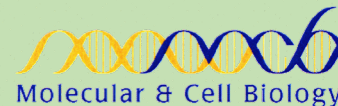


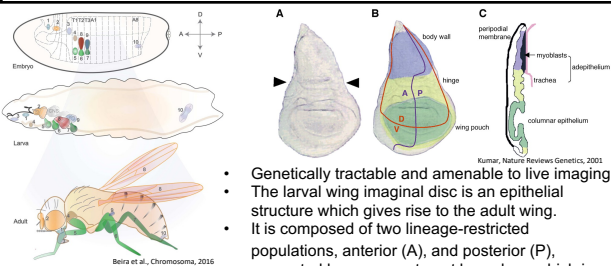
Regulation of Hedgehog signaling and compartment boundary maintenance by membrane potential.



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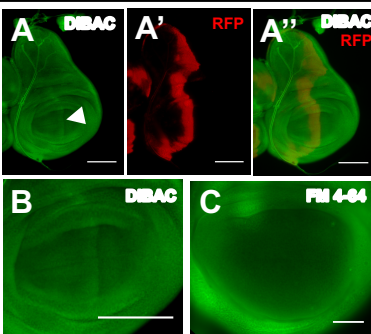


D. melanogaster is an ideal system for investigating developmental morphogenesis.



Membrane potential is patterned in the third instar wing disc.

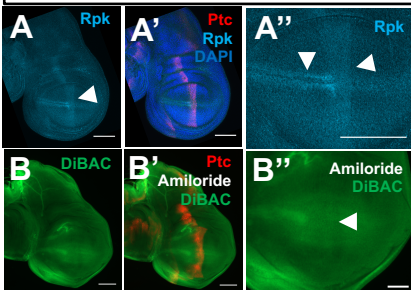
ptc>RFP



- Live imaging in the membrane potential reporter dye DiBAC reveals a region of relative depolarization anterior to the compartment boundary (A, B). Increased DiBAC fluorescence indicates more depolarized tissue.
- The depolarized population expresses the Hh target gene *Ptc* (A-A'').
- Staining with voltage-insensitive membrane dye FM 4-64 does not recapitulate pattern of DiBAC staining (C).

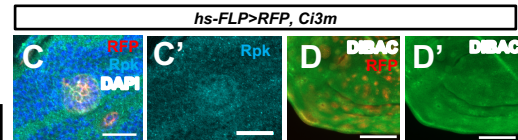
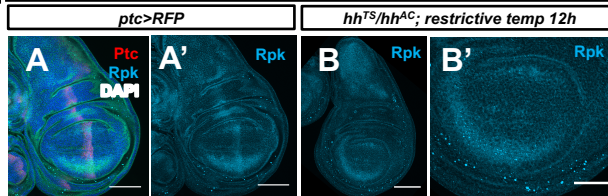
Expression of the endogenous ion channel Rpk is patterned, and contributes to depolarization anterior to the compartment boundary.

ptc>RFP



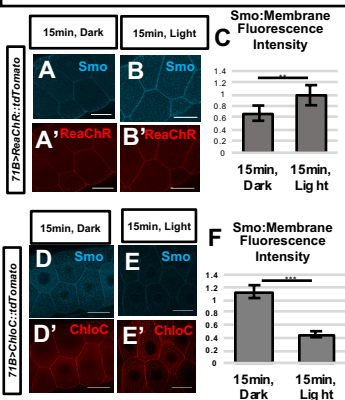
- The Epithelial Na⁺ Channel Rpk is enriched anterior to the compartment boundary, and at the dorsoventral compartment boundary in A cells (A-A'').
- Treatment with the ENaC antagonist amiloride eliminates increased DiBAC fluorescence anterior to the compartment boundary (B-B'').
- Rpk antibody is a kind gift from Dan Kiehart.

Hh signaling regulates patterned Rpk expression and membrane depolarization.



- Reduction of Hh signaling using a temperature-sensitive allele results in decreased Rpk enrichment anterior to the compartment boundary (A-B').
- Clones expressing a constitutively-active Ci show increased Rpk expression (C, C'), and DiBAC staining (D, D'), suggesting they are more depolarized. Scale bar 25µM C, C', and 50µM D, D'.

Manipulating membrane potential modulates Hh signaling.

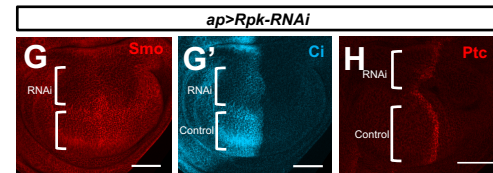


Optogenetic experiments

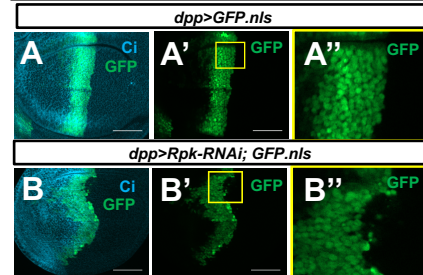
- Activation of depolarizing channelrhodopsin ReaChR results in enriched Smo membrane localization (A-C).
- Activation of hyperpolarizing channelrhodopsin ChloC results in decreased Smo membrane localization (D-F).
- Data compared using an unpaired *t* test. ** indicates *p*<0.01, *** indicates *p*<0.001.

Genetic experiments

- Knockdown of Rpk in the dorsal compartment of the disc results in a reduction in antibody staining for Smo (G), activated Ci (G'), and Ptc (H). Scale bars 50µM.

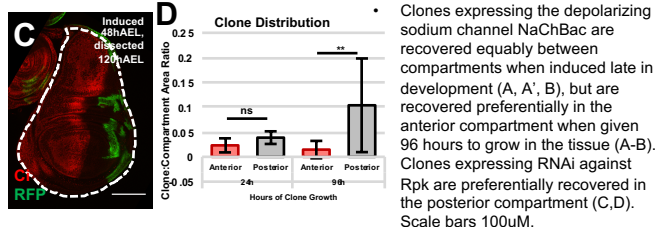
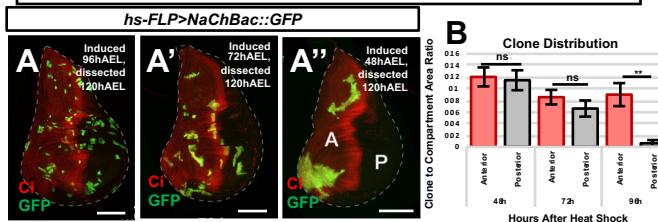


Rpk is required for compartment boundary integrity.



- Knockdown of Rpk anterior to the compartment boundary results in a wiggler compartment (B-B''), as compared with control discs (A-A''). Scale bars 50µM.

Membrane potential confers compartment-specific fitness advantage.



- Clones expressing the depolarizing sodium channel NaChBac are recovered equally between compartments when induced late in development (A, A', B), but are recovered preferentially in the anterior compartment when given 96 hours to grow in the tissue (A-B). Clones expressing RNAi against Rpk are preferentially recovered in the posterior compartment (C,D). Scale bars 100µM.

Conclusions.

- Hh signaling and membrane depolarization are self-reinforcing anterior to the compartment boundary.
- Rpk is necessary for maintenance of the compartment boundary
- Clones of cells with altered membrane potential have compartment-specific survival advantages.

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