



L'œil de la Drosophile pour l'étude du développement et de l'apoptose

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Apoptosis and Neurogenetic

LBMC UMR5239 ENS Lyon

<http://www.ens-lyon.fr/LBMC/ApoDroso/>

Drosophila is a powerful genetic model

- Gene conservation and little gene redundancy

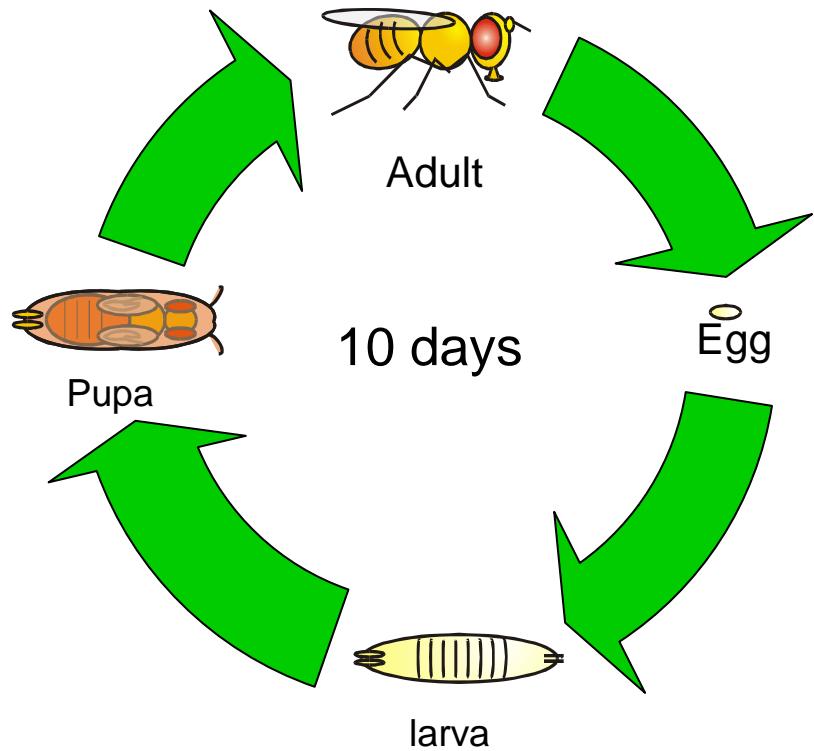
- Short generation time

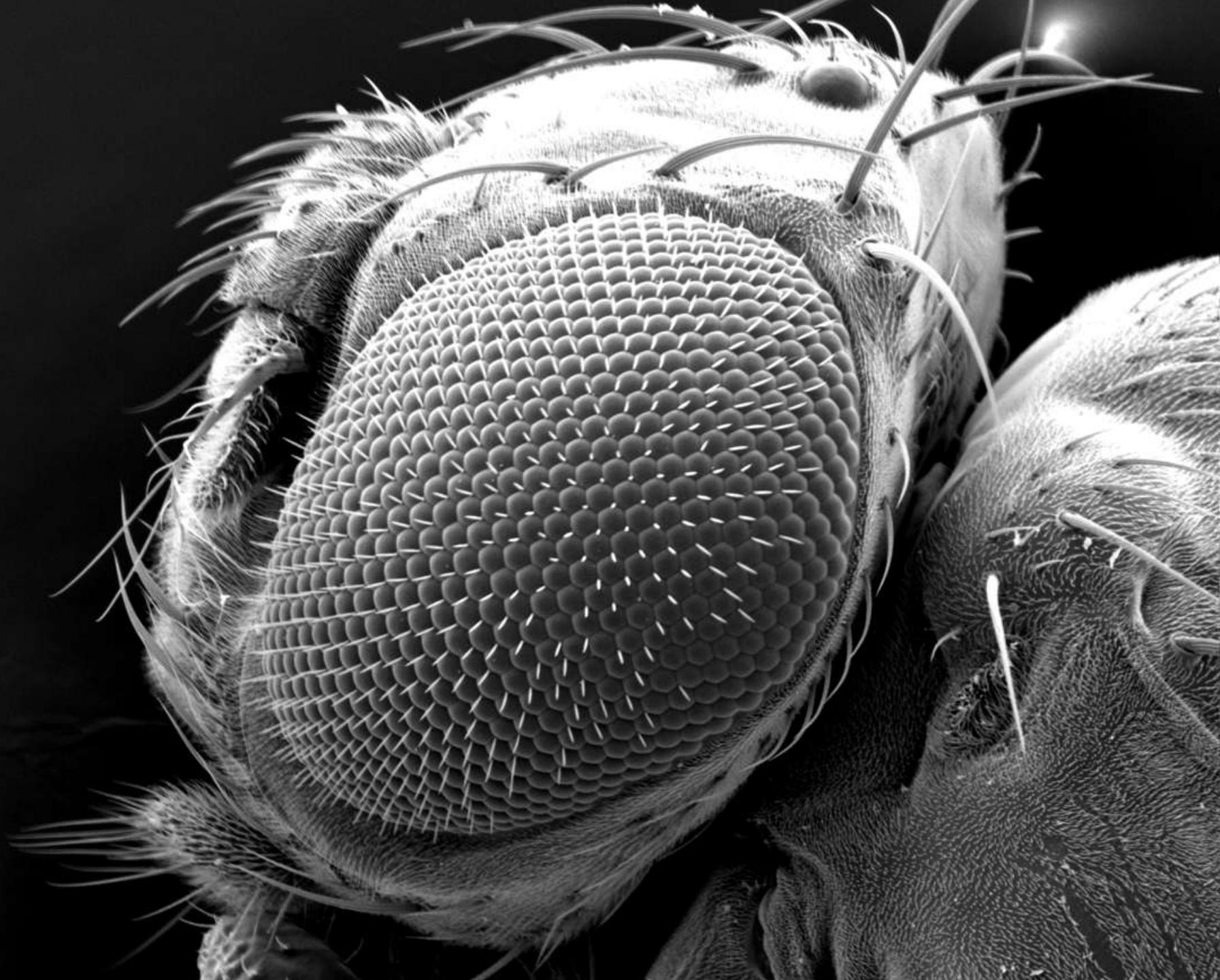
- Easy gene manipulation :

- transgenesis

- gene invalidation

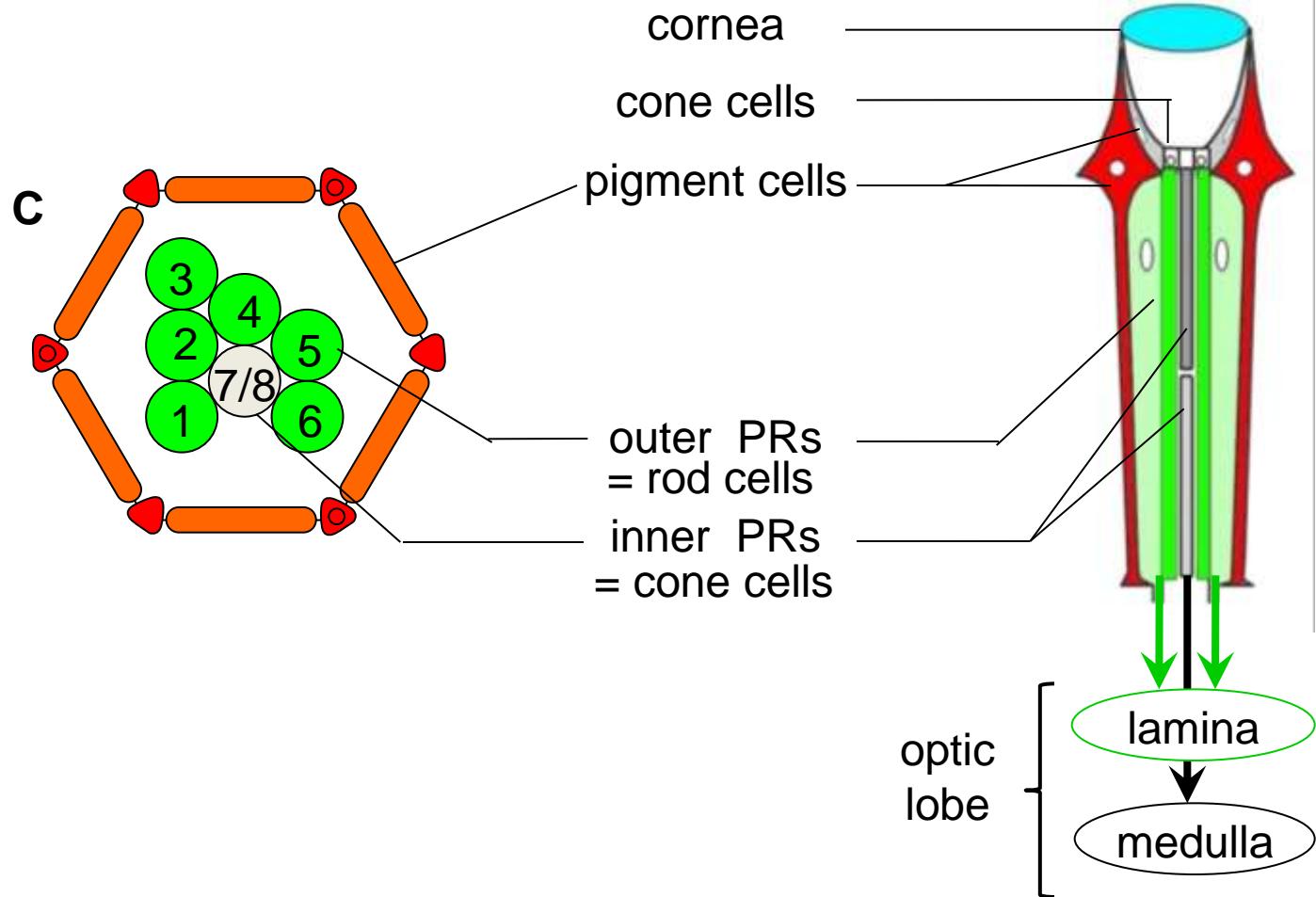
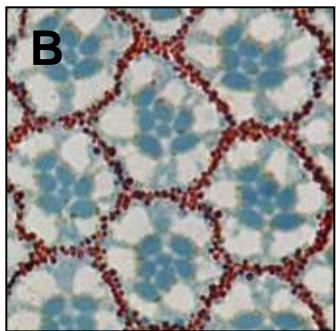
- Genetic screens





The Drosophila compound eye

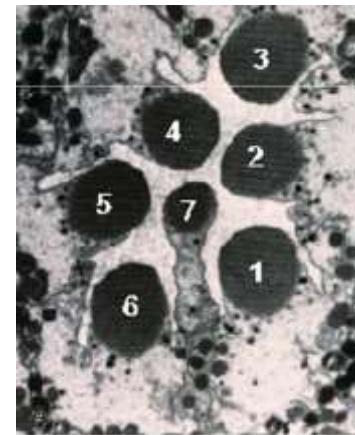
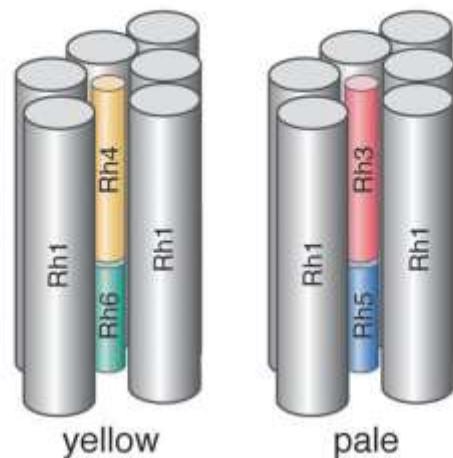
Organization of the *Drosophila* eye



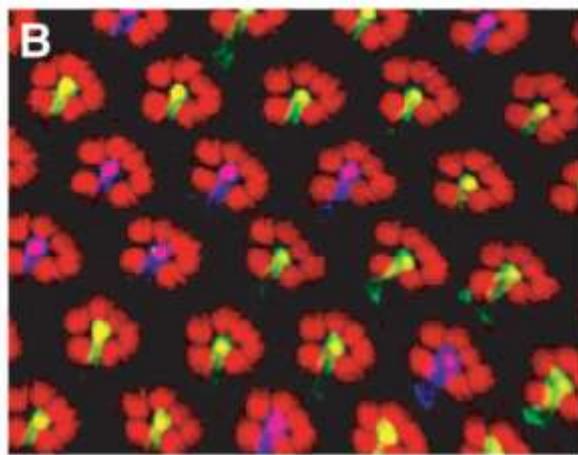
Outer/inner Photoreceptors

A

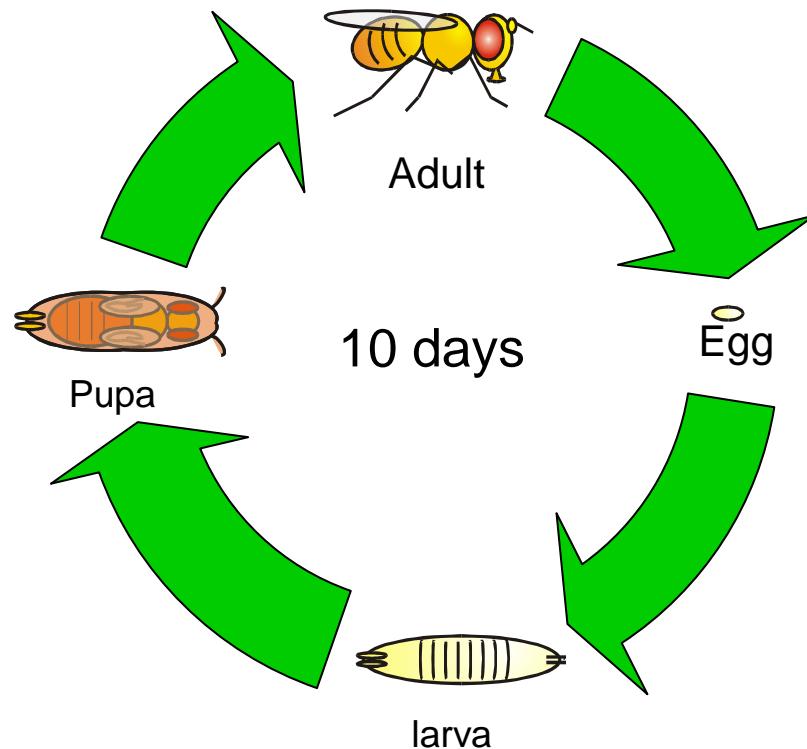
wild type



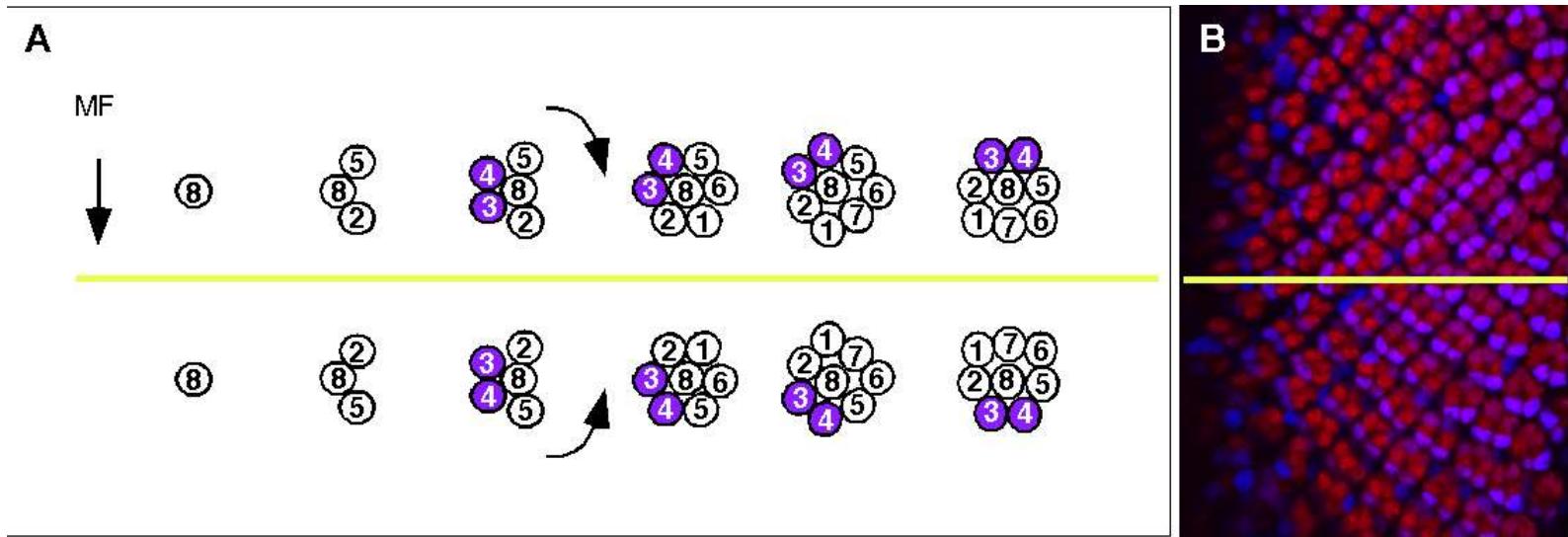
B



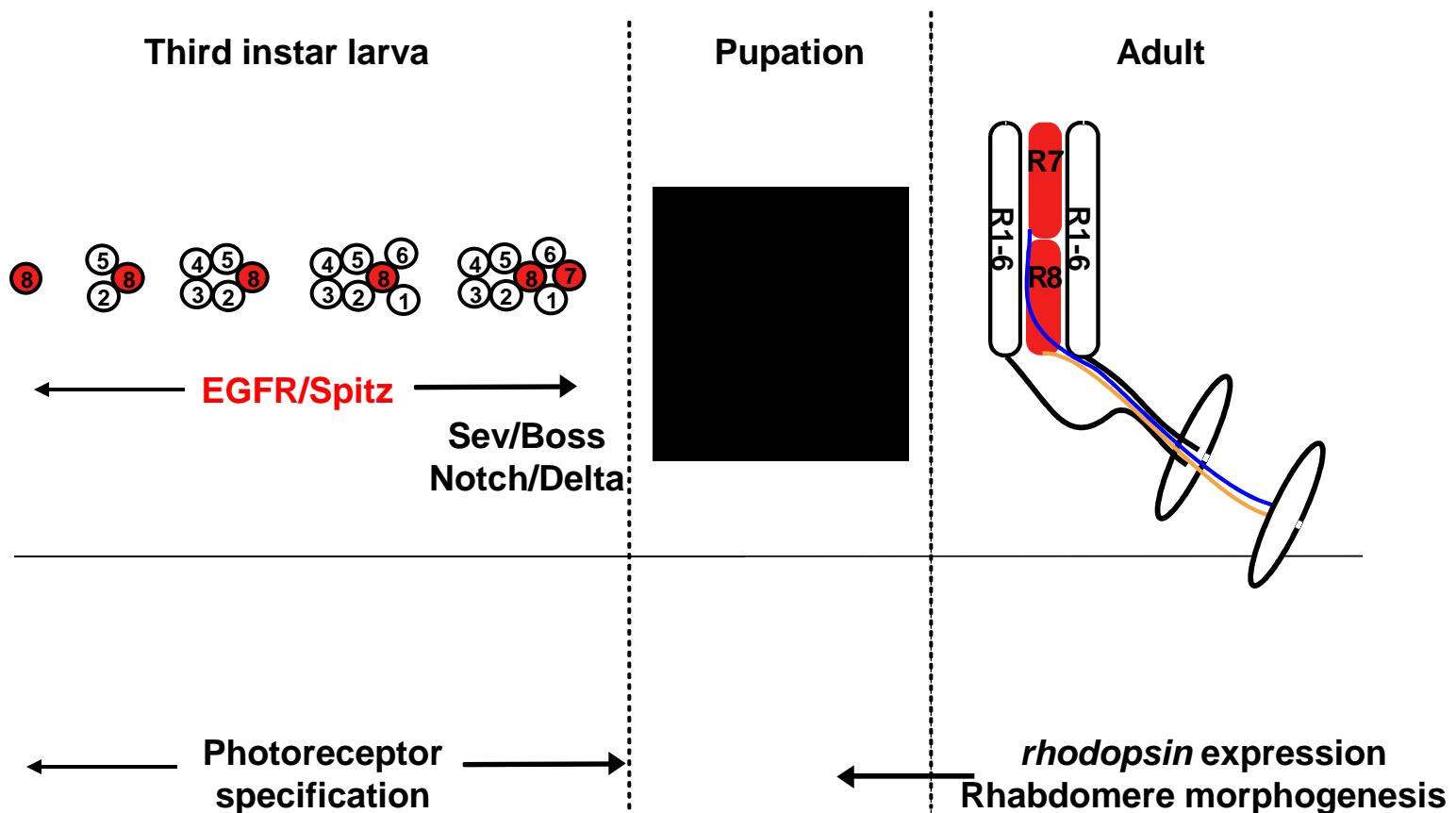
The Drosophila life cycle



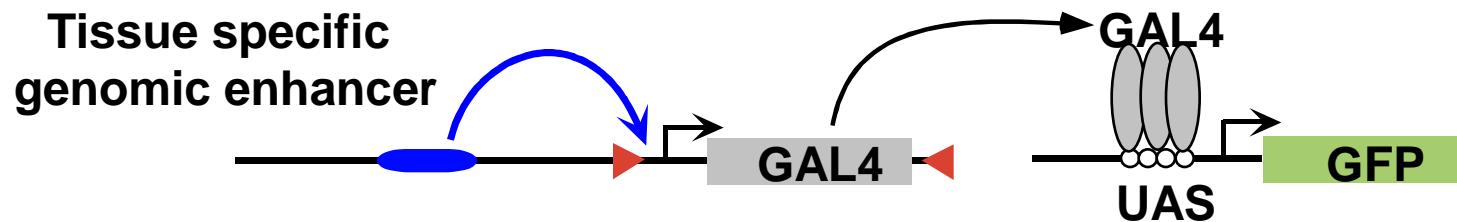
Photoreceptor differentiation and establishment of planar cell polarity



Photoreceptor specification and differentiation

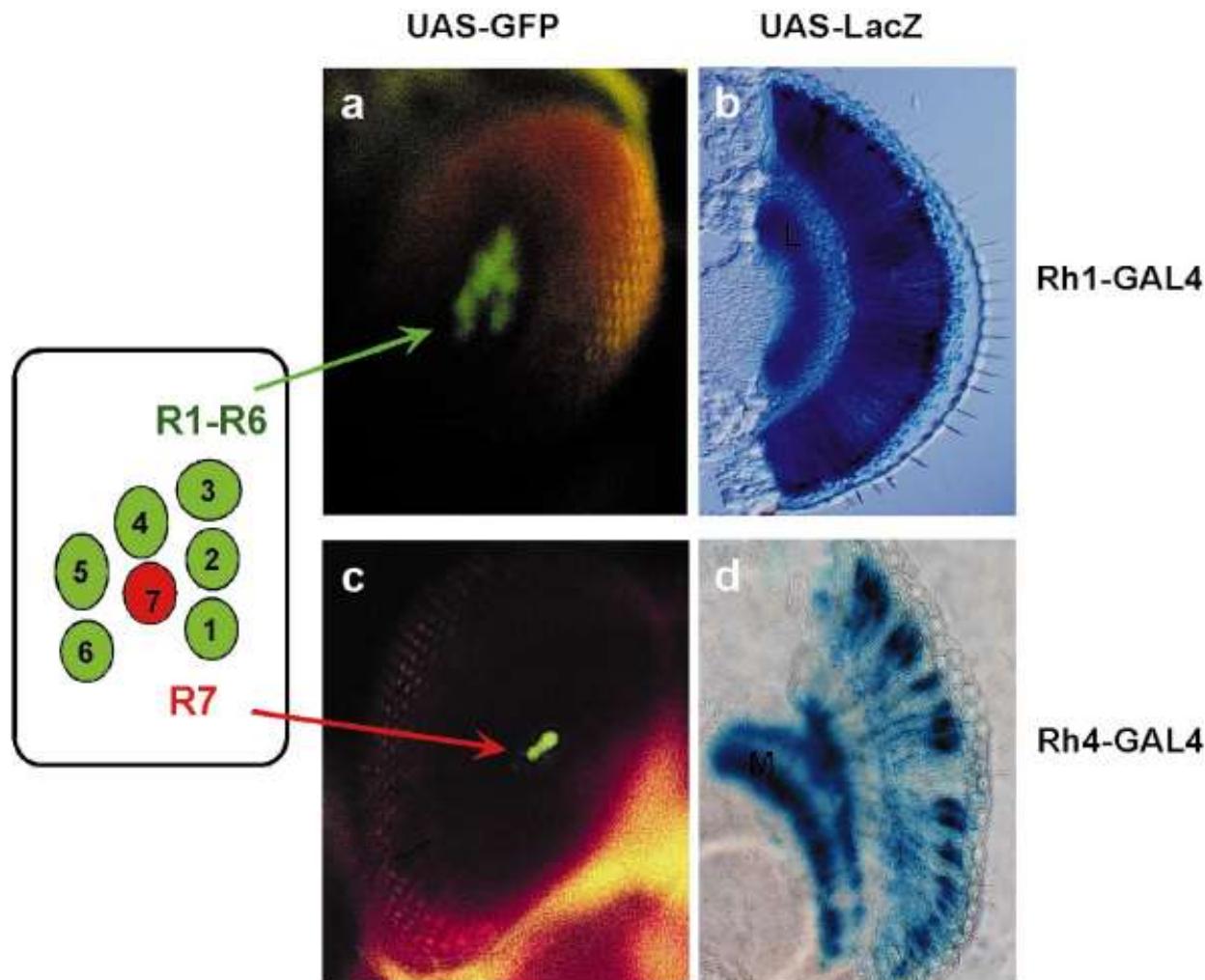


A GFP enhancer trap screen for genes expressed in adult photoreceptors

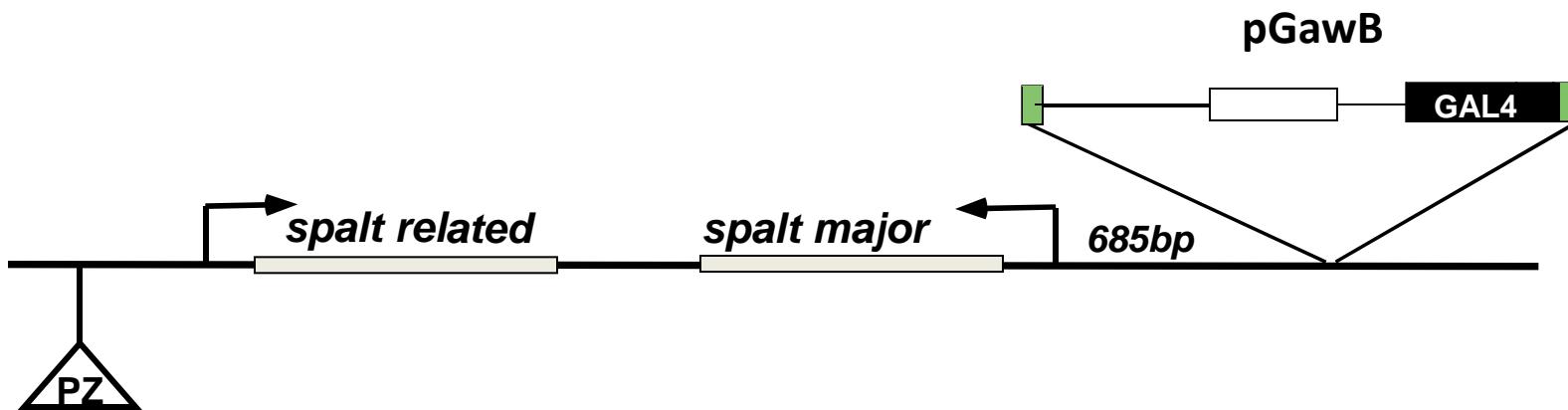
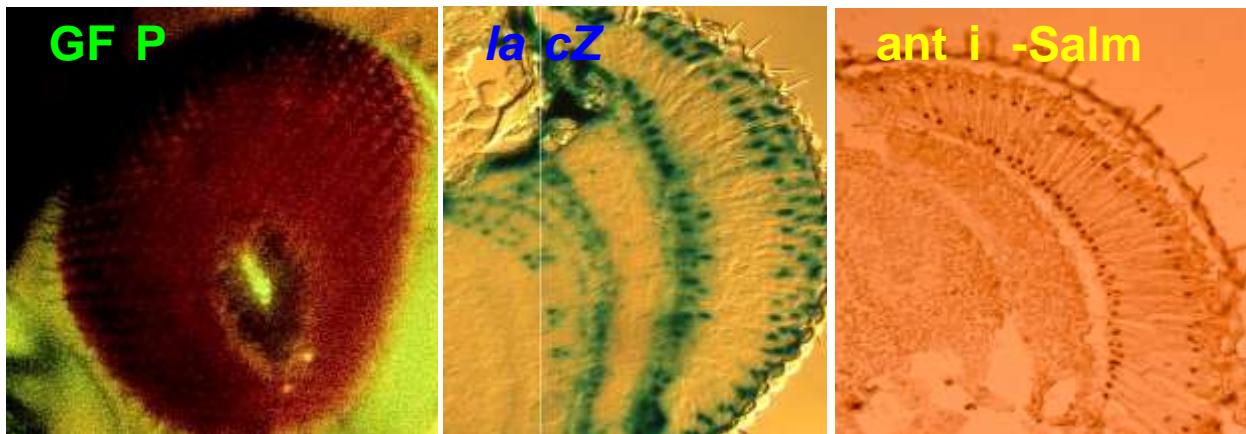


- 150 000 flies observed for a GFP expression in the retina
- 2730 lines with a GFP expression in adult Drosophila
- 16 lines with a specific photoreceptor expression

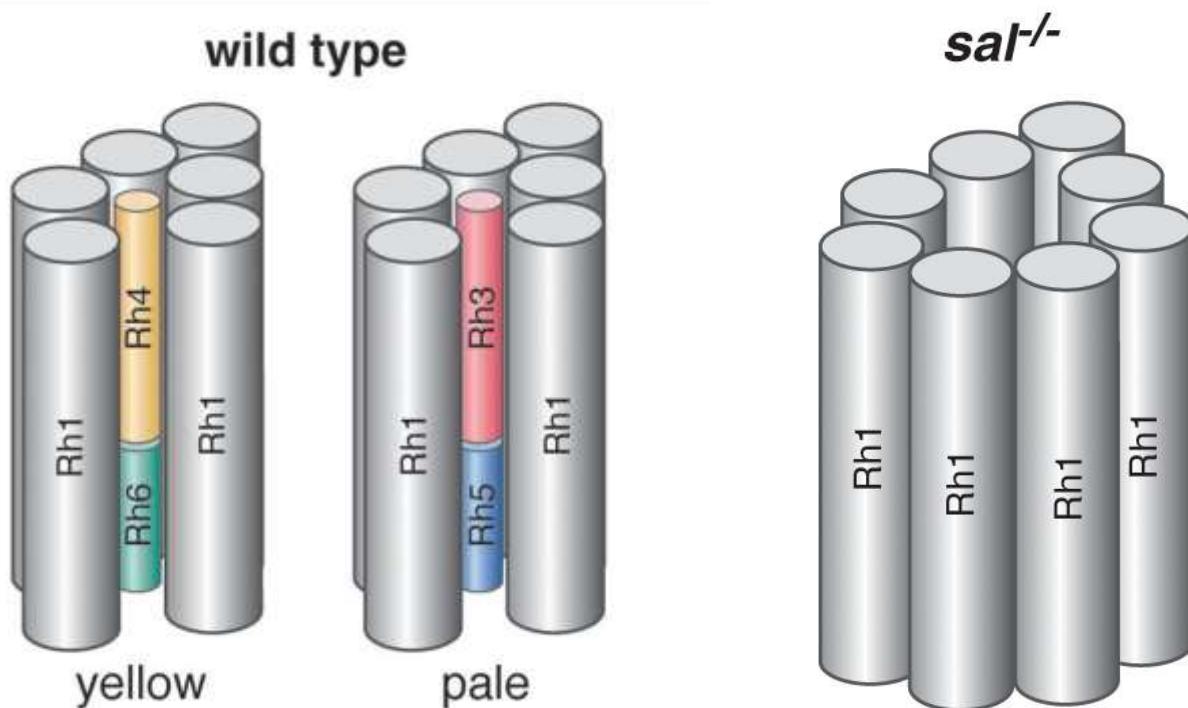
Rhodopsins are expressed in photoreceptor subtypes



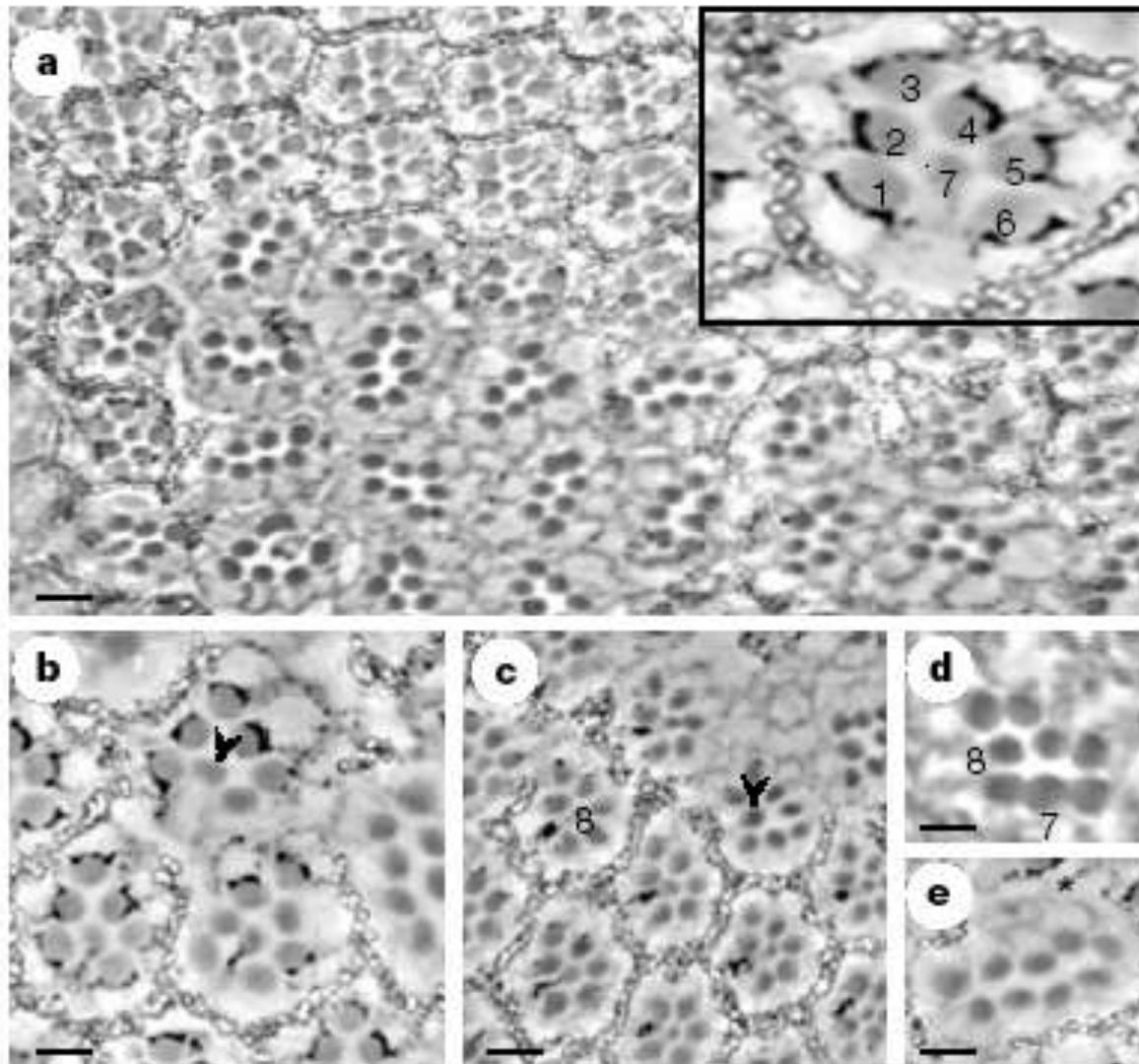
spalt insertion - expression in R7 and R8



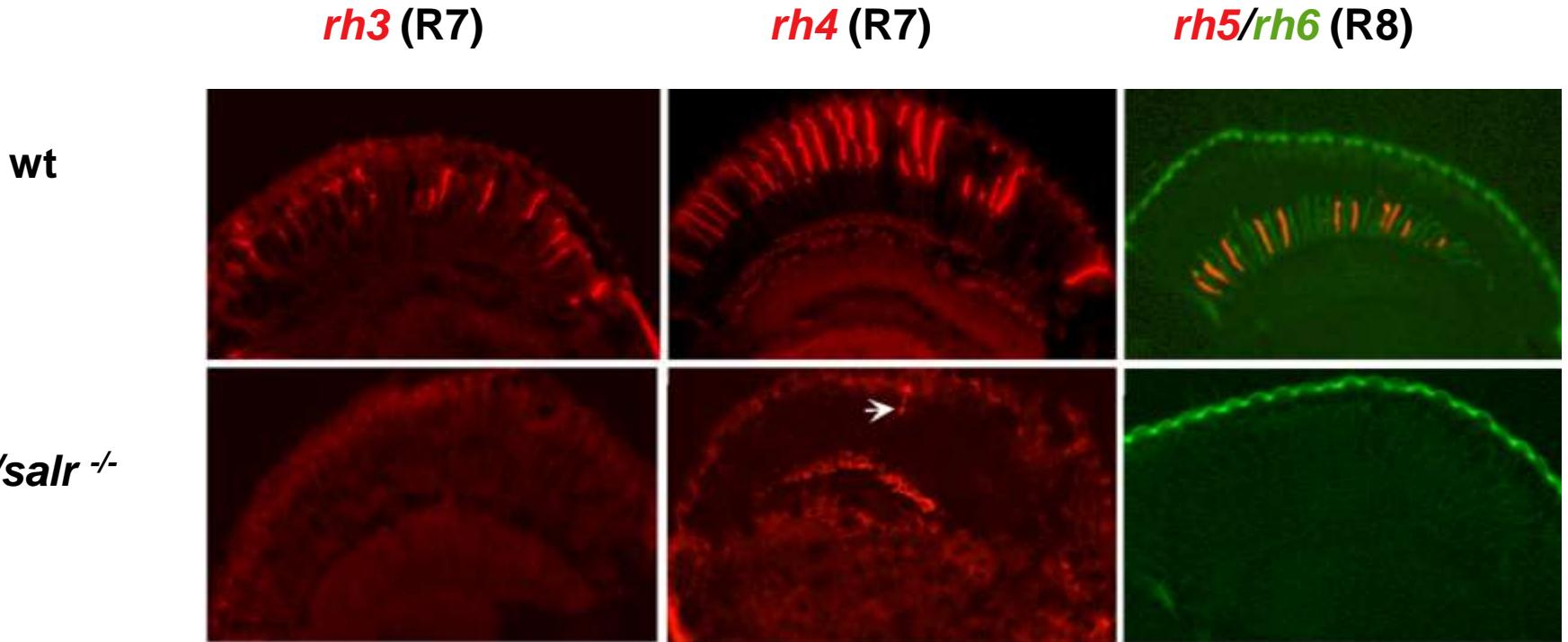
Spalt is required for R7/R8 differentiation



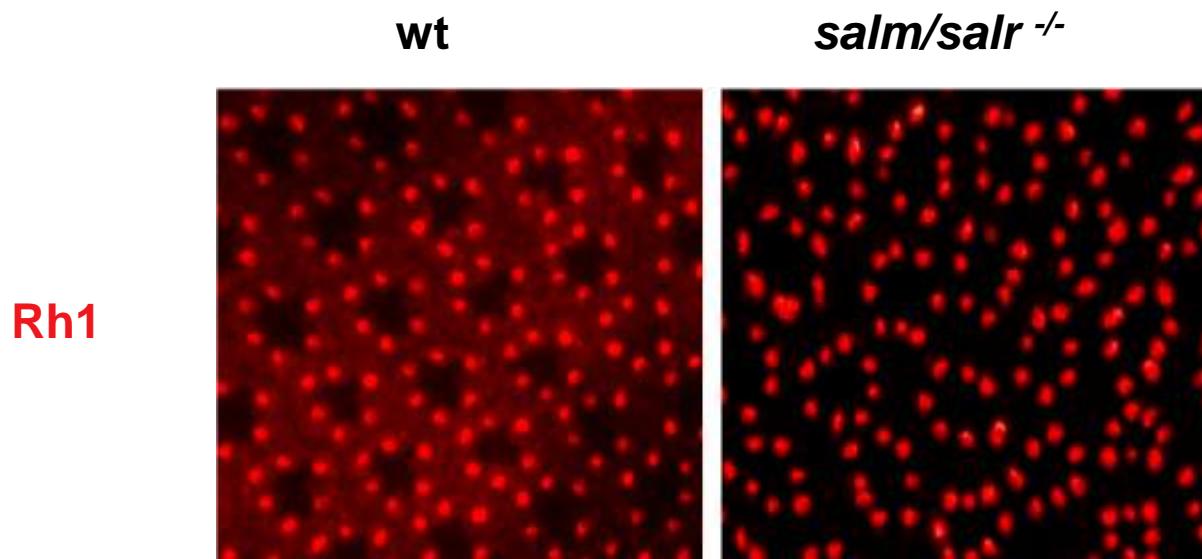
In *salm/salr*^{-/-} R7 and R8 acquire R1-6 subtype rhabdomere morphology



***salm/salr* are required for *rhodopsin* expression in R7 and R8**

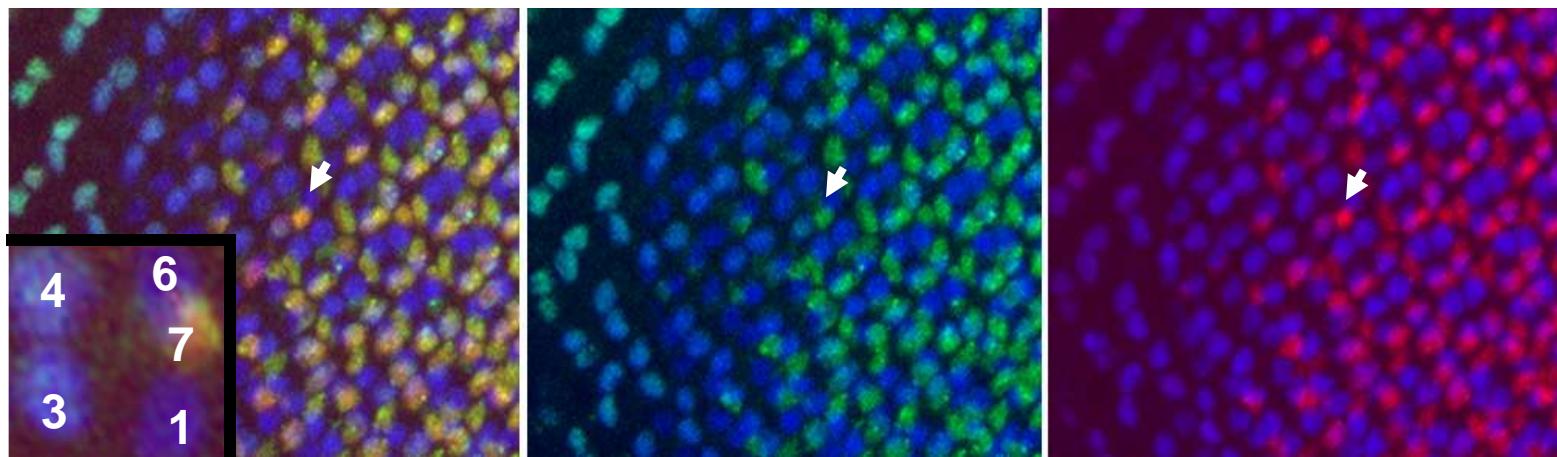


R7 and R8 are transformed into R1-R6 in *salm/salr*^{-/-} retina

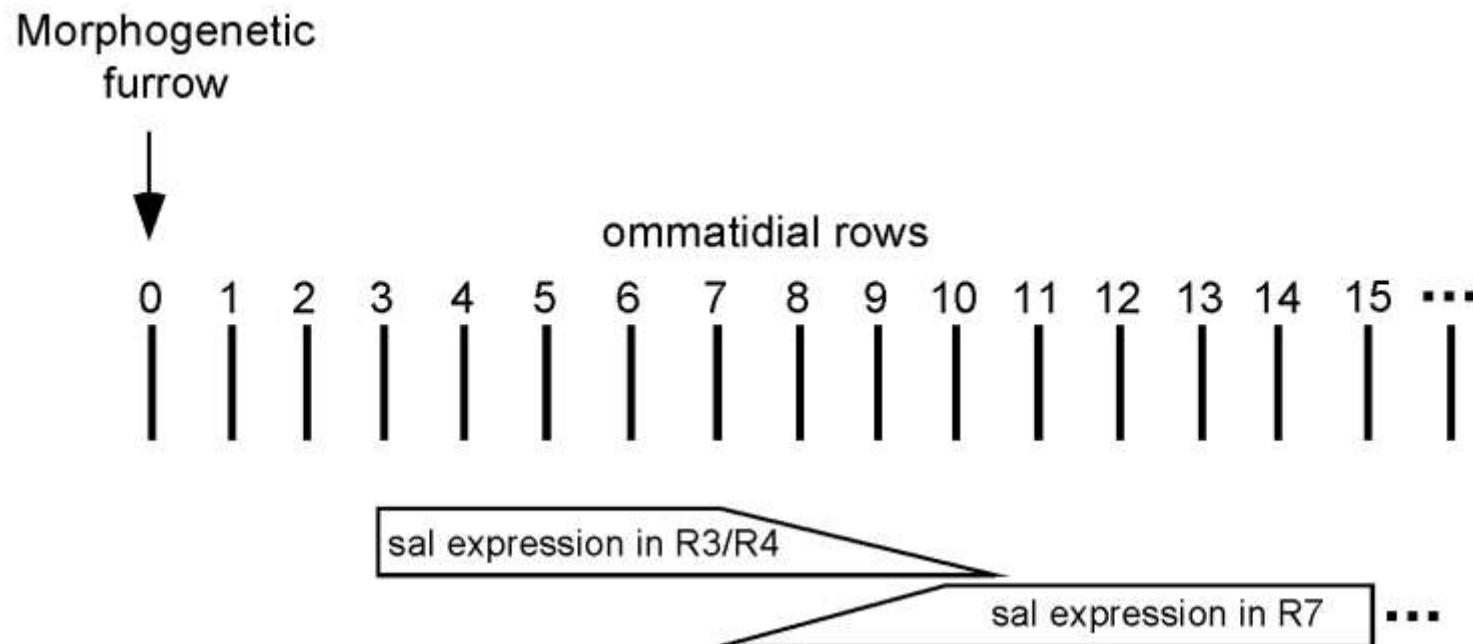


salm is expressed in R3/R4 and R7 in the larval eye imaginal disc

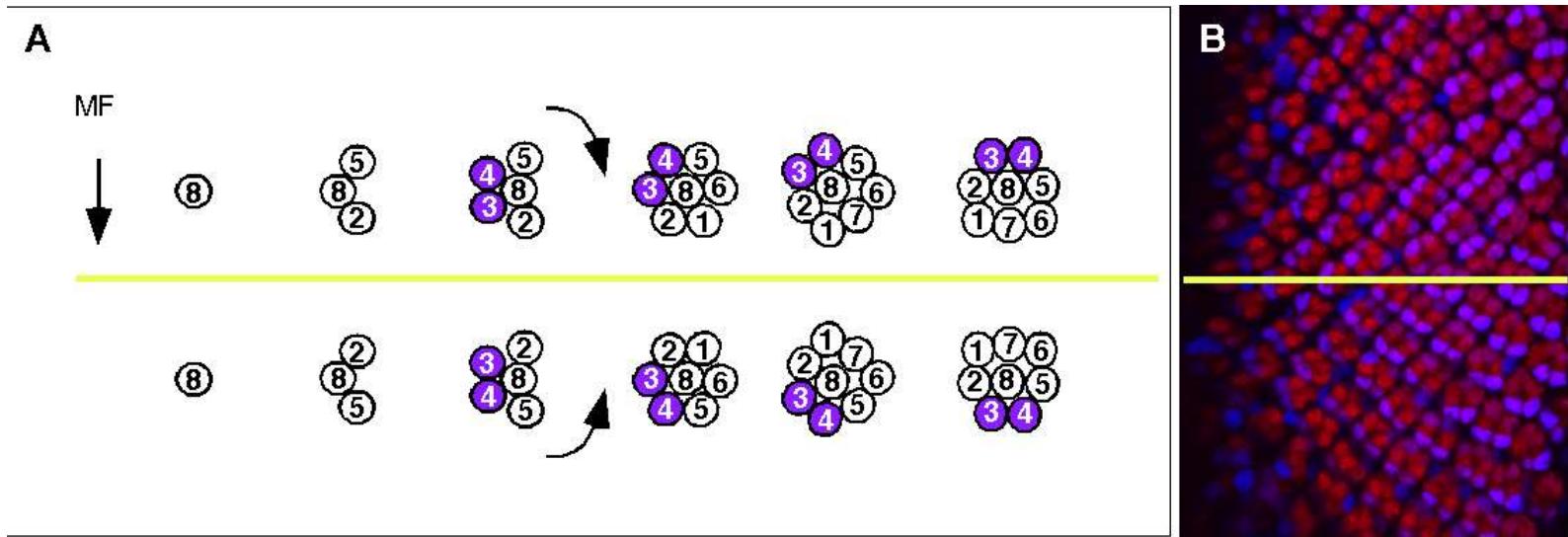
Salm
Svp
(R3,4, 1, 6)
Pros (R7,cc)



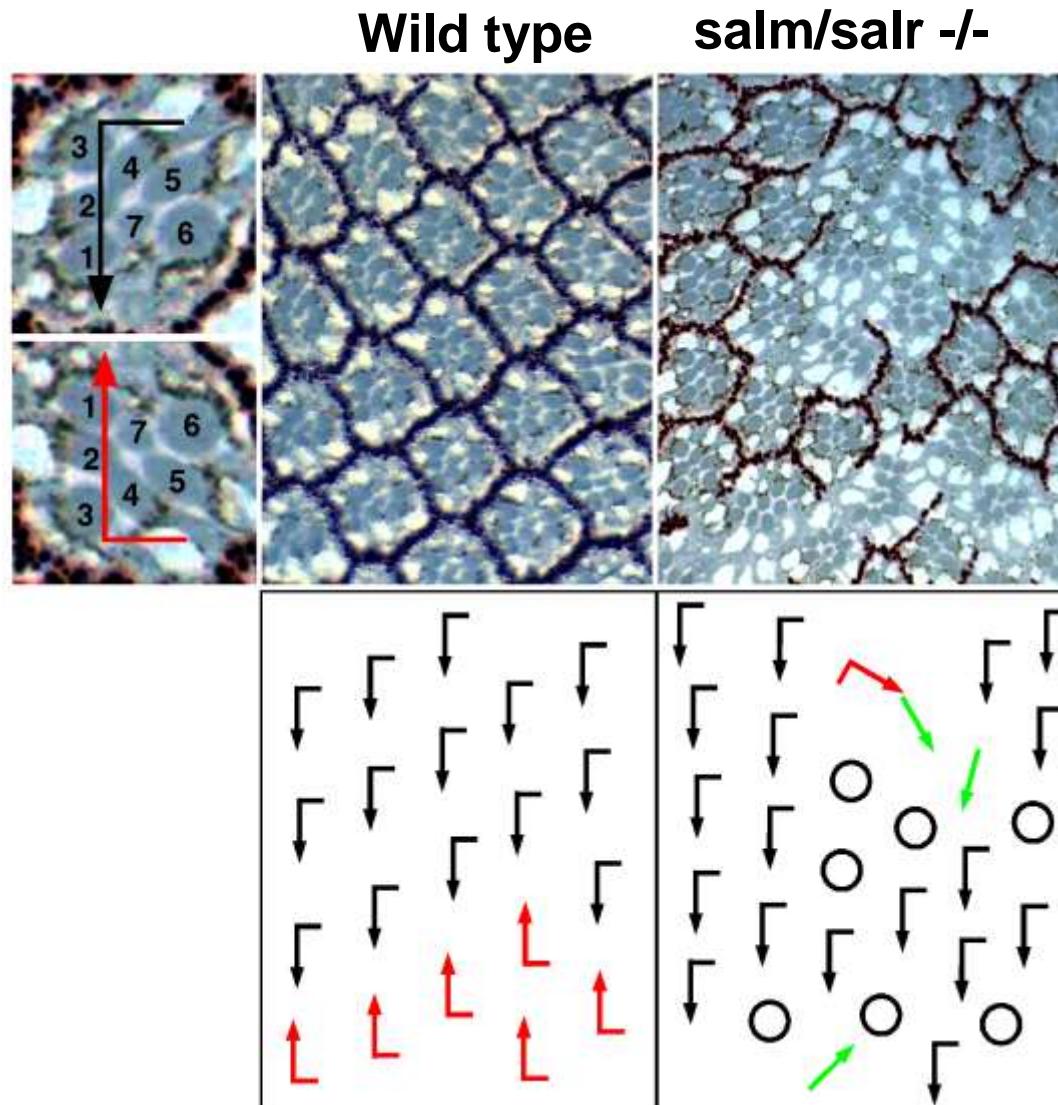
***salm* is expressed in R3/R4 and R7 in the larval eye imaginal disc**



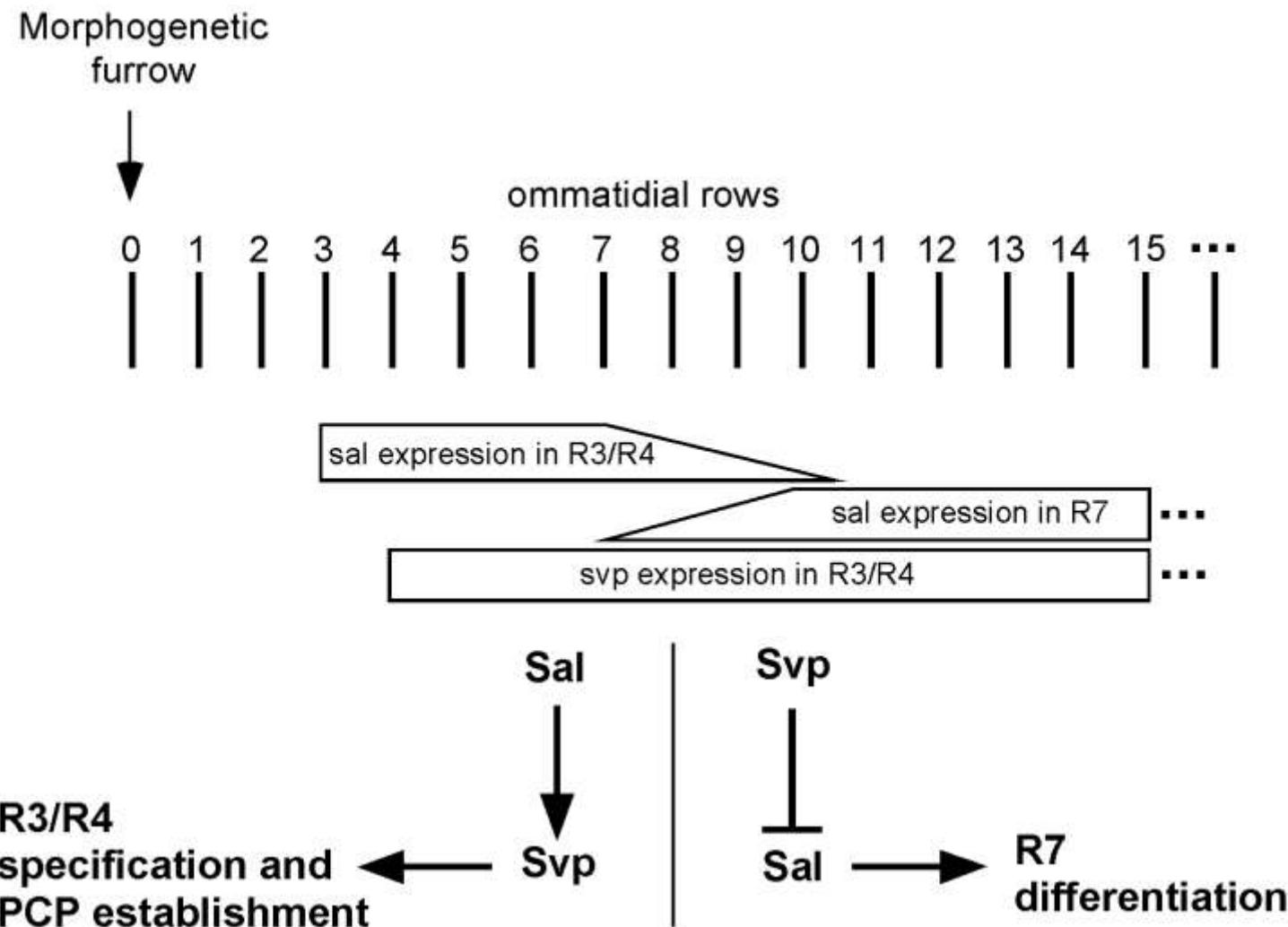
Photoreceptor differentiation and establishment of planar cell polarity



spalt mutant ommatidia have a defect in planar cell polarity



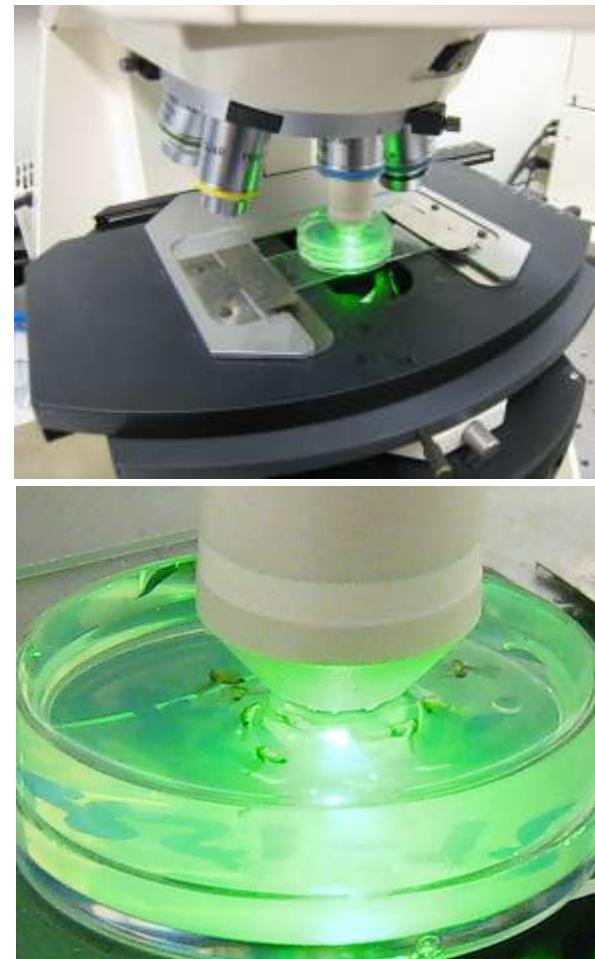
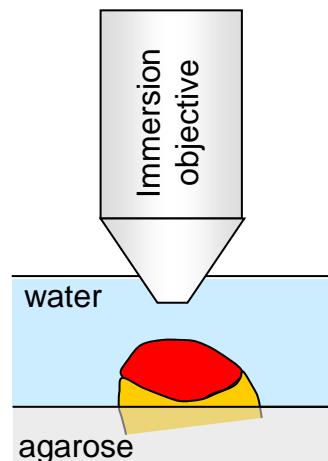
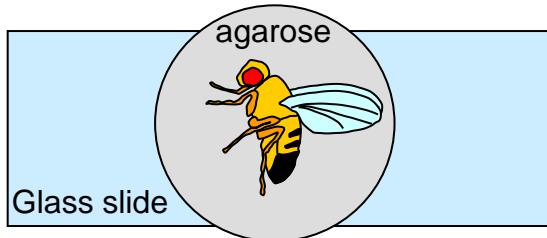
Sequential roles of spalt in R3/R4 and R7 differentiation

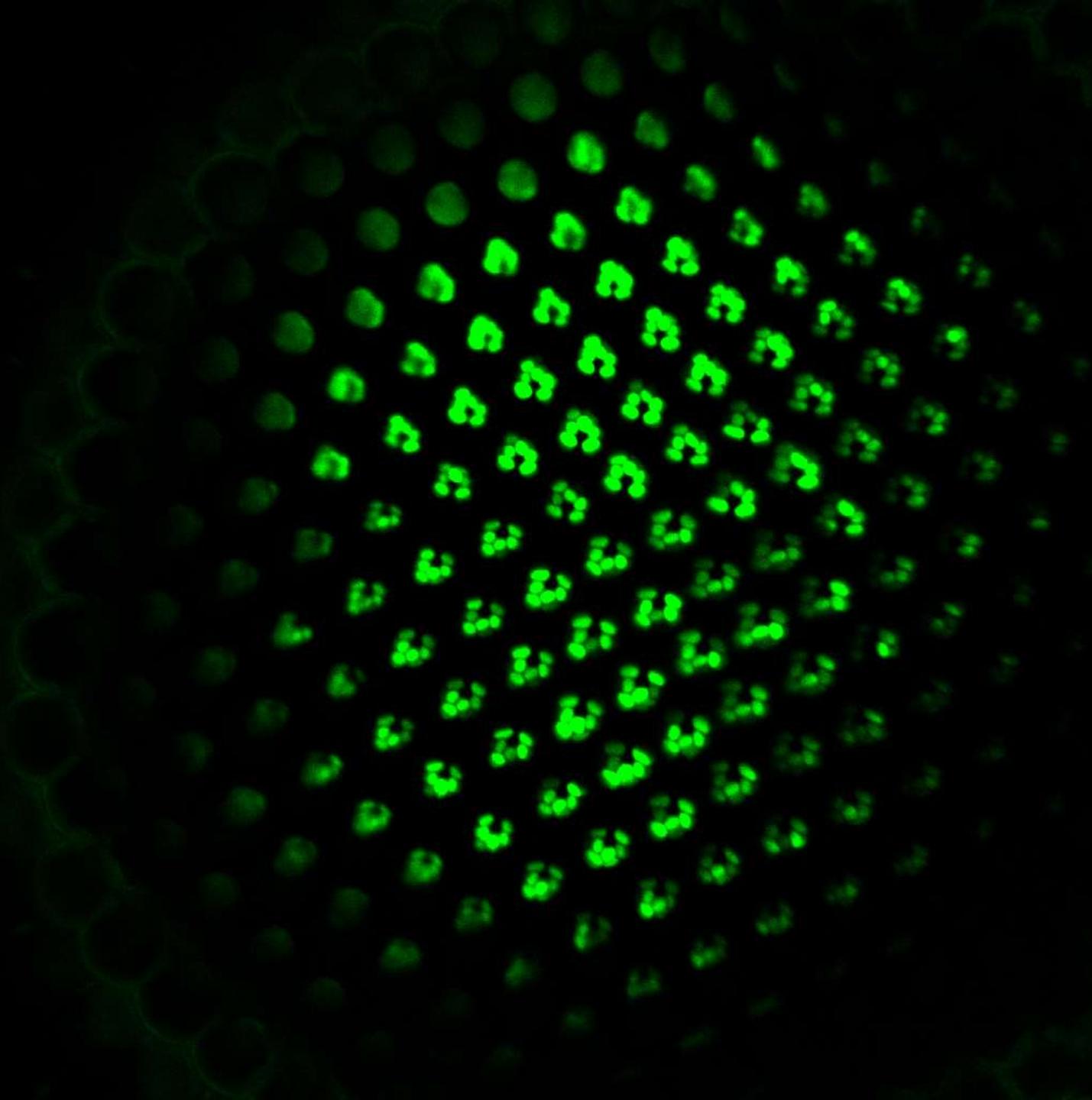


***Identification of new regulators of development
and apoptosis in the Drosophila retina***

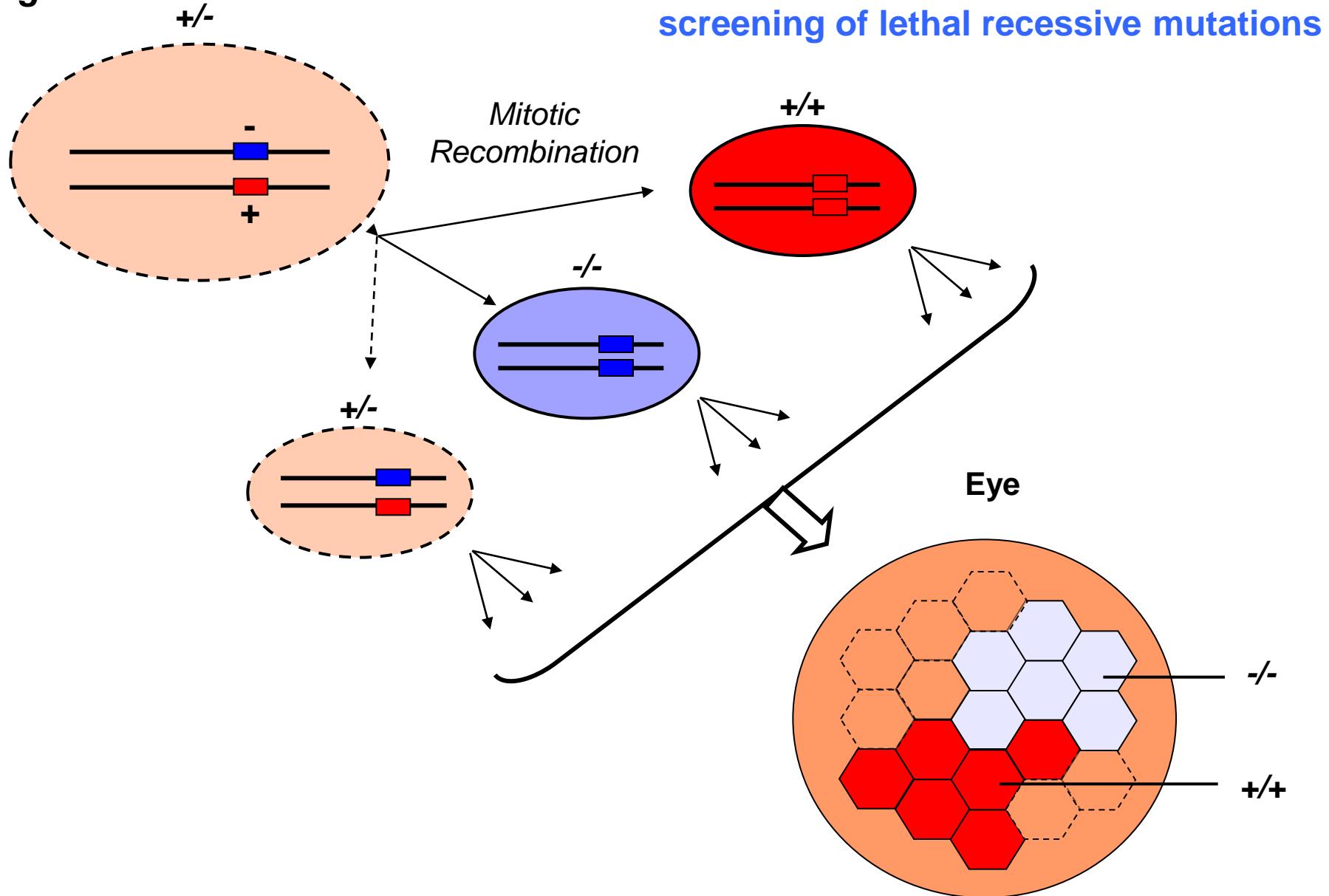
Screen strategy

Cornea neutralization and PR-specific expression of fluorescent protein (Pichaud & Desplan, 2001)

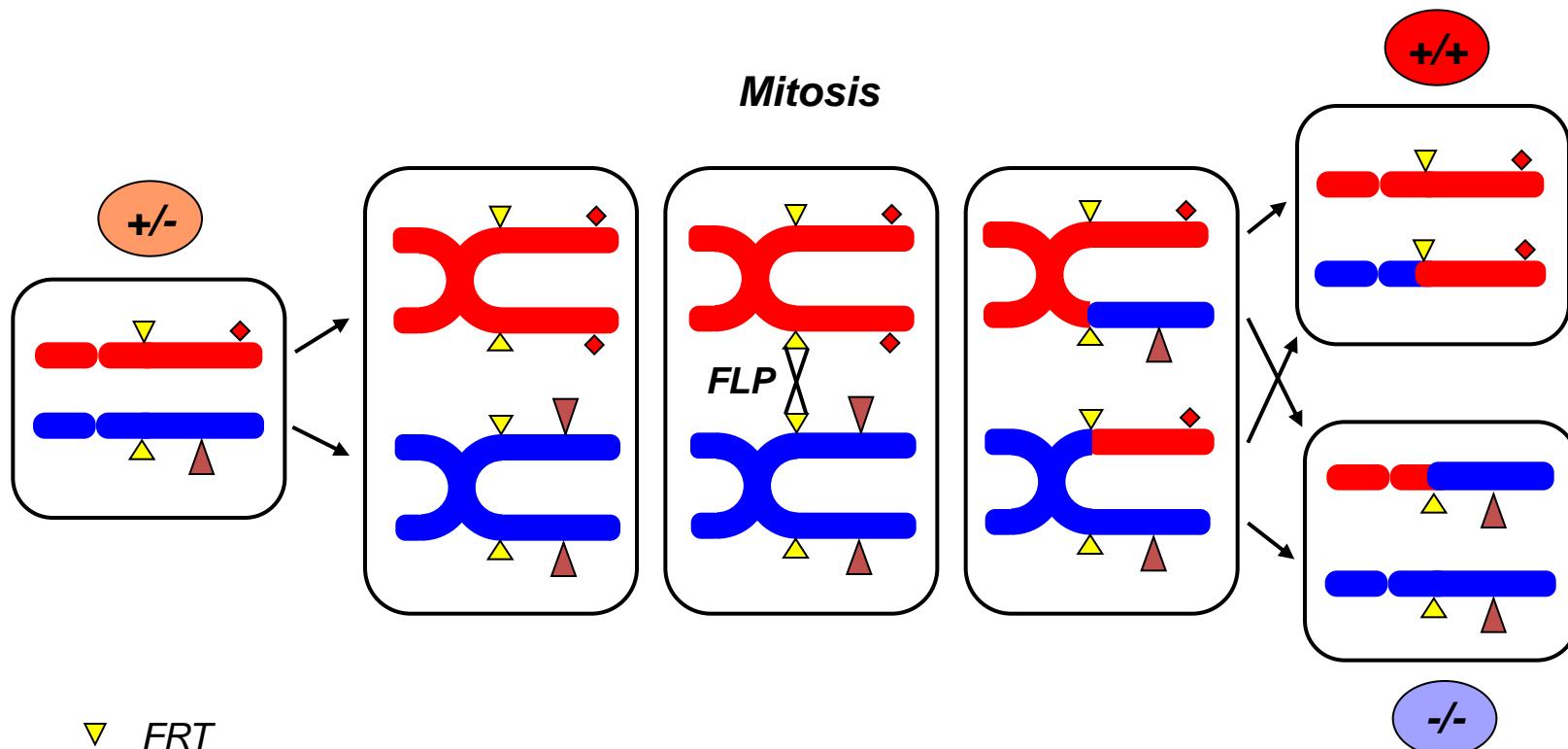




Mitotic recombination and recessive lethal mutant clone genesis in non vital organs



FRT/FLP technique and mitotic recombination



▼ *FRT*

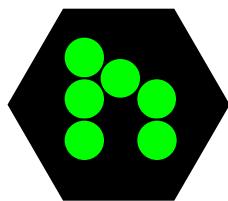
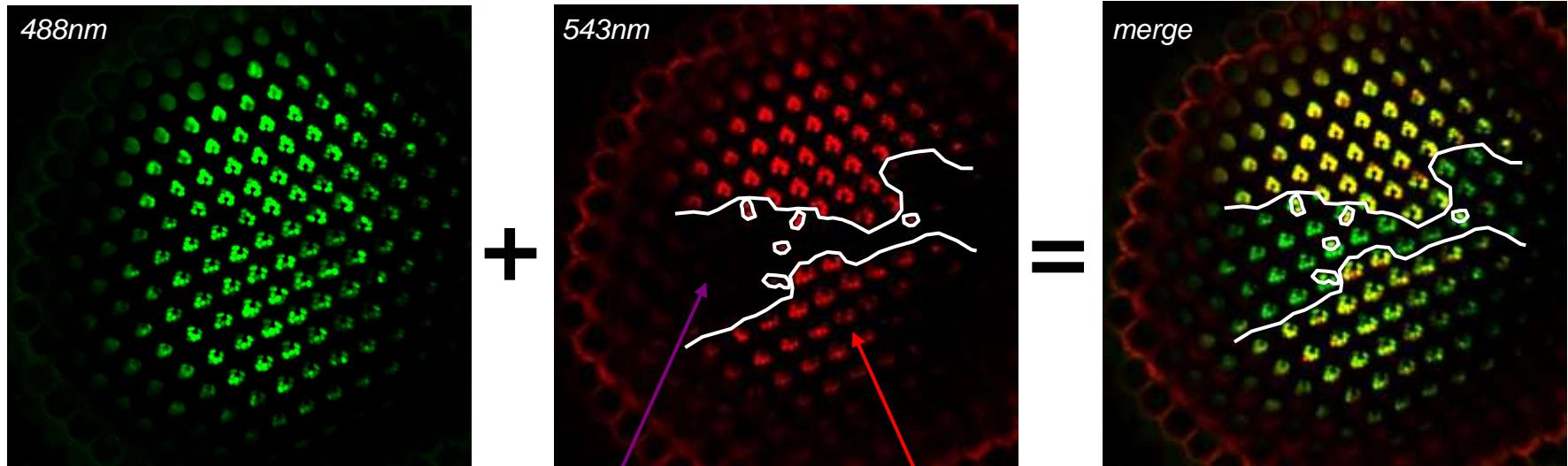
▲ *mutation*

◆ *Phenotypic marker, for example red fluorescent protein*

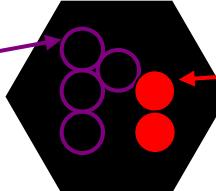
The Tomato/GFP FLP/FRT method of PR visualization

Mitotic recombinaison, cornea neutralization and confocal microscope

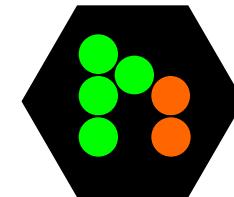
*Rh1-Gal4,ey-FLP; UAS-GFP; FRT rh1-tdTomato/FRT gene**



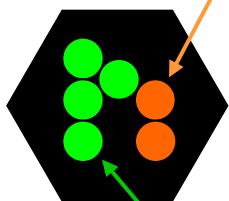
Mutant
PRs -/-



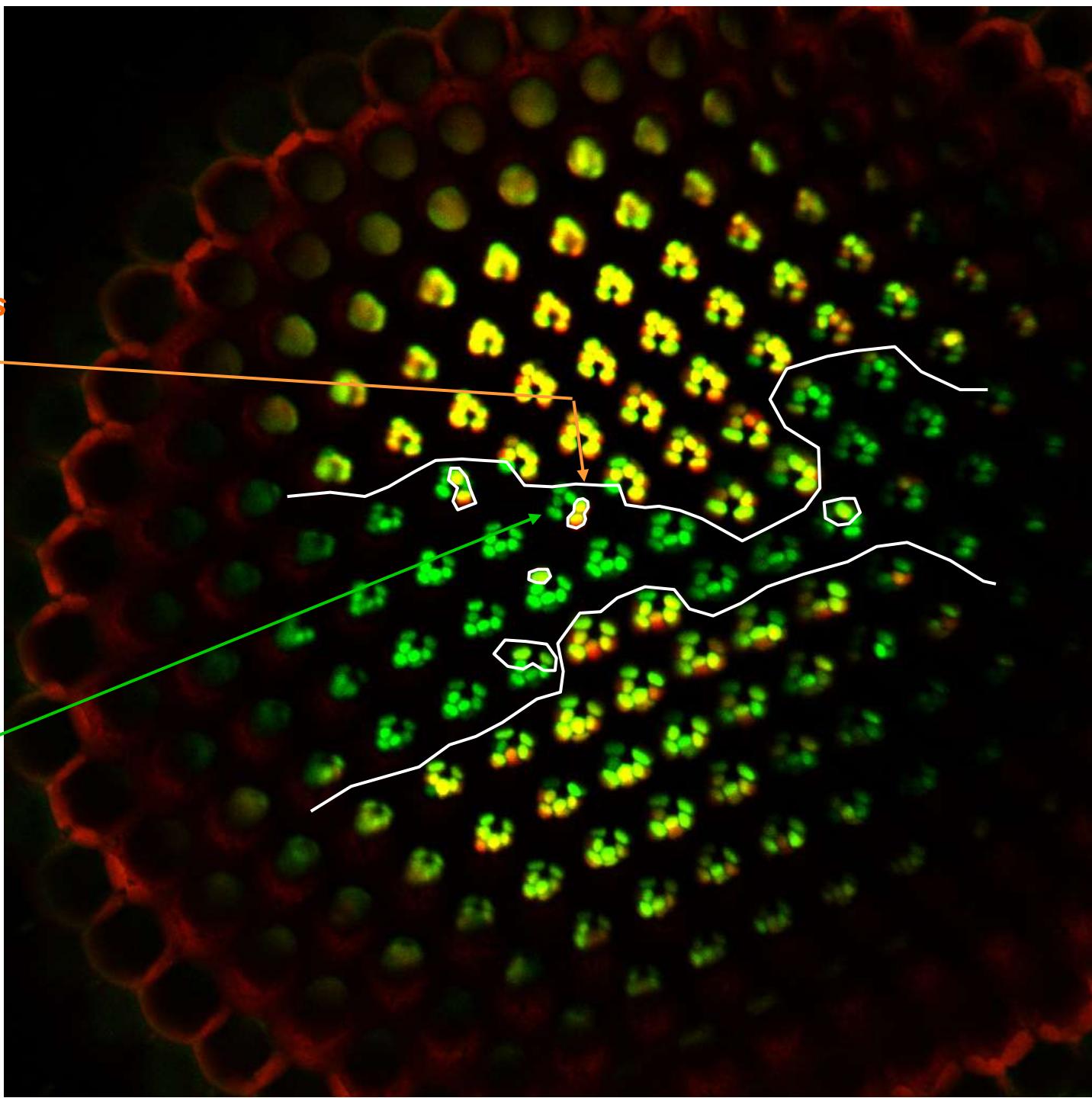
Wild type and
Heterozygous
PRs



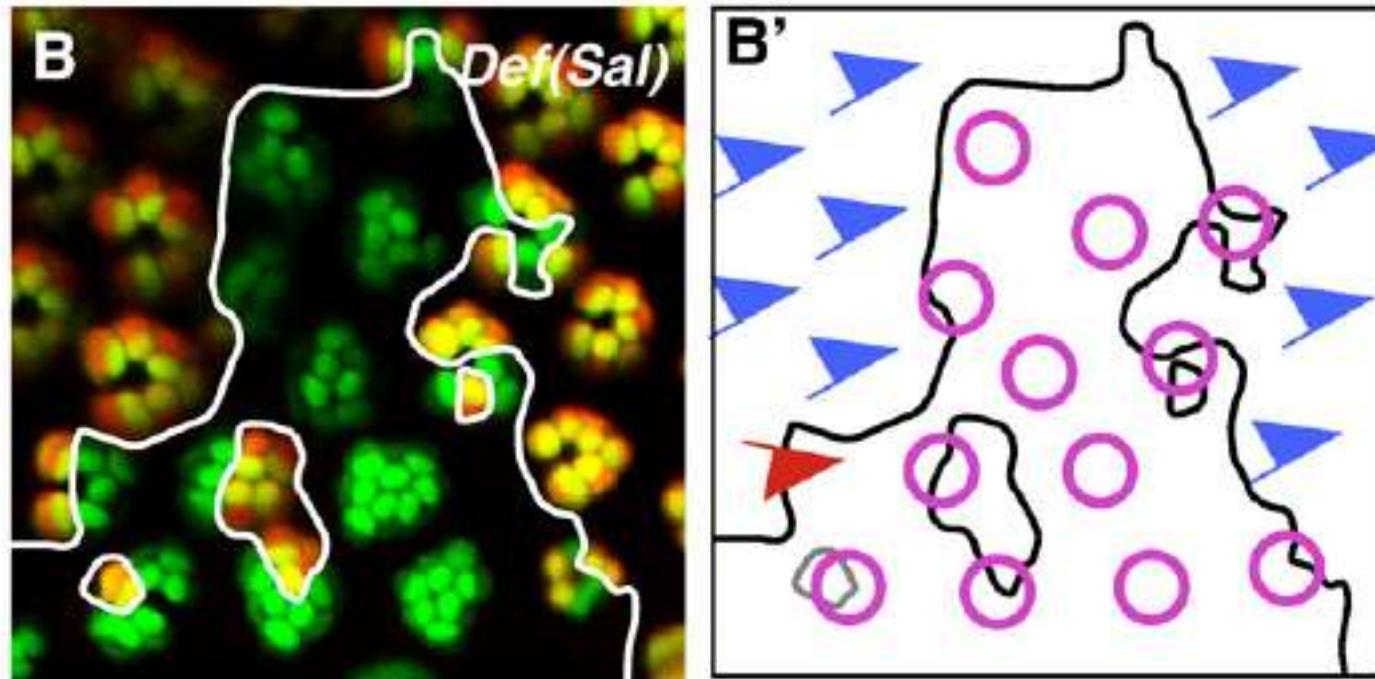
*Wild type and
Heterozygous PRs*



Mutant PRs -/-



Spalt mutants analyzed with Tomato/GFP FLP/FRT method

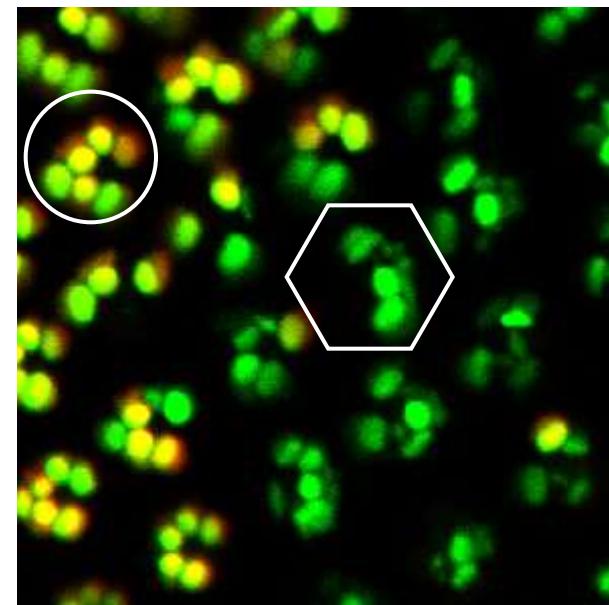
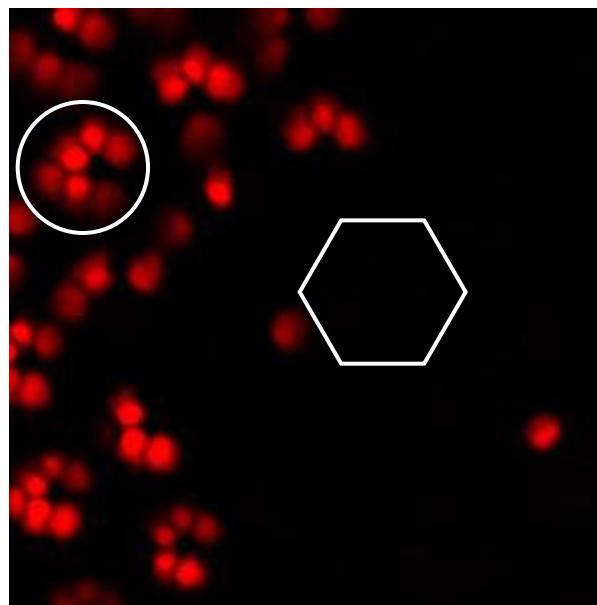
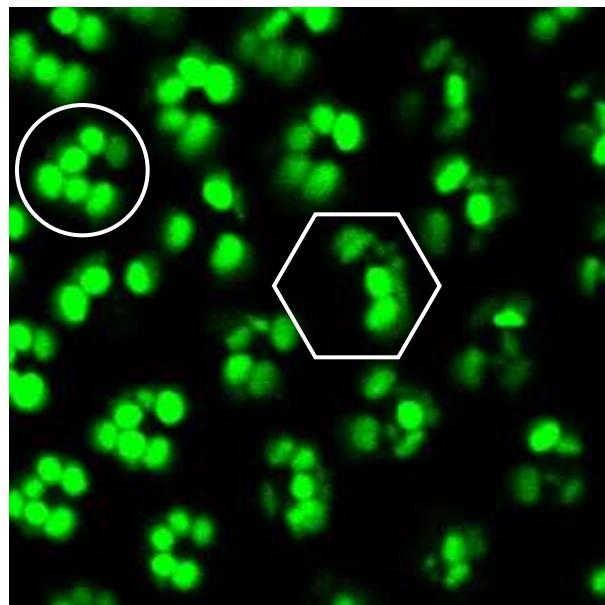


141 mutations with loss of photoreceptors

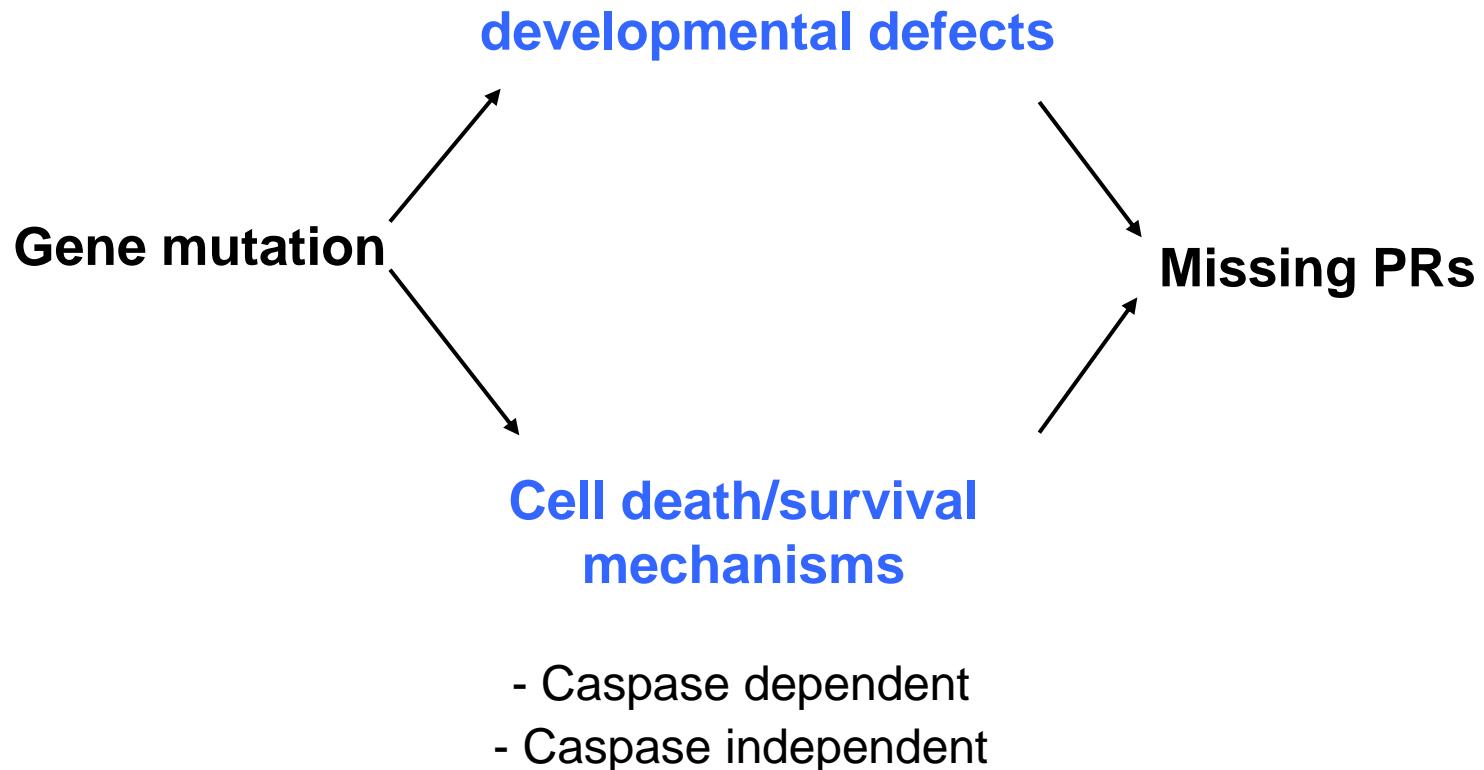
Fatty acid transport protein *Fatp* : missing PRs

+/-

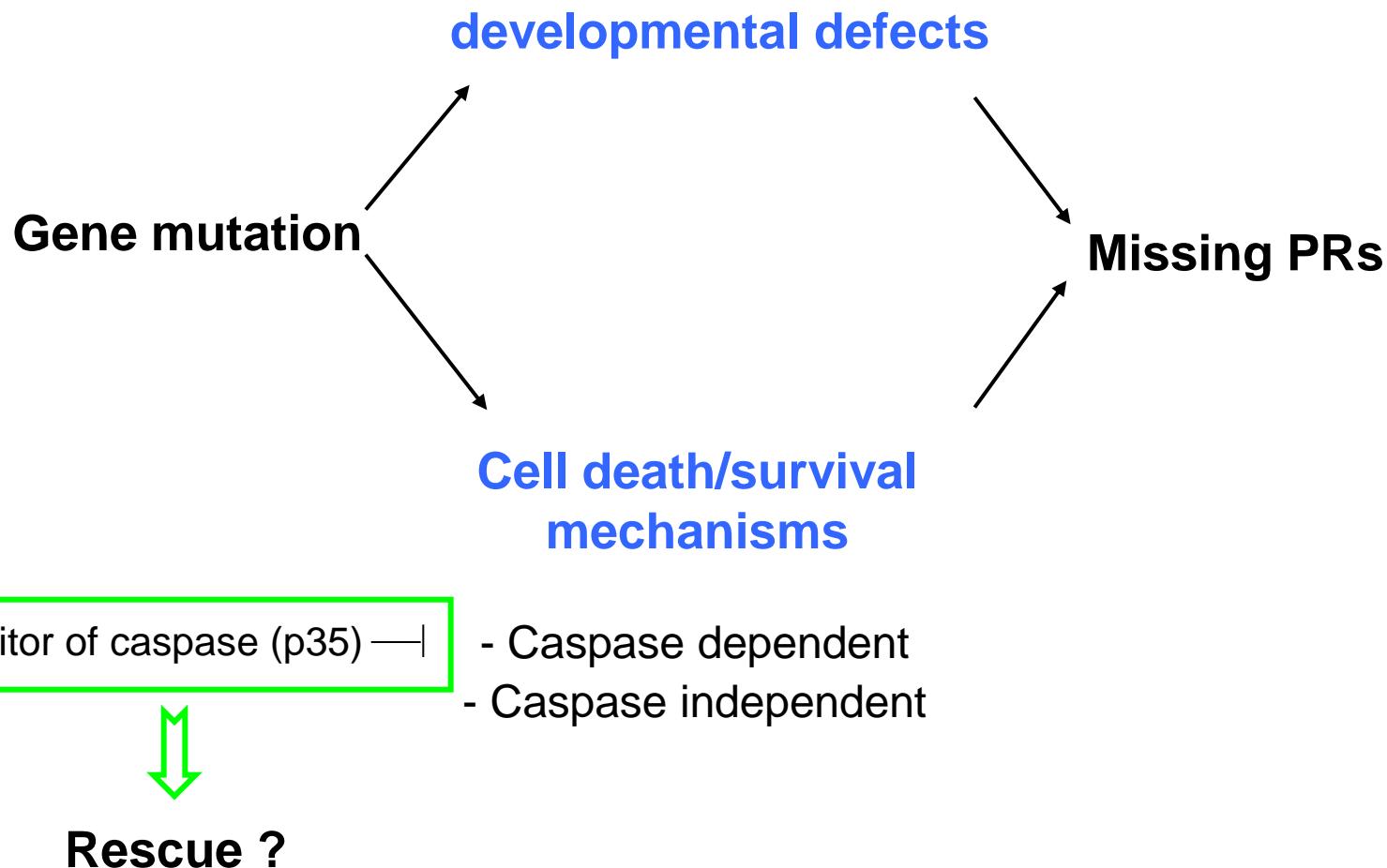
-/-



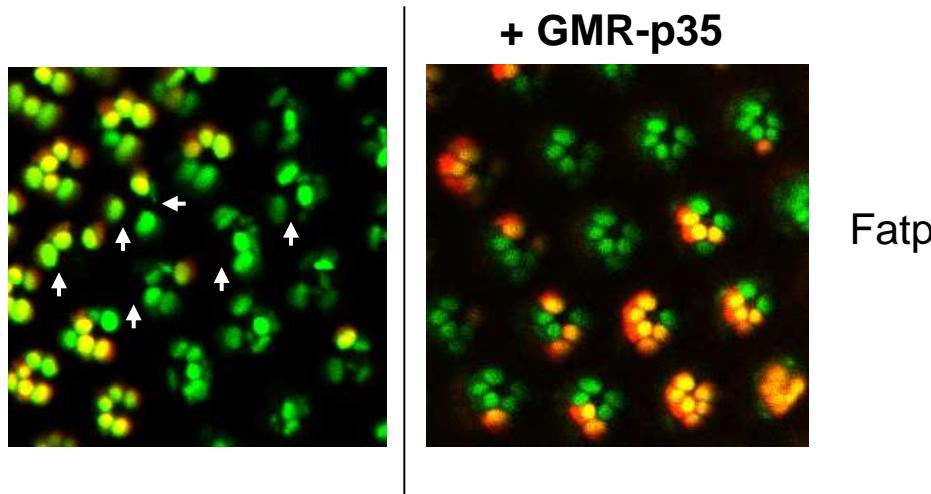
Focusing on mutations leading to caspase dependent cell death



Focusing on mutations leading to caspase dependent cell death



Genes which mutation is rescued by the expression of an inhibitor of caspase



Fatp

Name	function
<i>I(3)06536^{i2E5}</i>	unknown
CG9523	Adénylyltransferase
<i>I(2)k09828</i>	unknown
<i>fatp</i>	Fatty acid transport protein

Characterization of Fatp mutation-induced Neurodegeneration

Fatp : Fatty acid transport protein

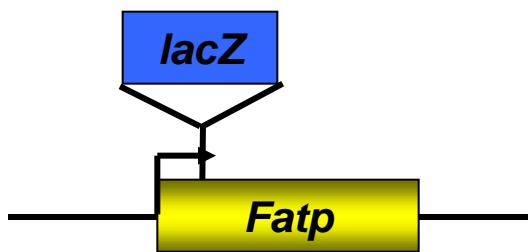
- Unknown in drosophila
- Mammalian homologs ($\approx 50\%$ identity):
 - * Fatp1 : obesity, thermogenesis
 - * Fatp4 : Ichthyosis syndrom

-Molecular activity :

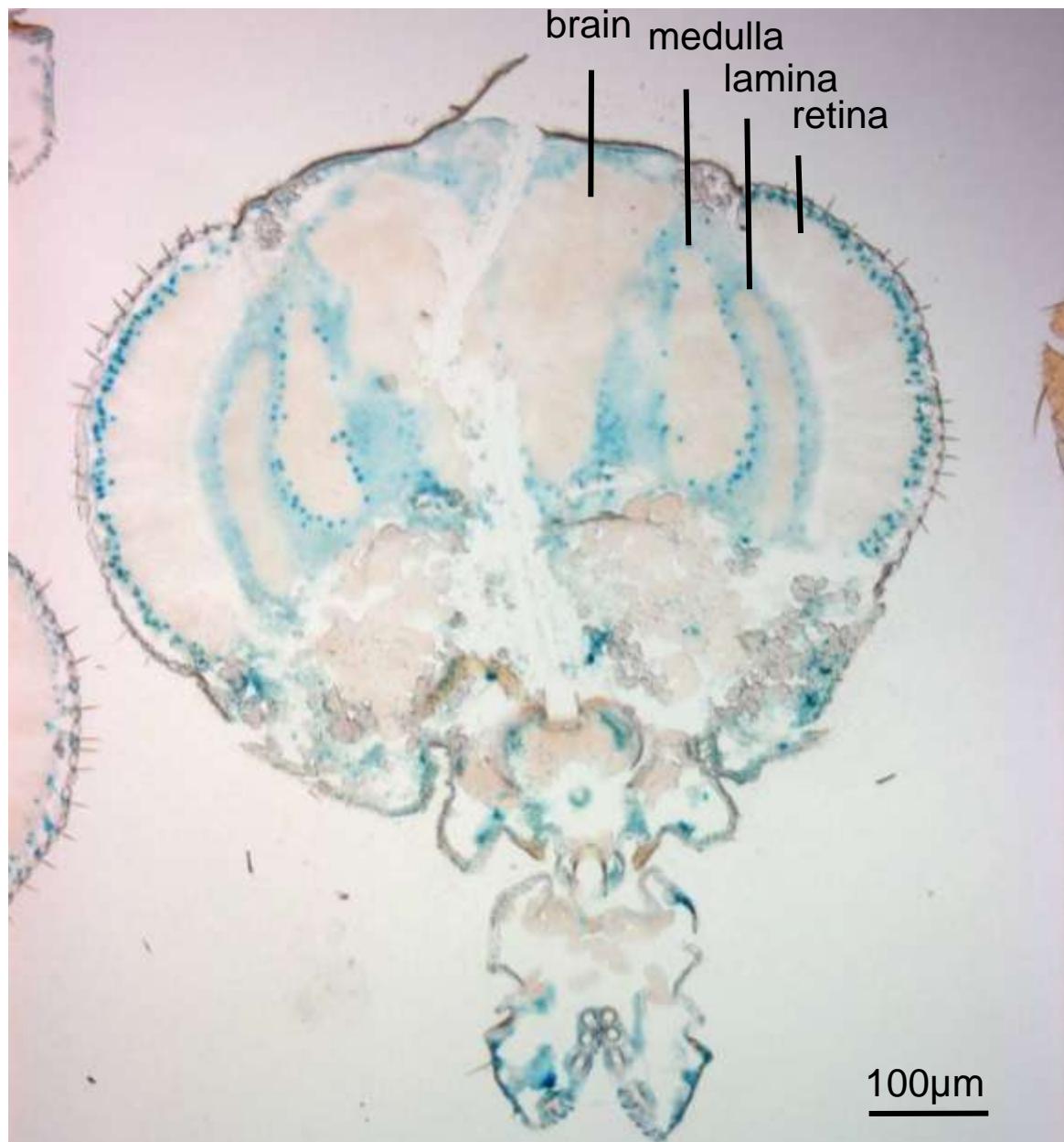
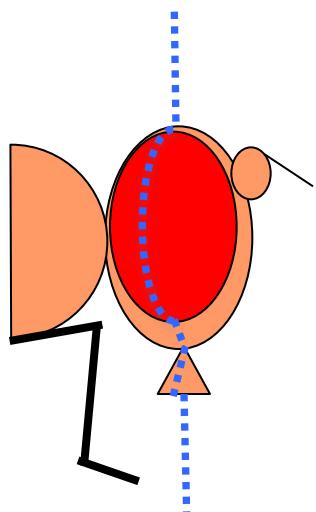
- * Fatty acid transporter
- * Acyl coA synthetase

Fatp expression pattern

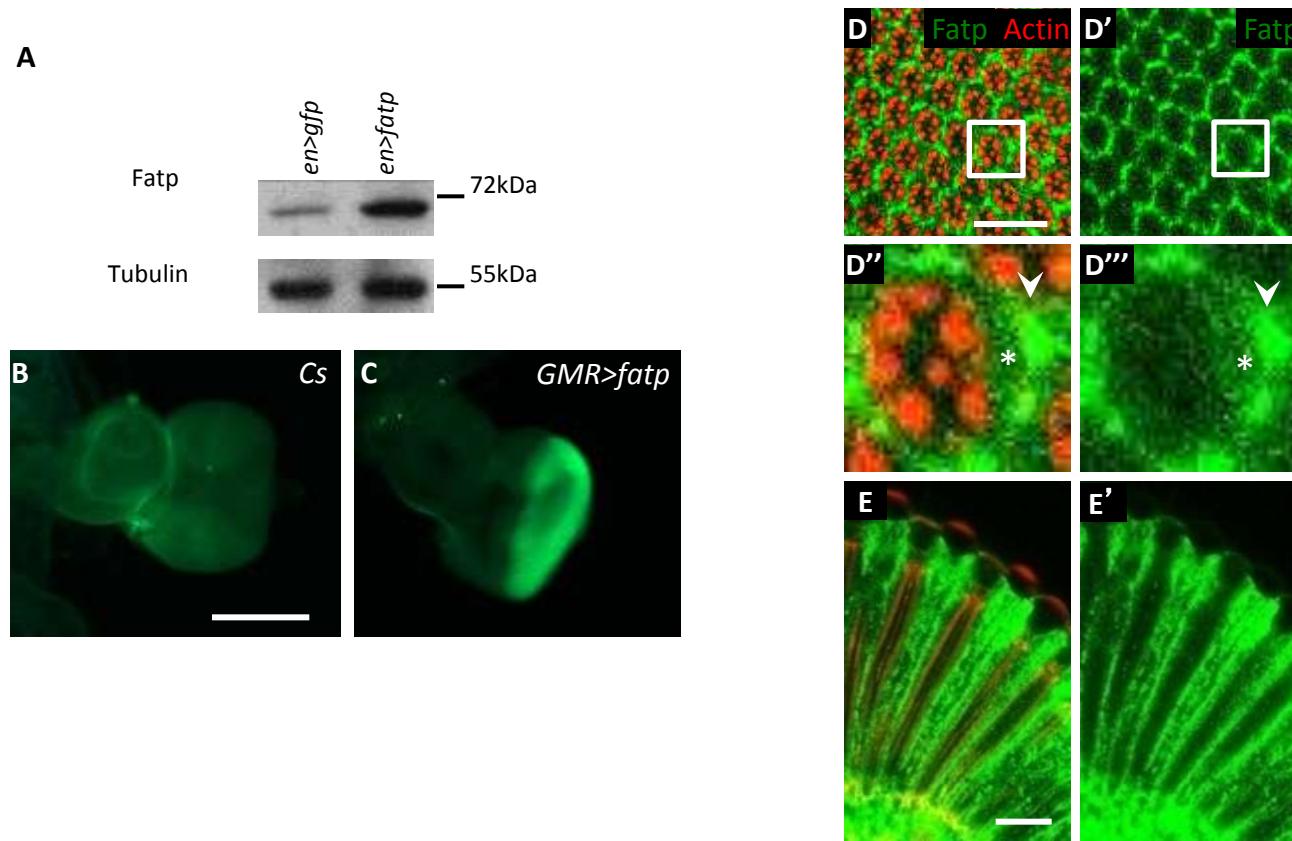
PlacW insertion in *Fatp* gene



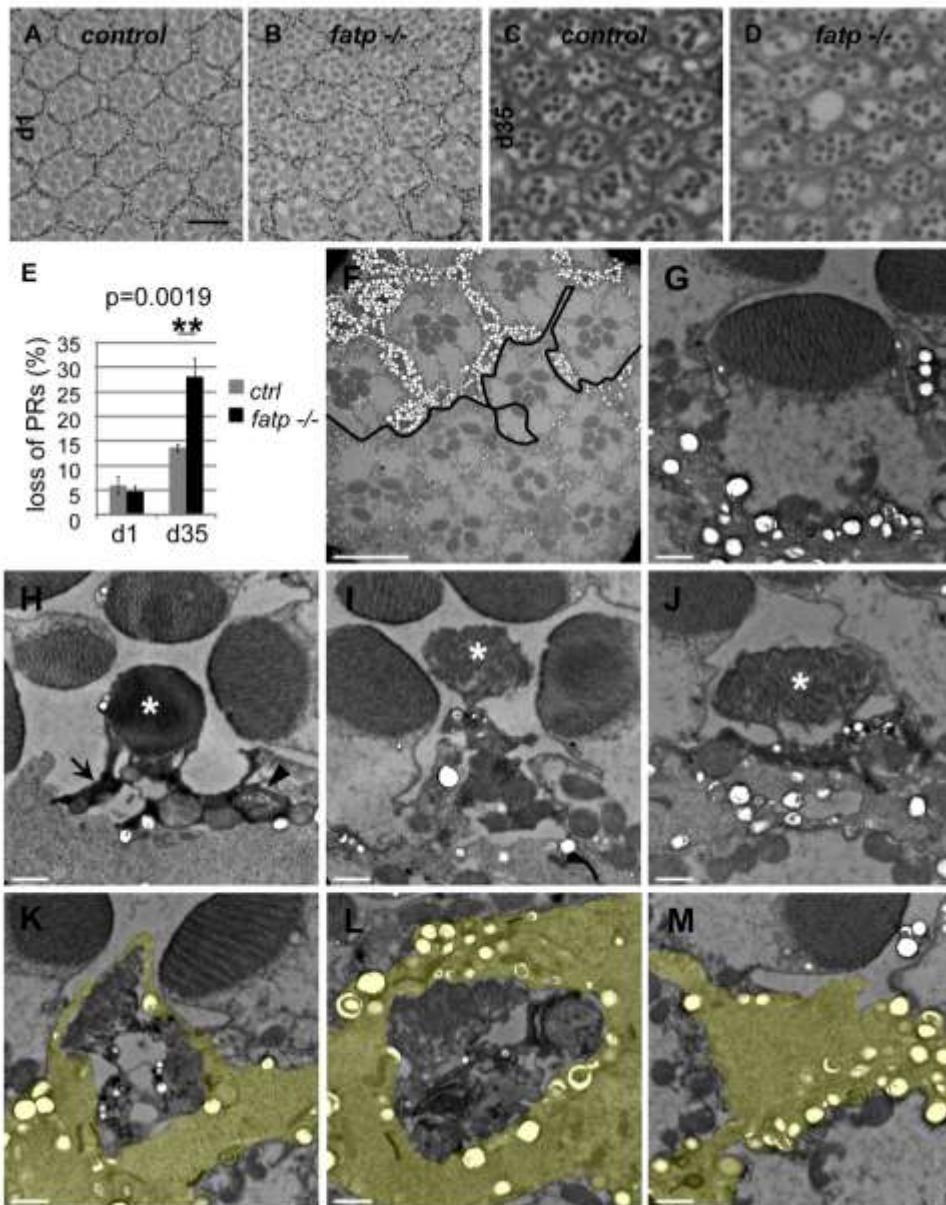
Horizontal cryosection



Fatp is expressed in the adult retina

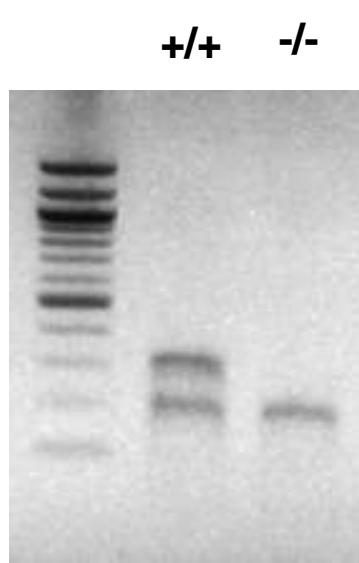


Histological analysis of fatp mutant retina



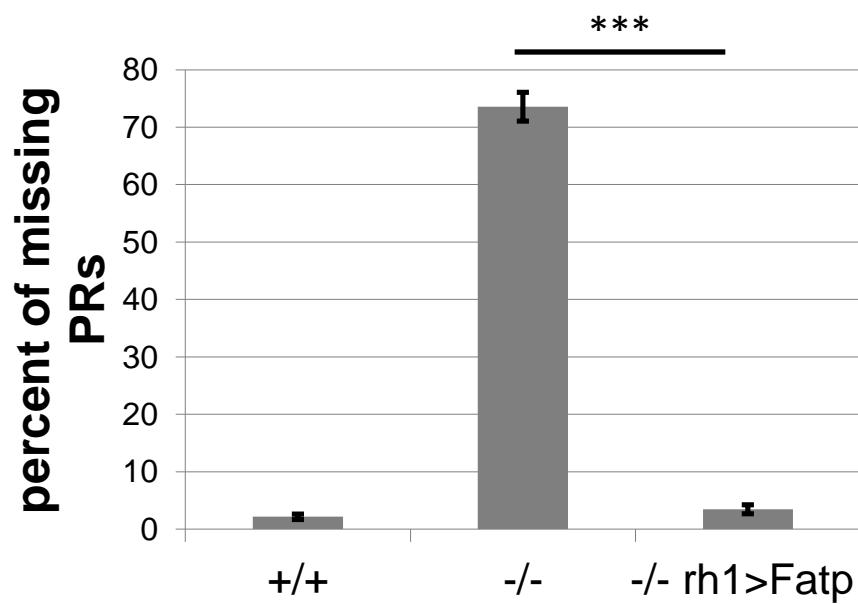
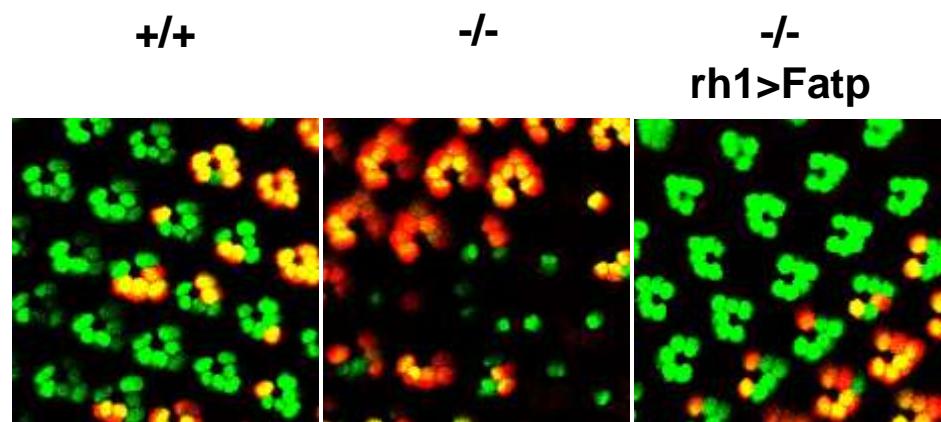
Fatp mutation affects fatp expression

RT-PCR

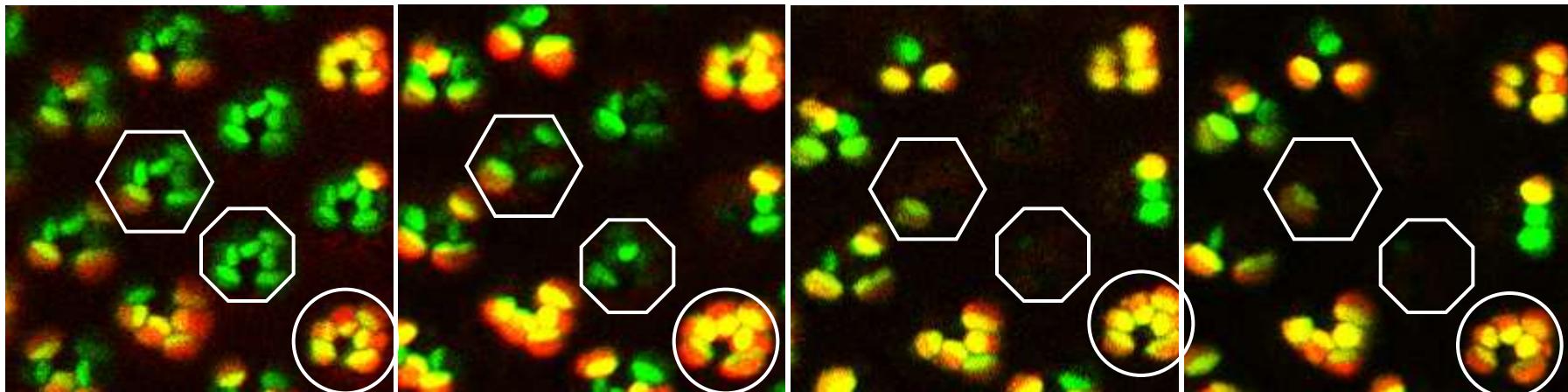


Ectopic *fatp* expression rescues *fatp* mutation

+/+ -/-
15 days



Fatp mutation induces progressive photoreceptor degeneration during adulthood

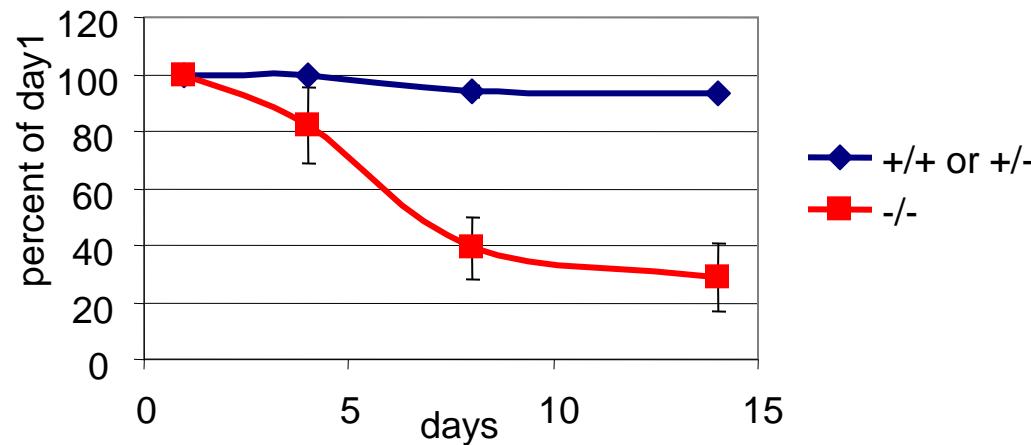
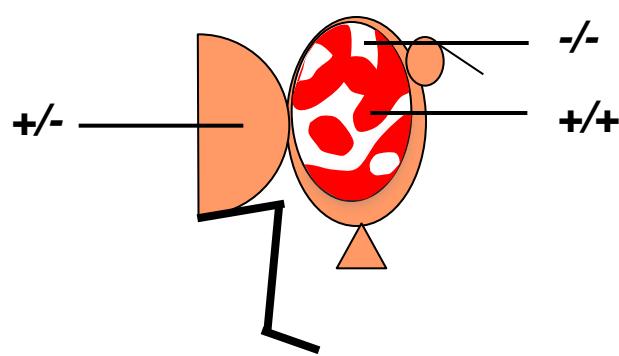


1d

4d

8d

14d

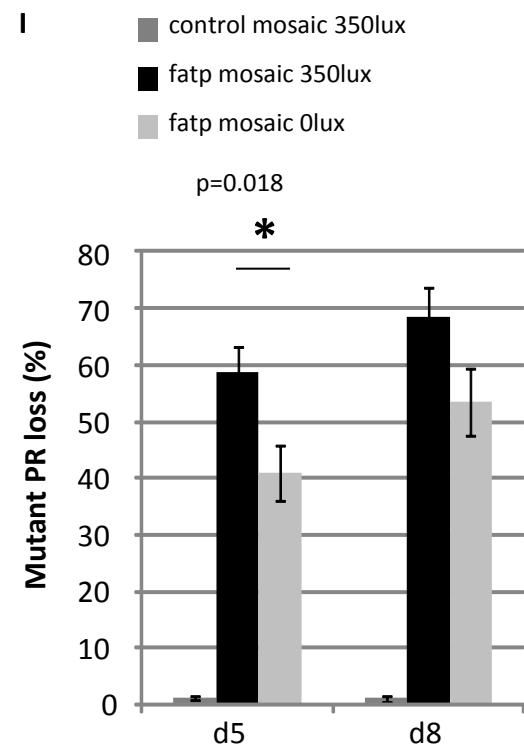
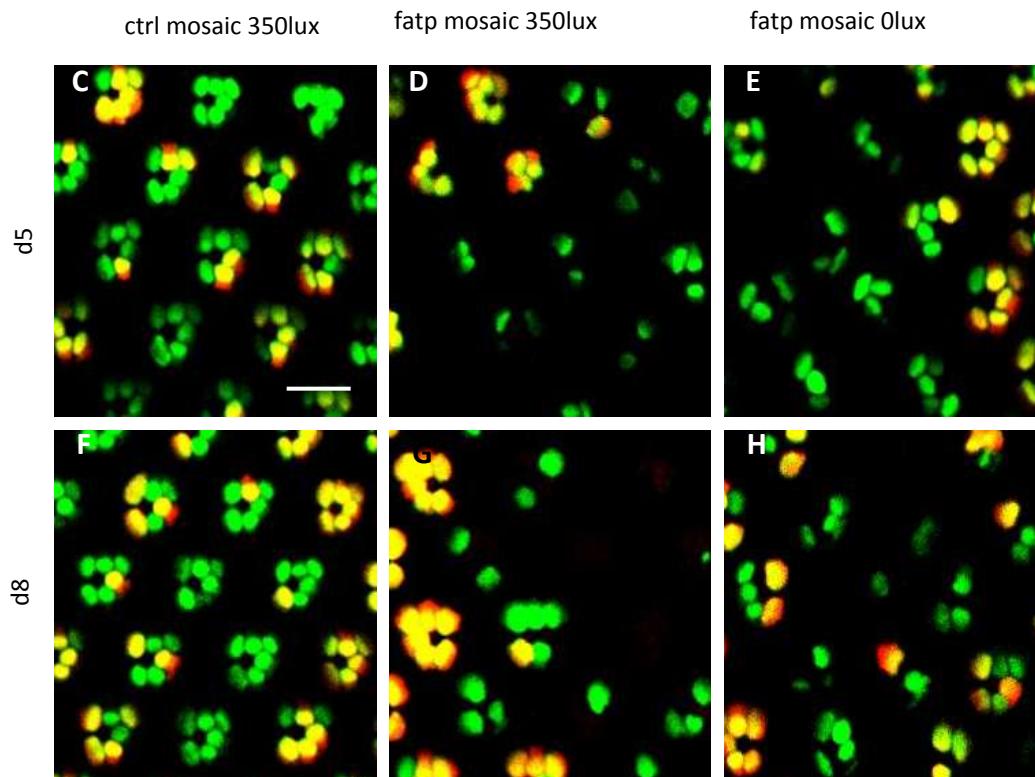


Confirmation of the progressive neurodegeneration:

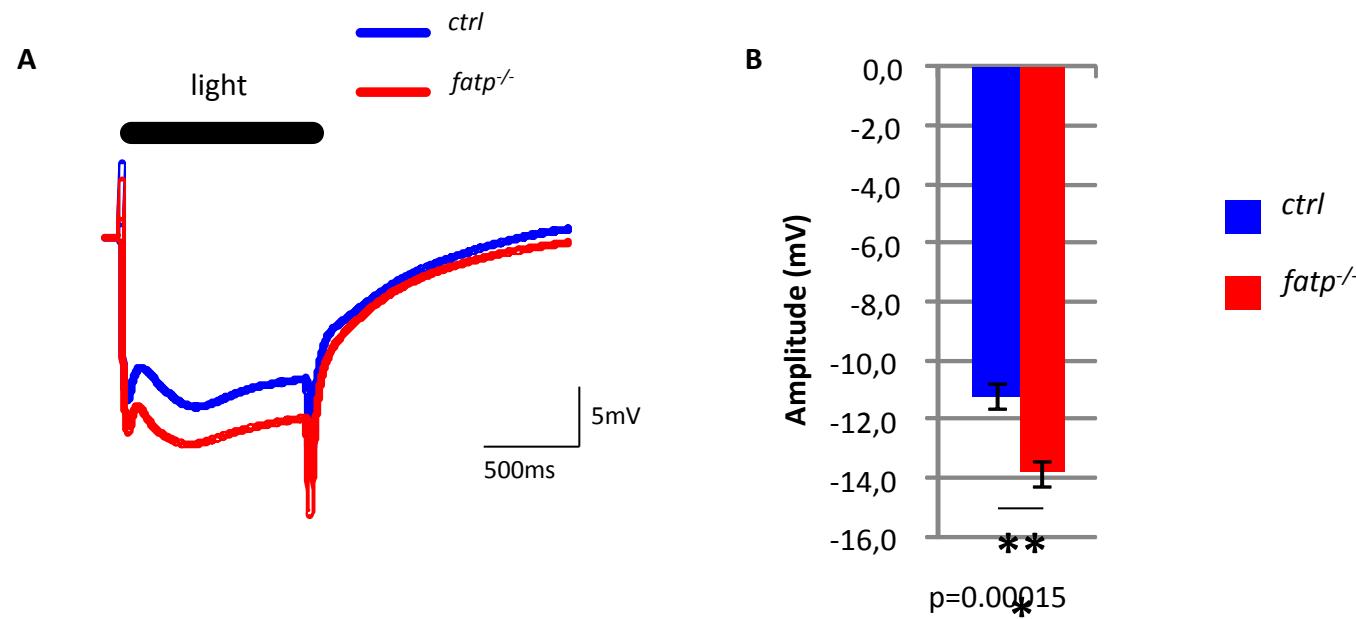
- Resin-embedded section

- RNAi

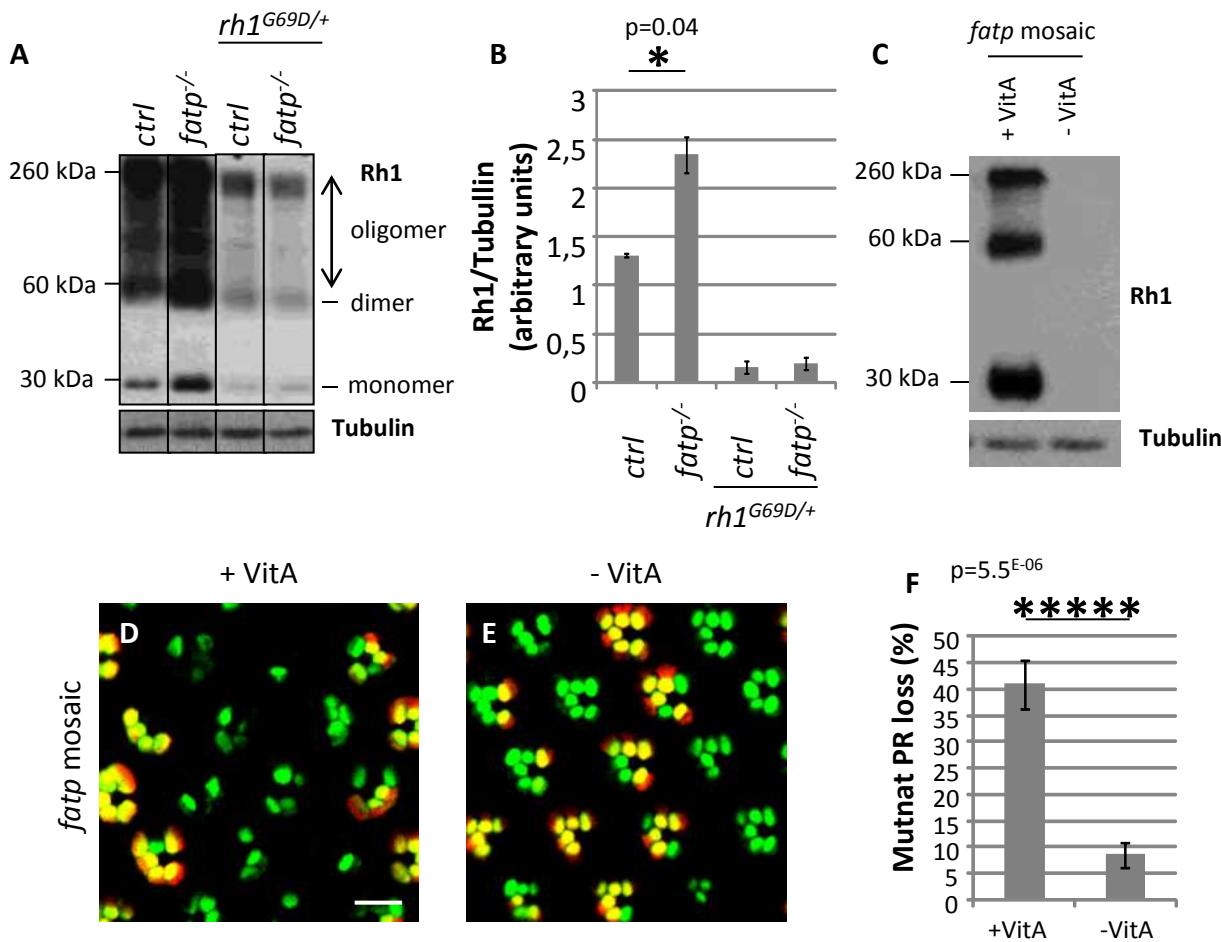
Photoreceptor degeneration is light dependent in fatp mutant retina



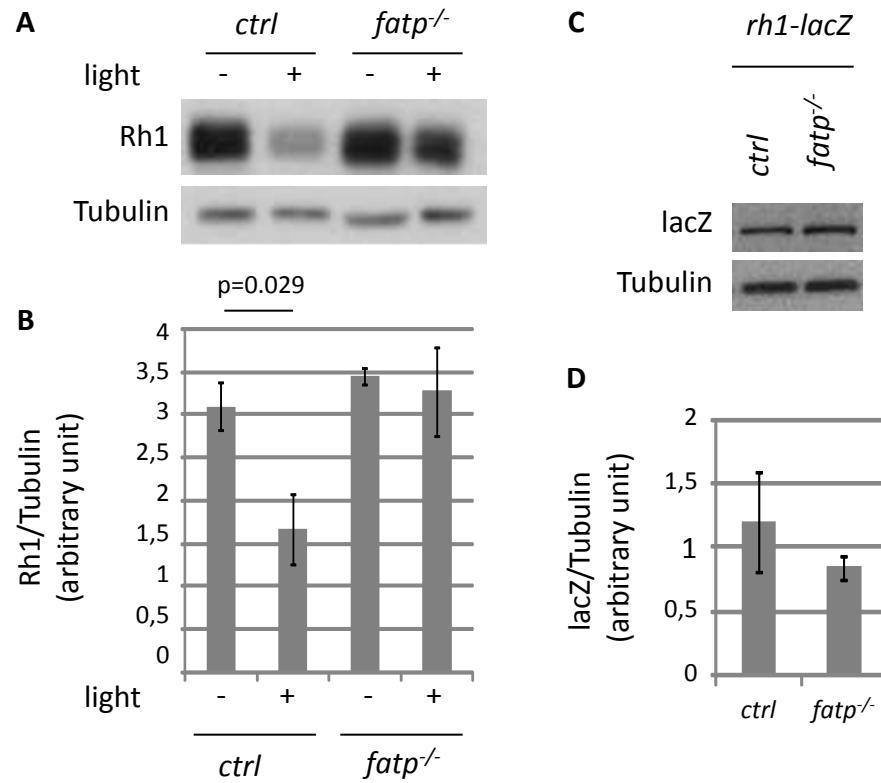
*Visual response is increased in *fatp* mutant retina*



*Rh1 expression is increased in *fatp* mutant retina*



***Rh1* degradation is compromised in *fatp* mutant retina**



Conclusions Part II

- **Fatp mutation:**

- ¤ affect fatp expression in the retina
 - ¤ Is responsible for PR degeneration

- **Fatp mutation induced degeneration :**

- ¤ adult onset
 - ¤ progressive
 - ¤ caspase dependent, rescued by caspase inhibitors
 - ¤ light independent
 - ¤ PR degeneration due to an increased Rh1 and increased visual response



Acknowledgments



Lab members

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Marion Robin

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Antoine Ducuing

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César Mendes Ph.D

Pedro Domingos PhD

Alexis Gambis PhD

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Phototransduction in Drosophila

