basally enriched overtime



Endocytosis assay to follow surface SNAP-SMO
(A) Endocytosis assay: surface SNAP-SMO labelling (green circles) followed by fixation or chase and fixation. Orange boxes represent cell junctions.
(B, C) Confocal Z average projections of wing discs expressing SNAP-SMO labelled as in (A). After 30 minutes-chase, a relative depletion of the apical population and an enrichment of the basal one are observed.Yellow line: A/P boundary. Ap – Apical, Ba – Basal. 21 no chase discs, 15 chase discs.

Phosphorylation of SNAP-SMO promotes its basal accumulation and apical depletion





Phosphorylation of SMO by PKA/CKI and FU promotes its basal enrichment in presence of HH.
(A, B) Confocal Z sections of the wing disc expressing surfaced-labelled SNAP-SMO (A) and a mutant mimicking the phosphorylation of its PKA/CKI and FU sites (B).
Discs large (DLG) protein marks the septate junctions.
Ap – Apical, Ba – Basal.
(C,D) Quantification showed that SNAP SMO PKA SD FU SD is apically depleted (C) and basally enriched (D) compared to SNAP-SMO in presence and absence of HH (posterior compartment).
T-test: p = 0.0001 (****), 21 SNAP-SMO discs, 16 SNAP-SMO PKA-SD FU-SD discs.

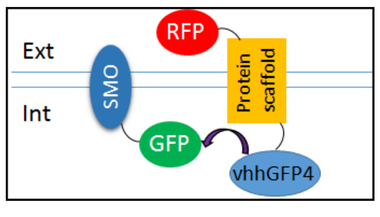
Perspectives

My current goals are:

(i) to consolidate the data on SMO trafficking

(ii) to understand how the PKA/CKI and Fused kinases promotes the basal redistribution of SMO

(iii) to understand how this basal localisation of SMO critically regulates its signalling activity by trapping SMO using the GRAB system



Grab system. Fusing the GFP-nanobody (vhhGFP4) to a tagged (RFP) transmembrane protein that has a specific Ap/B distribution (protein scaffold) will determine the localization of GFP marker. When co-expressed with SMO-GFP or FU-GFP, it will force its localization to a specific plasma membrane domain.