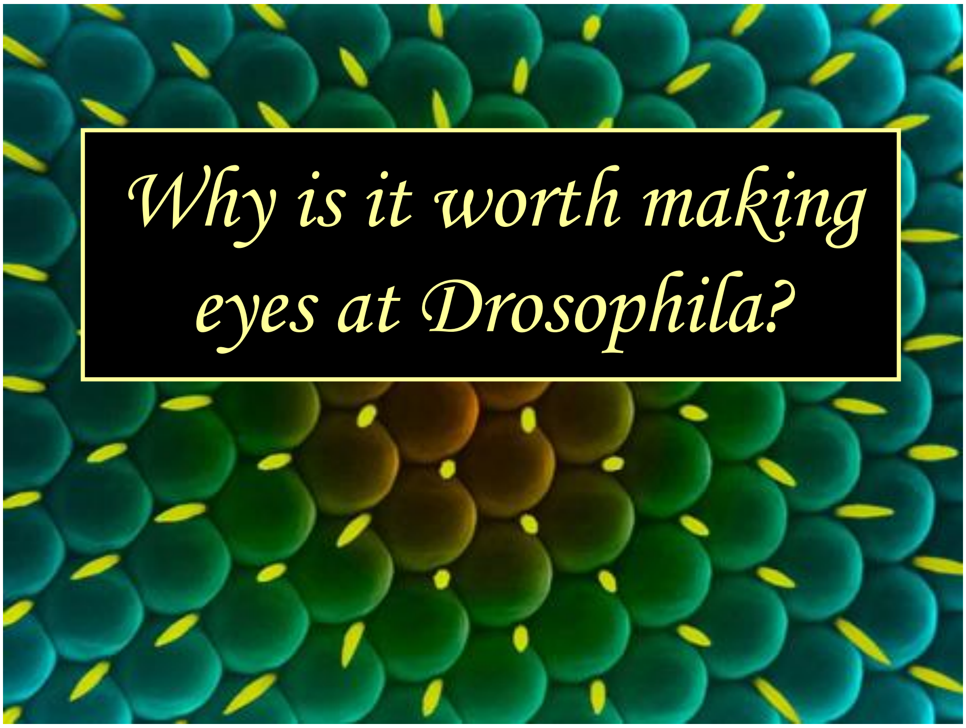
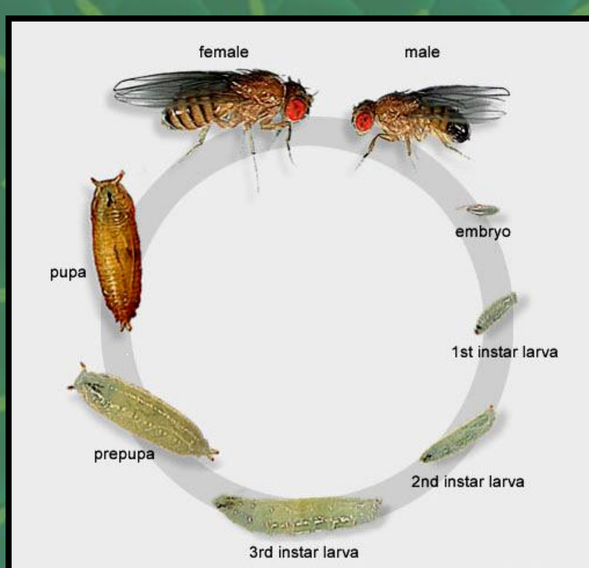


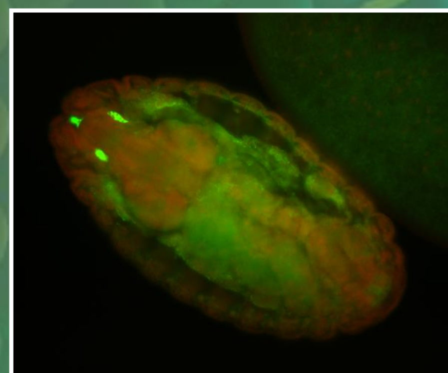
Life cycle of *Drosophila melanogaster*



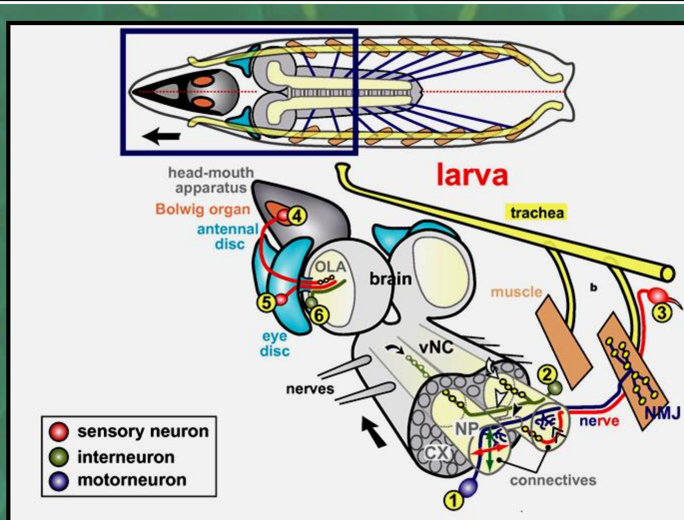
Bolwig's organ

Niels Bolwig (1911 – 2004)

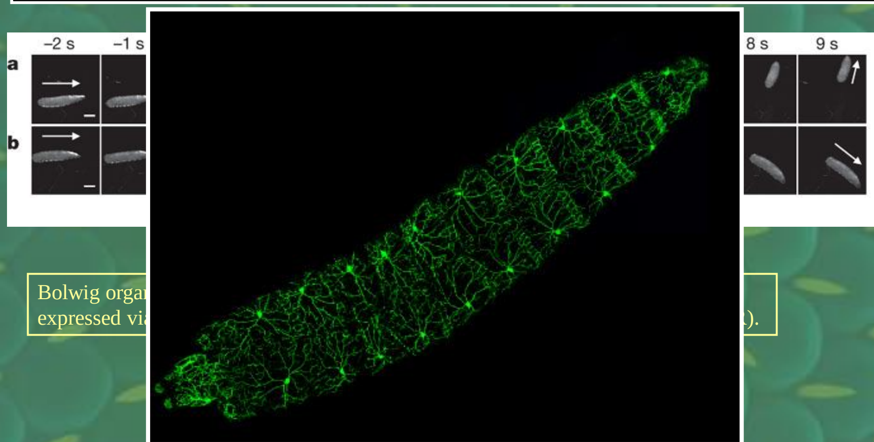
GMR: glass multiple reporter promoter elements, has been commonly utilized to express target transgenes, specifically in the developing eye



Bolwig's organ in the larva



Maggots are all eyes

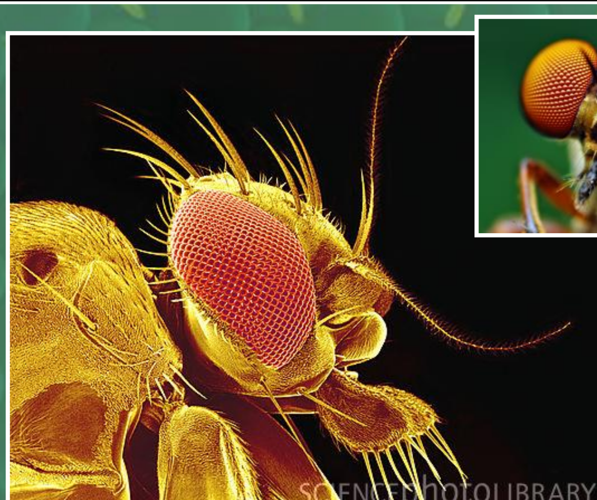


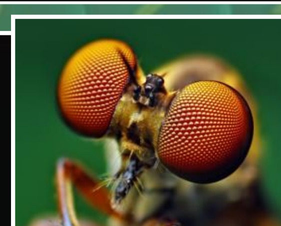
Bolwig orga...
expressed vi...).

The authors conclude:
Our study has uncovered unexpected light-sensing machinery, which could be critical for foraging larvae to avoid harmful sunlight, desiccation and predation.

Xiang et al,, Nature 2010

Let’s see the compound eye!

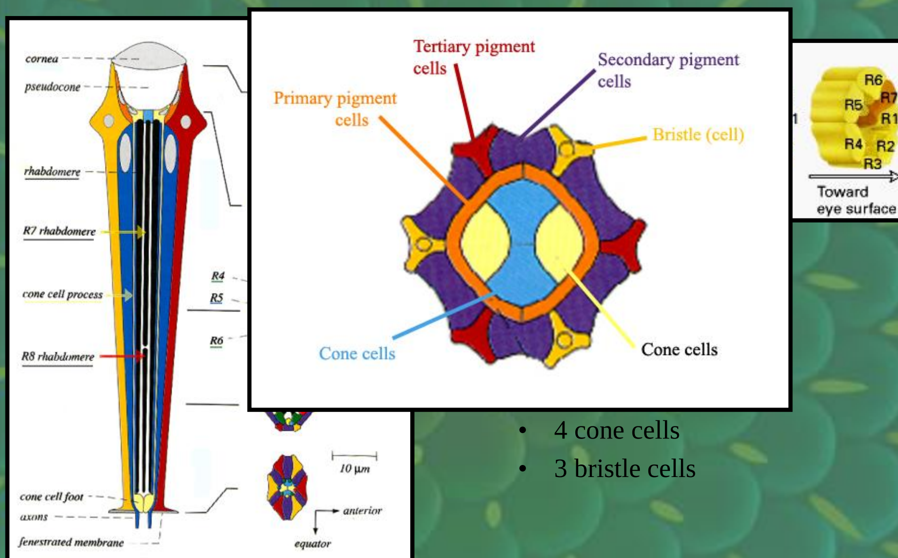




Dragonfly: 10 000 ommatidium

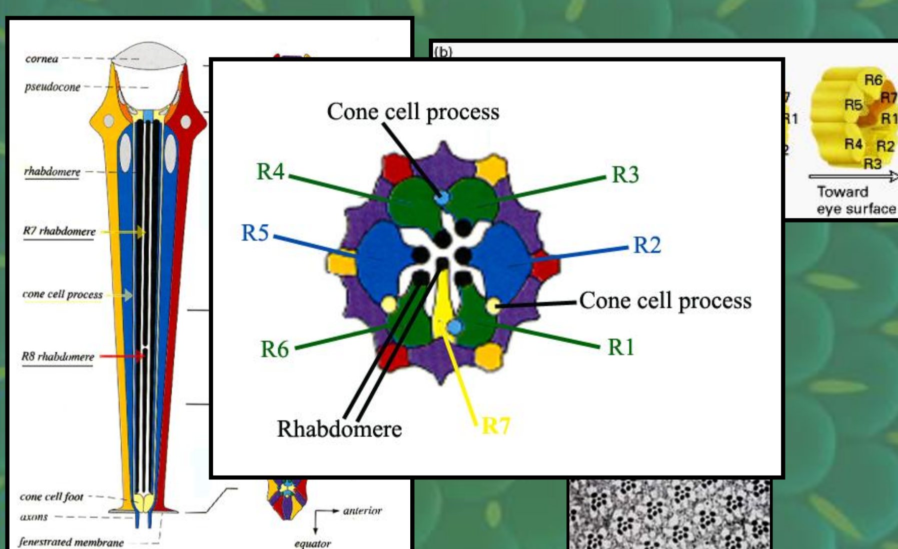
Drosophila: 750-800 ommatidium

An ommatidium of Drosophila eye

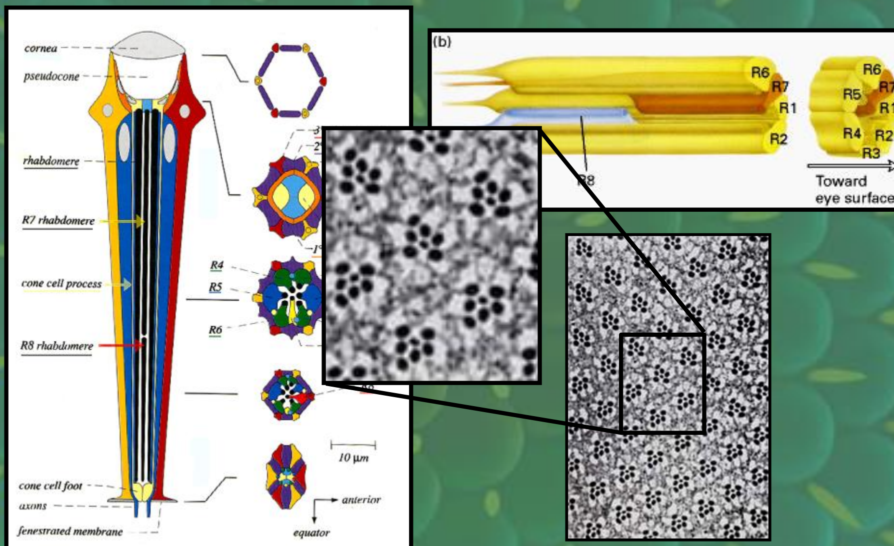


- 4 cone cells
- 3 bristle cells

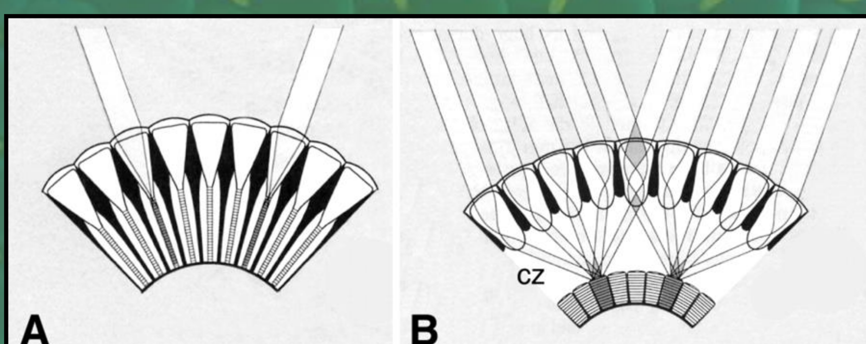
An ommatidium of Drosophila eye



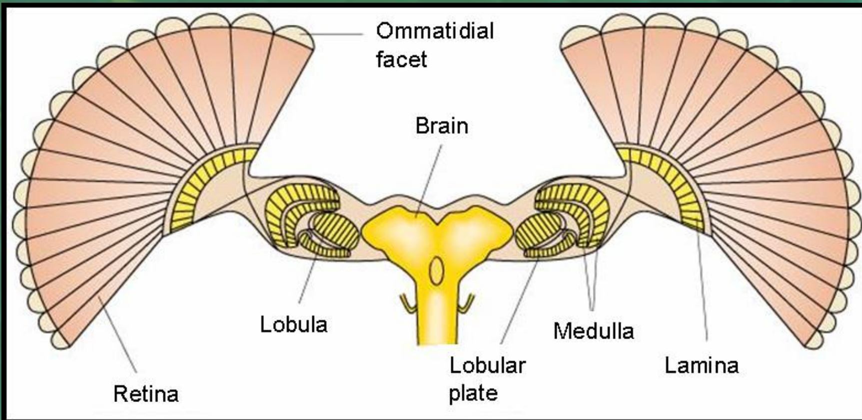
An ommatidium of Drosophila eye



Apposition and superposition eyes



Cross-section of eyes and brain



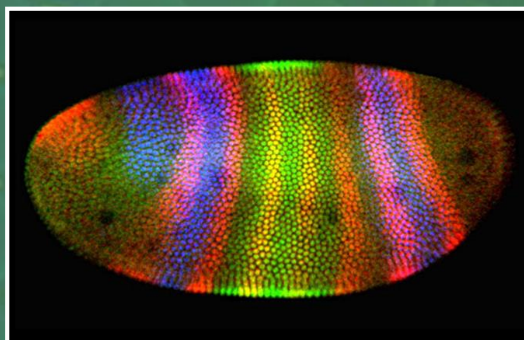
The compound eye as the model

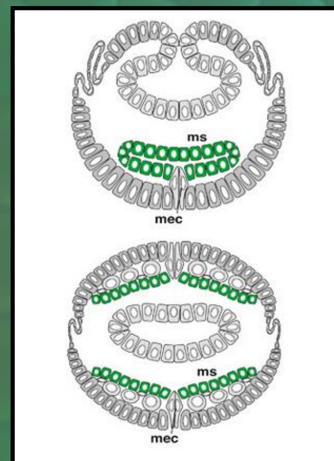
Making cells different from one another and assigning them to the right places are central to organ formation.

In the *Drosophila* visual system, the compound eye two themes emerge: the interplay between cell signaling and cell competence generates diversity of cell types and selective cell adhesion determines diversity of cellular patterns.

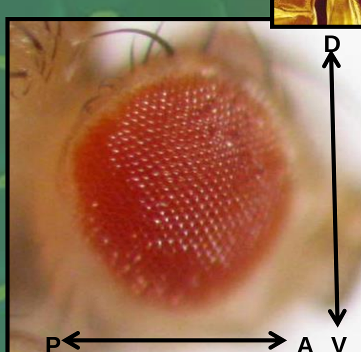
Sujin Bao, 2010

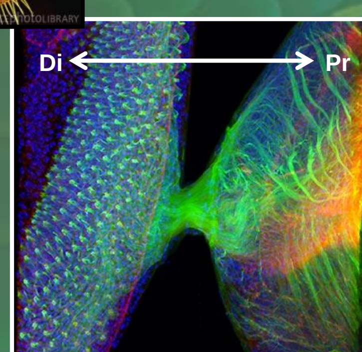
Axis polarity: in 2D



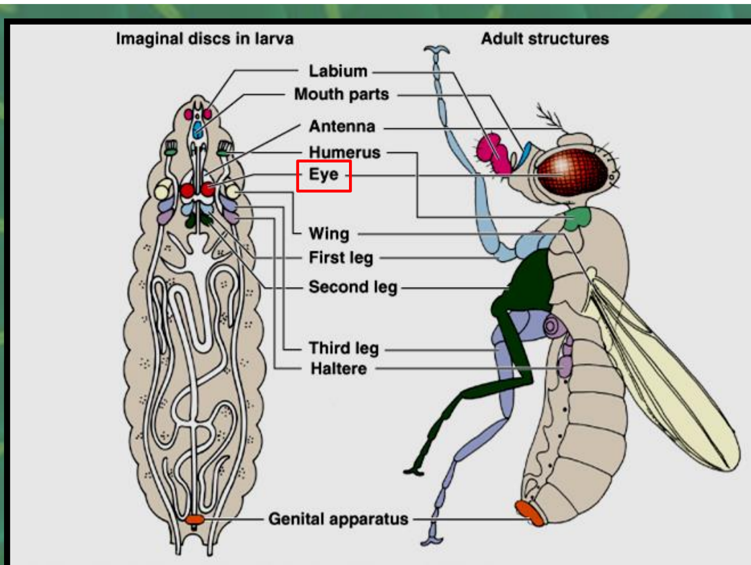


Organization of eye in 3D

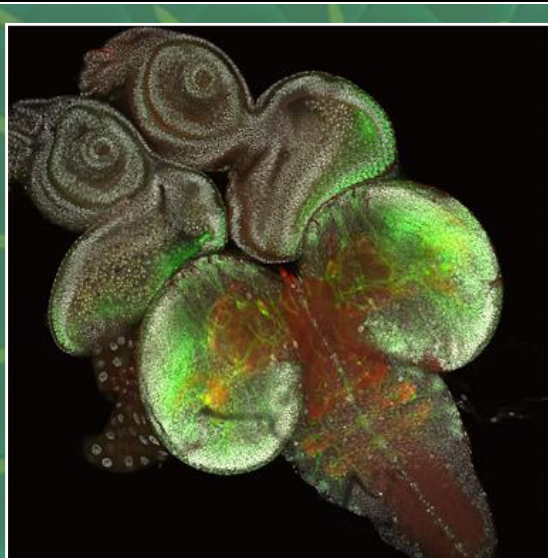




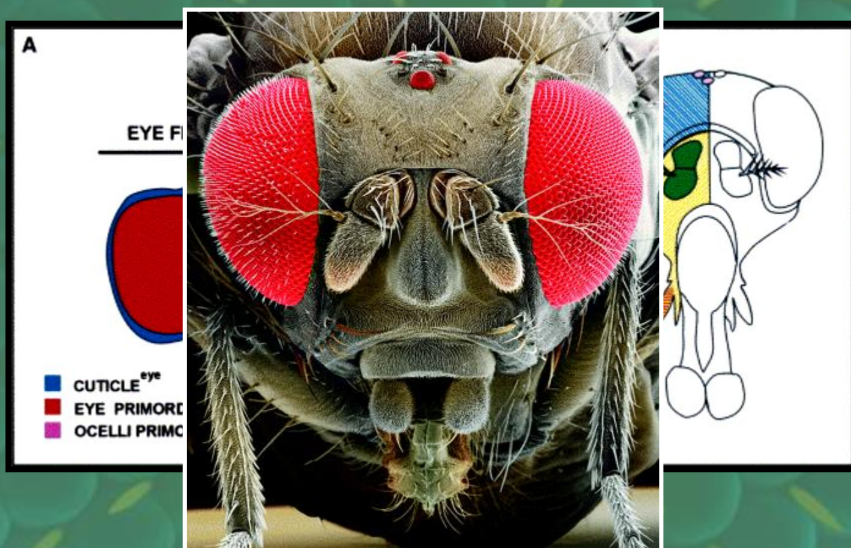
Imaginal discs



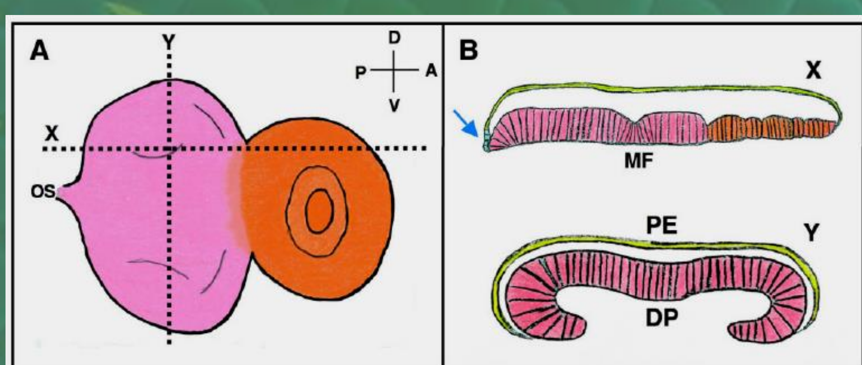
The eye-antenna discs and the brain



Derivates of E-A disc



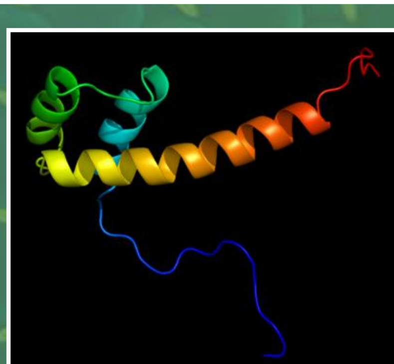
Sections of eye-antenna disc



Steps from the disc to the eye

- Activity of master genes determines the eye fate of the disc
- Dorsoventral polarity of the eye
- The random cell division synchronized
- Antero-posterior polarity
- Morphogenetic furrow forms
- Distal-proximal polarity
- Notch signaling activates the proneural genes
- The proneural clusters of 12 cells form
- The cells of peripodial membrane designe the R8 cells
- Determination of R2-R5, R3-R4, R1-R6 cells
- Selection of R7 cell
- Expression of various rhodopsins by different R cells
- Differentiation of non-neural cells
- Formation of the rhabdom
- Apoptosis in the eye

Master (switch) genes : *ey* and *toy*



PAX 6

Four *Drosophila* Pax6 orthologues:
eyeless (ey)
twin of eyeless (toy)
eyegone (eyg)
twin of eyegone (toe)

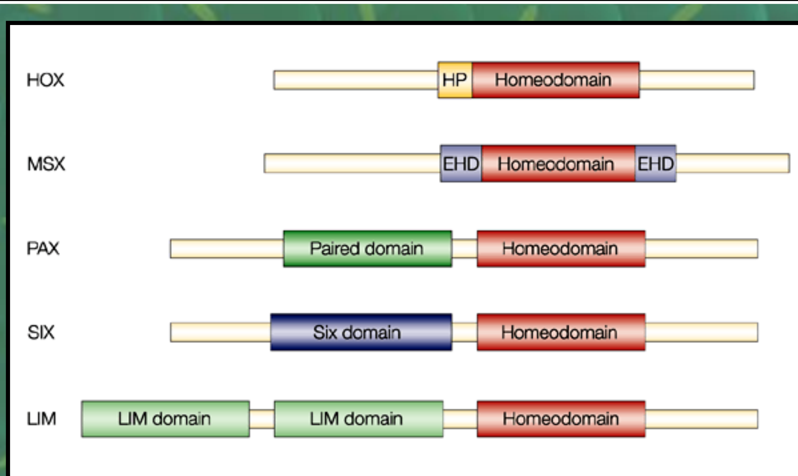
Eyeless (ey)

Mildred Hoge, 1915

Ectopic overexpression of *ey*

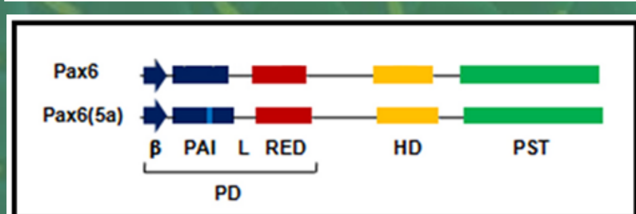
Ey: paired box (pax) gene family, tissue specific transcriptional factors

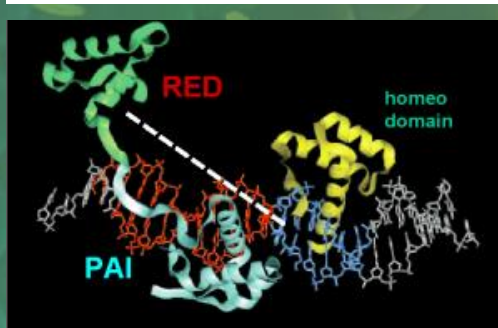
Homeobox genes and Homeodomain proteins



Homeobox: 180 bp, hox domain: 60 amino acids ⟶ DNA binding (enhancers)
Paired box:384 bp,

Pax6 from afar



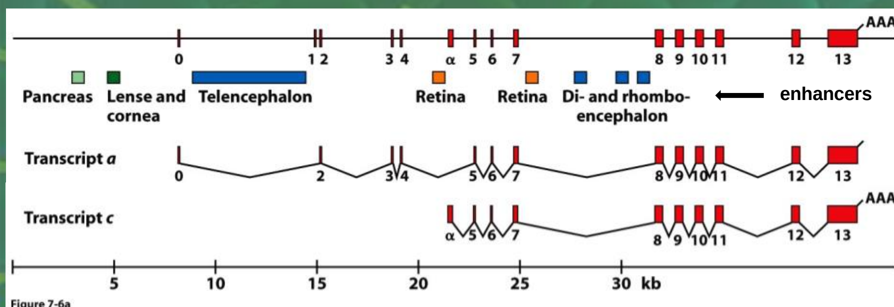


Hanson, Ped. Res., 2003

PAI and RED: two helical parts of paired domain
L: linker
HD: homeodomain
PST: proline, serine, threonine-rich domain (transactivation)
5a: alternative exon

Pax6 from a short distance

Different Pax 6 enhancers are active in different tissues: Results in different sites of transcription initiation and alternative splicing in different tissues



3 promoters: P0, P1 and Pα

<u>Transcript *c*:</u>

detected in retina
lacks exons 0-4
contains exon α
exon α is unique to vertebrates

Pax-6 othologs

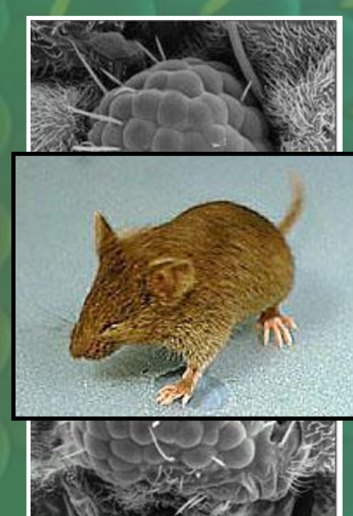
Mouse *Pax6* gene:
GTATCCAACGGTTGTGTGAGTAAAATTCTGGGCAGGTATTACGAGACTGGCTCCATCAGA

Fly *eyeless* gene:
Genetic similarity to mouse: 76.66%
Protein similarity to mouse: 100%
GTATCAAATGGATGTGTGAGCAAAATTCTCGGGAGGTATTATGAAACAGGAAGCATACGA

Shark eye control gene:
Genetic similarity to mouse: 85%
Protein similarity to mouse: 100%
GTGTCCAACGGTTGTGTCAGTAAAATCCTGGGCAGATACTATGAAACAGGATCCATCAGA

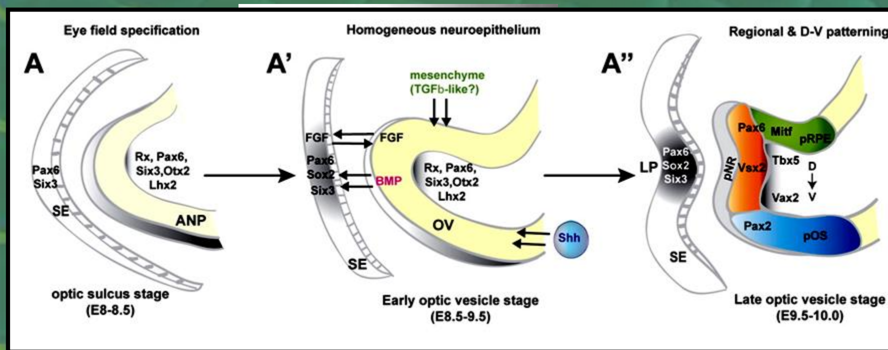
Squid eye control gene:
Genetic similarity to mouse: 78.33%
Protein similarity to mouse: 100%
GTCTCCAACGGCTGCGTTAGCAAGATTCTCGGACGGTACTATGAGACGGGCTCCATAAGA

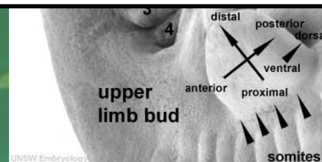
Flatworm eye control gene:
Genetic similarity to mouse: 71.66%
Protein similarity to mouse: 100%
GTGTCTAATGGTTGTGTTAGTAAAATACTTTGCCGATATTATGGAACAGGTTCTATTAAA



Aniridia

Pax6 in mammalian eye development



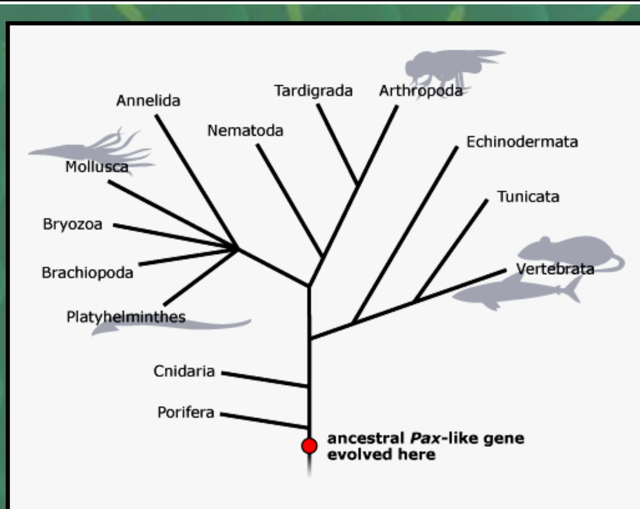


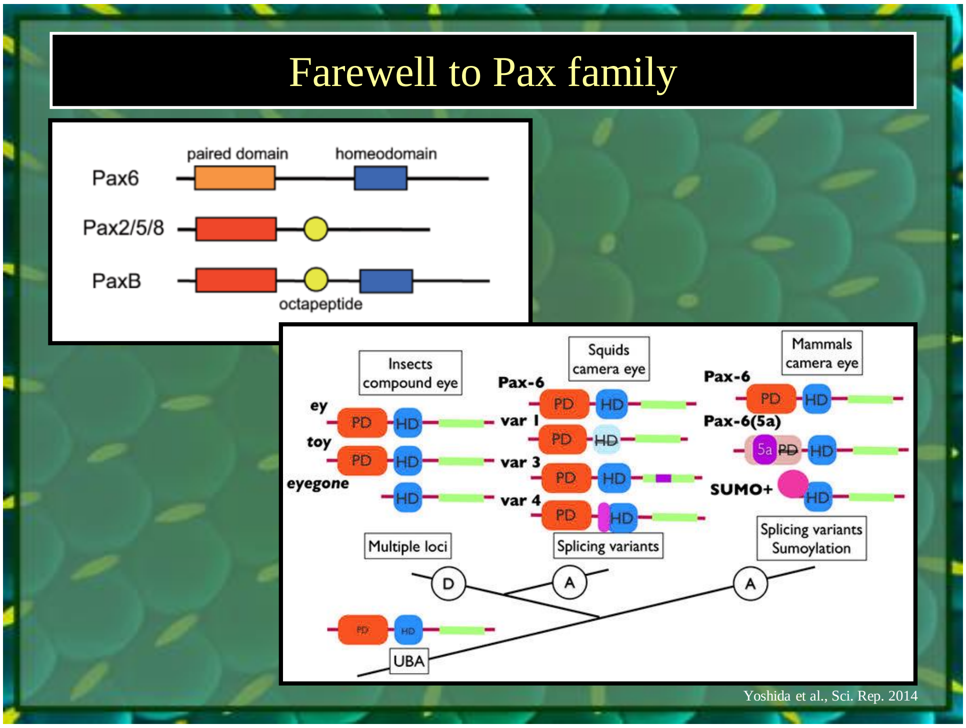
SOX: Sry-related HMG box
Tbx: T-box transcription factor
Vax: ventral anterior homeobox

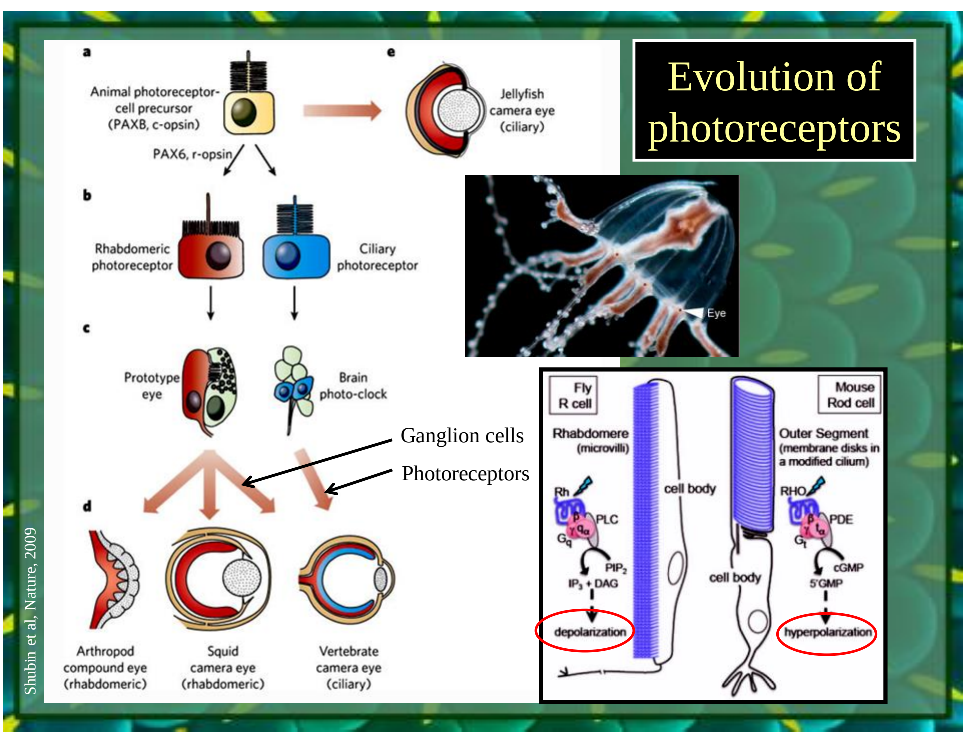
ANP: anterior neural plate
LP: lens placod
OV: optic vesicle
SE: surface ectoderm
pOS: presumptive optic stalk
pNR: presumptive neural retina
pRPE: presumptive retinal pigment epithelium.

Yun et al., Development, 2009

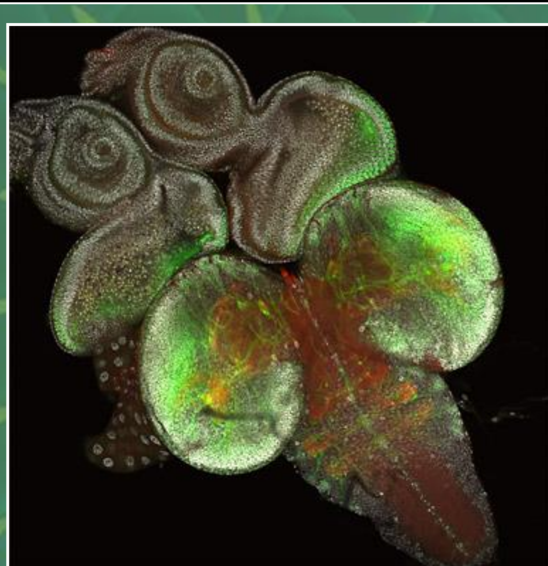
Evolution of Pax-6




Farewell to Pax family
paired domain
homeodomain
Pax6
Pax2/5/8
PaxB
octapeptide
Insects compound eye
Squids camera eye
Mammals camera eye
ey
toy
eyegone
PD
HD
Pax-6
var 1
var 3
var 4
Pax-6
Pax-6(5a)
5a
SUMO+
Multiple loci
Splicing variants
Splicing variants Sumoylation
D
A
A
UBA
Yoshida et al., Sci. Rep. 2014

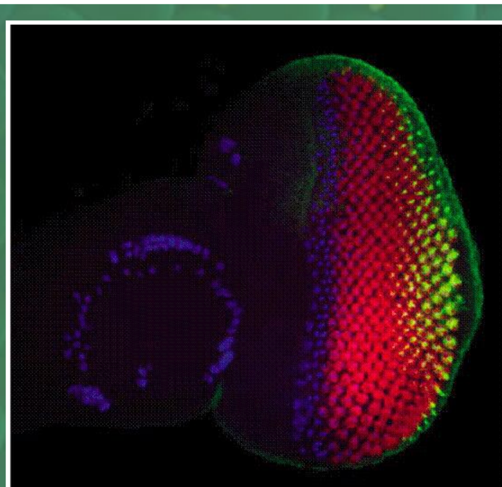

Evolution of photoreceptors
a
Animal photoreceptor-cell precursor (PAXB, c-opsin)
PAX6, r-opsin
e
Jellyfish camera eye (ciliary)
b
Rhabdomeric photoreceptor
Ciliary photoreceptor
c
Prototype eye
Brain photo-clock
d
Arthropod compound eye (rhabdomeric)
Squid camera eye (rhabdomeric)
Vertebrate camera eye (ciliary)
Ganglion cells
Photoreceptors
Eye
Fly R cell
Rhabdomere (microvilli)
cell body
Rh
PLC
PIP2
IP3 + DAG
depolarization
Mouse Rod cell
Outer Segment (membrane disks in a modified cilium)
cell body
RHO
PDE
cGMP
5'GMP
hyperpolarization
Shubin et al, Nature, 2009

The eye-antenna discs and the brain



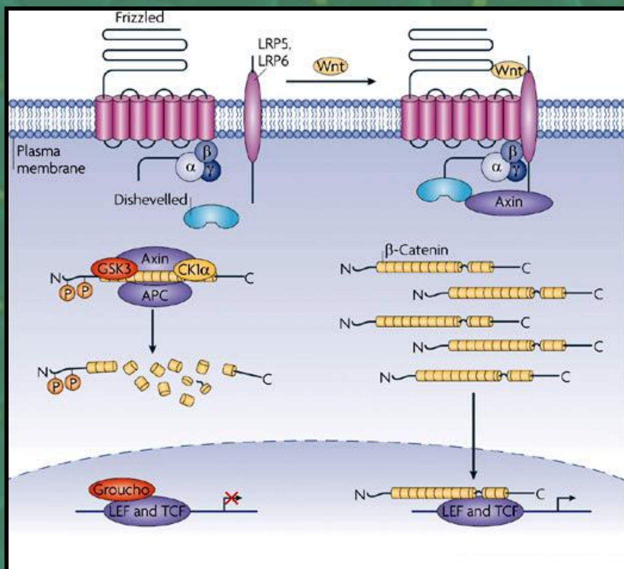
Cell determinations in the eye-antennal disc

anterior

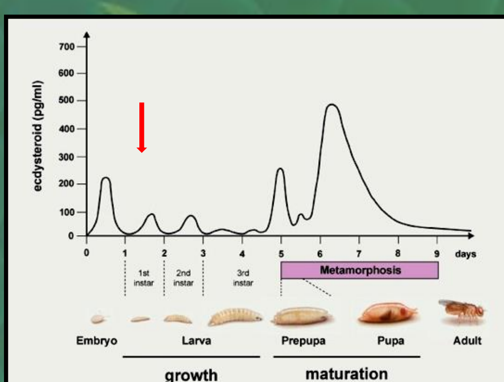


posterior

Wingless pathway



Undifferentiated phase

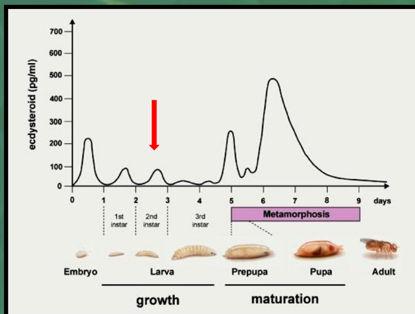


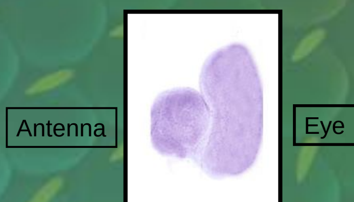
? → wg → hth, ext → string/cdc25 → Cell proliferation

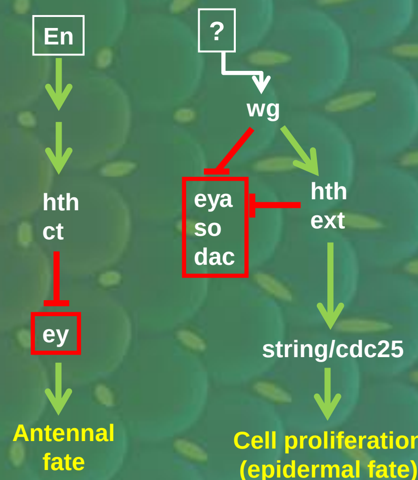
20-OH Ecdysone?

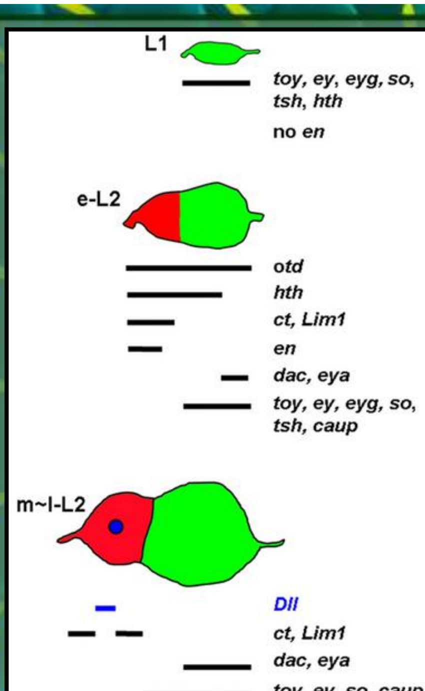
Wg(Wnt): wingless
Hth: homothorax, transcriptional co-factor
Ext: extradenticle, transcriptional factor

First step of segregation: antenna disc

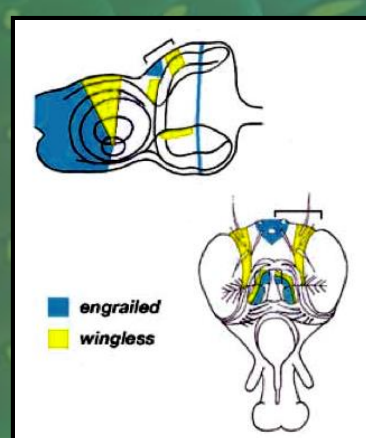






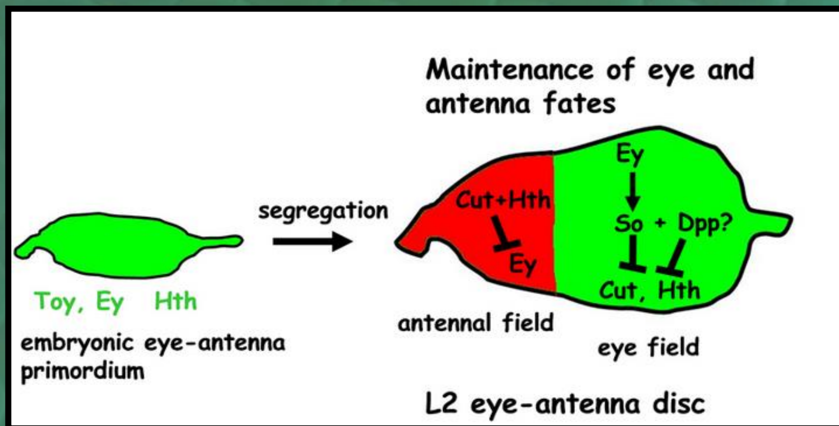


Segregation of the eye-antennal disc



Wang and Sun, Development, 2012

Current model of A-E segregation



Ct: cut
So: sine oculi
Dll: distalless

Tsh: teashirt
Hth: homothorax

Dpp: decapentaplegic
Dac: dachshund

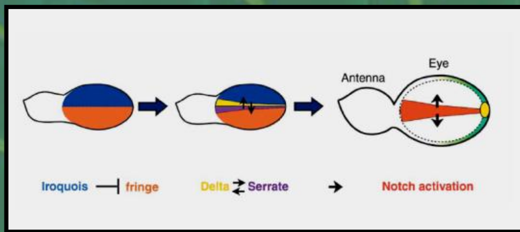
Wang and Sun, Development, 2012

Final step of segregation

En
↓
↓
hth
ct
⊥
ey
↓
Antennal fate

Ey, Eya, Dac
↓
so + dpp
⊥ ⊥
ct hth
↓
Eye fate

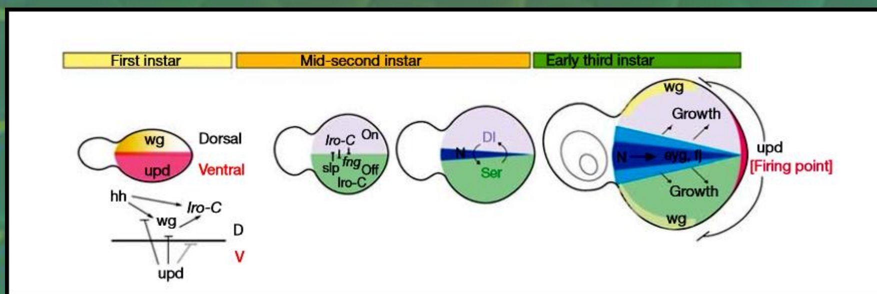
Dorsoventral polarity of the eye



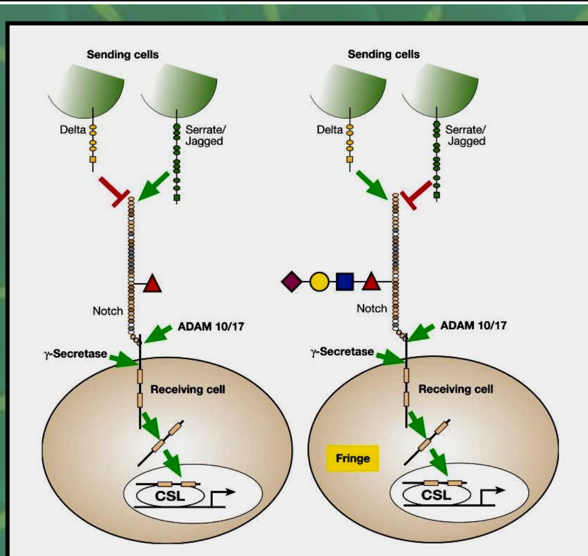
First planar axis

Iroquois: homeodomain transcription factor
Fringe: *N*-acetylglucosaminyltransferase

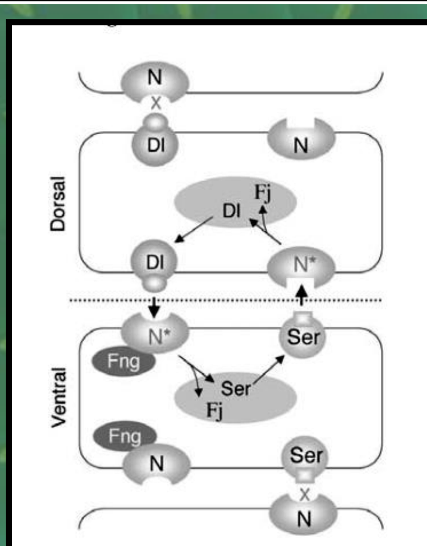
Unpaired, activates the JAK/STAT pathway
Delta/Serrate: Notch ligands



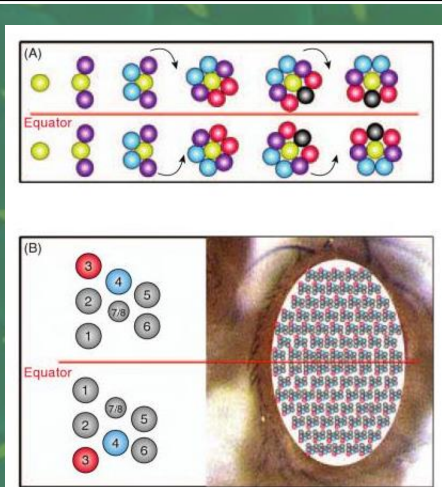
Variations on a theme :Notch/DSL signaling



Notch at the edge

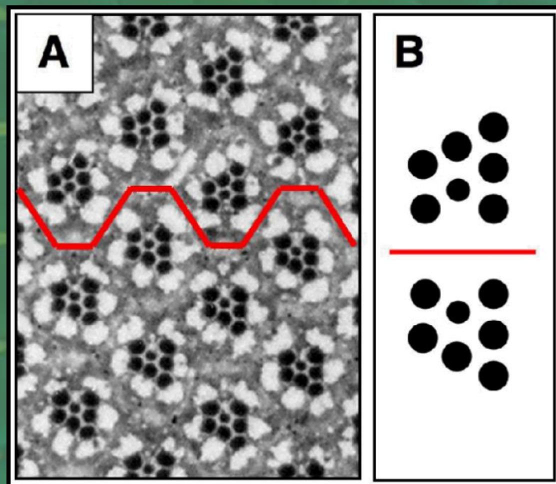


Rotation of clusters

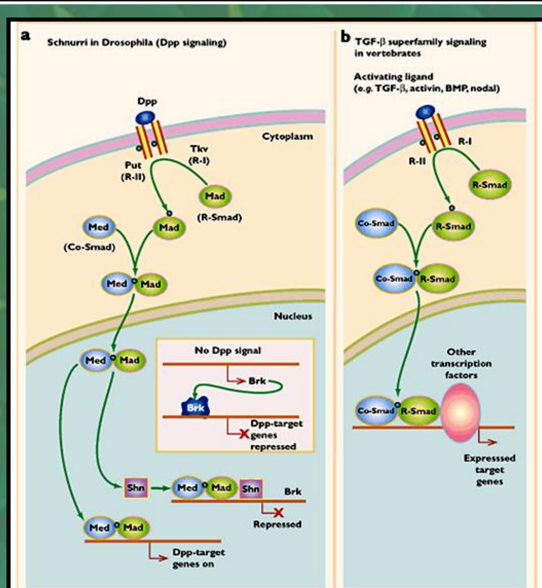


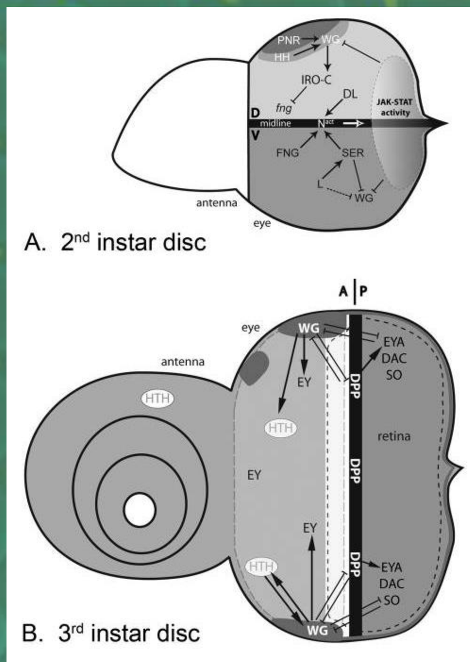
Nemo-like kinase – planar polarity

Dorsal and ventral ommatidia



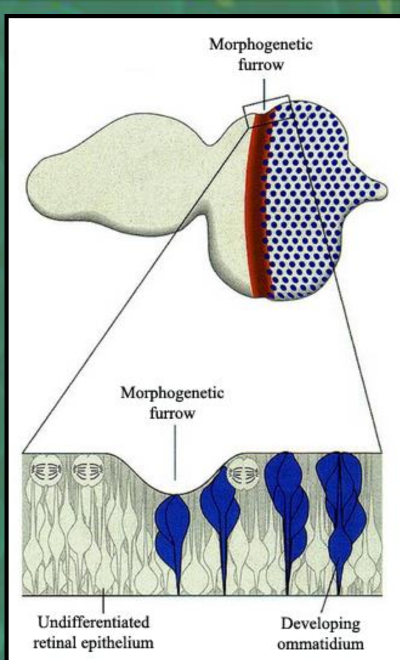
Dpp signaling



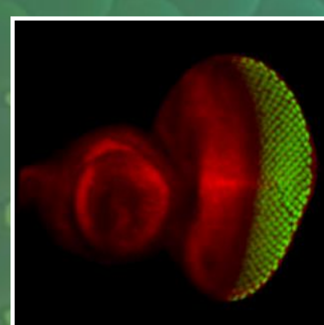


Second axis (P-A) formed by Dpp signaling

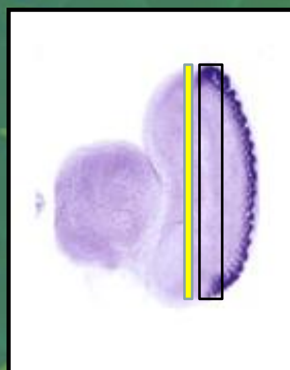
Legent and Treisman, Methods Mol Biol., 2008

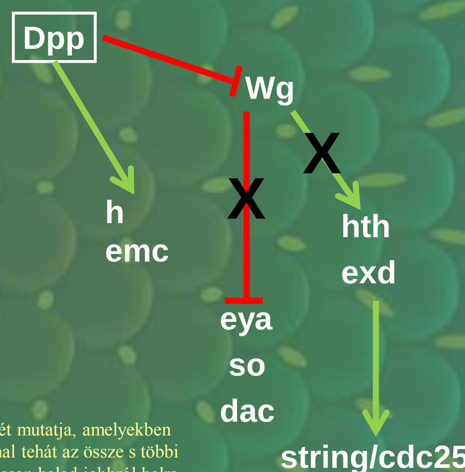


Morphogenetic furrow



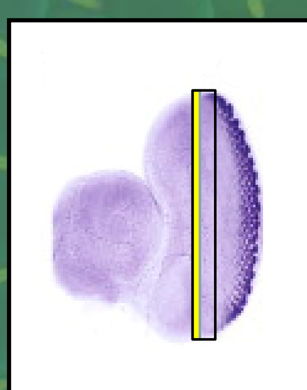
Early pre-proneuronal phase

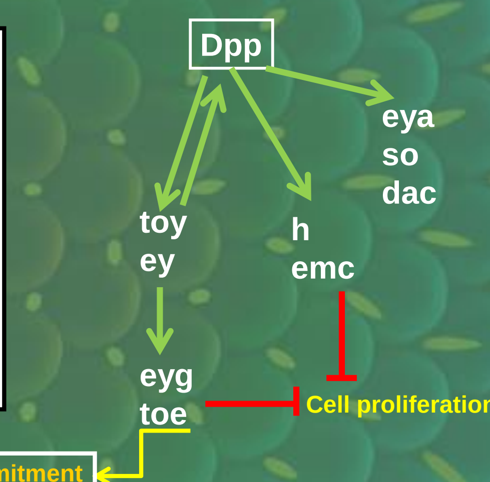




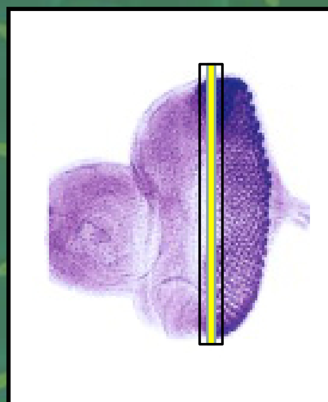
A képen a sárga vonal azoknak a sejteknek a helyét mutatja, amelyekben éppen a jobb oldali folyamatok zajlanak. Ez a vonal tehát az össze s többi ábrán azonos helyen áll, míg a MF-t jelző keret lassan halad jobbról balra (posterior végtől az anterior vég felé). Így az általa elhagyott sejtek egyre idősebbek és differenciáltabbak lesznek.

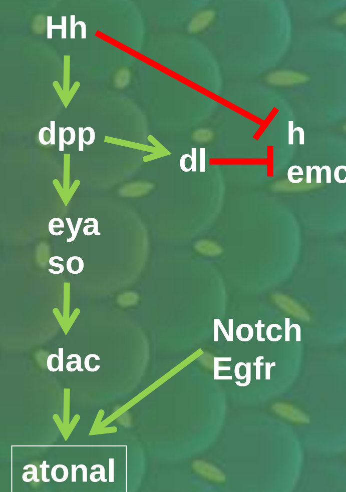
Late pre-proneuronal phase



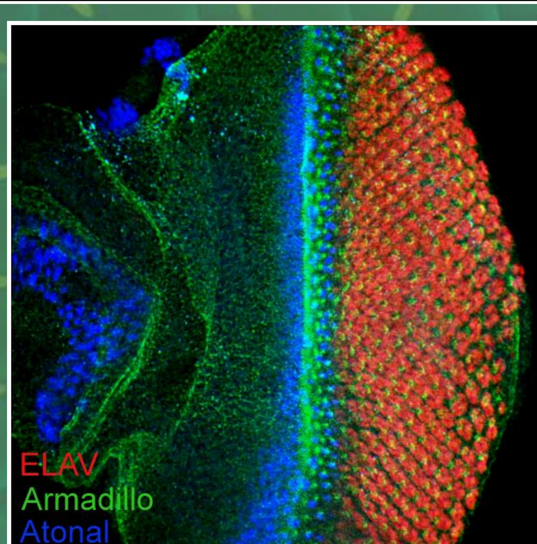


Proneuronal phase





Expression of *atonal*



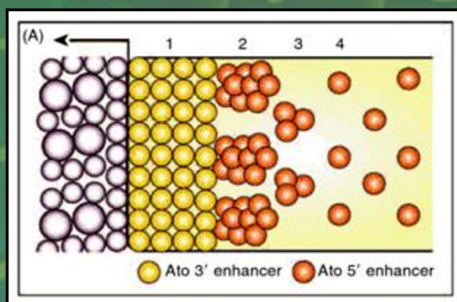
Enhancers of atonal

3' enhancer

5' enhancer

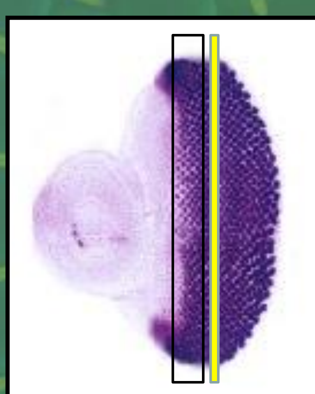
atonal gene

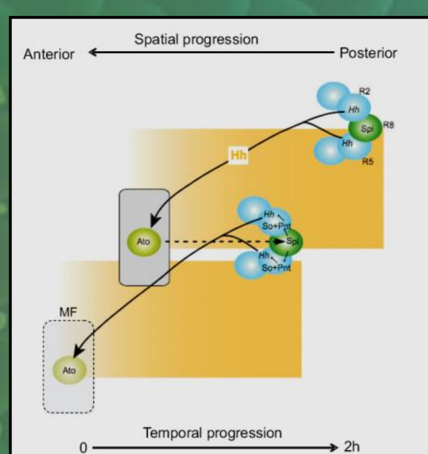
Notch
So
Ey
Hh



Notch
Hh
Ro
⇧
Egfr

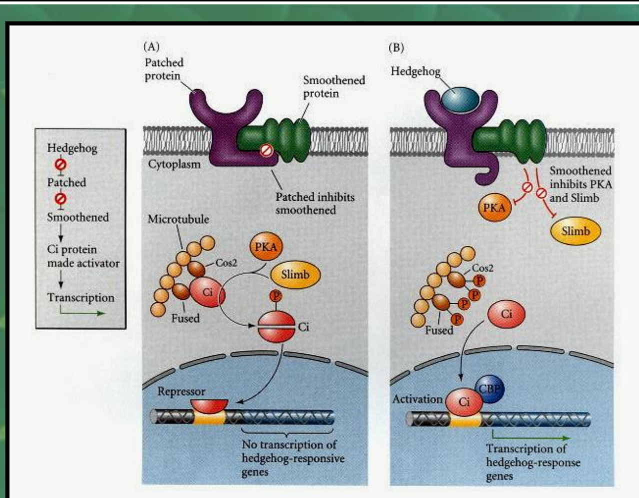
Neuronal development



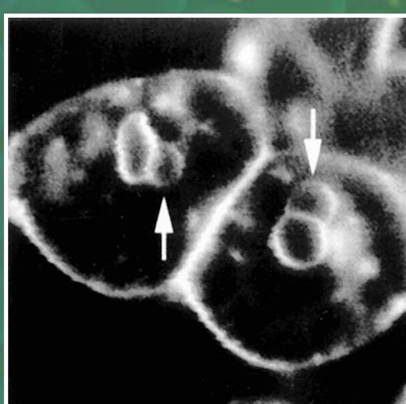


Hedgehog signaling

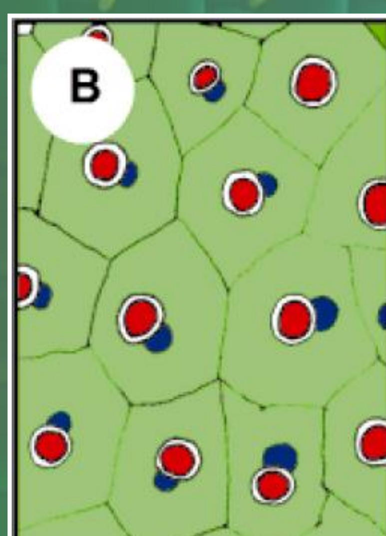
Hedgehog signaling antagonizes Notch: Mitotic cycles are instead of endoreduplications



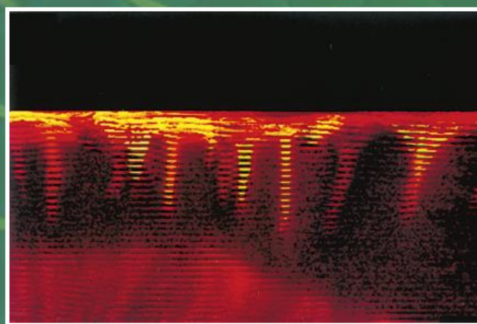
Perinuclear lobes

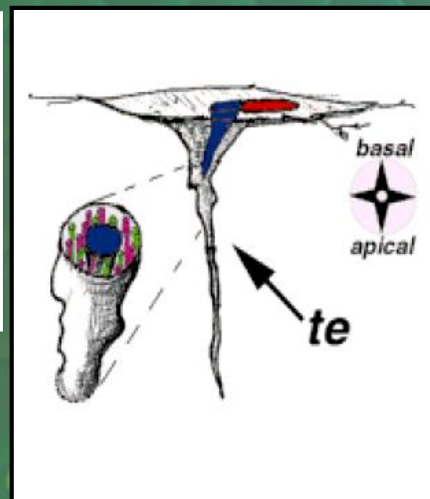


Gibson and Schubiger, 2000, Cell



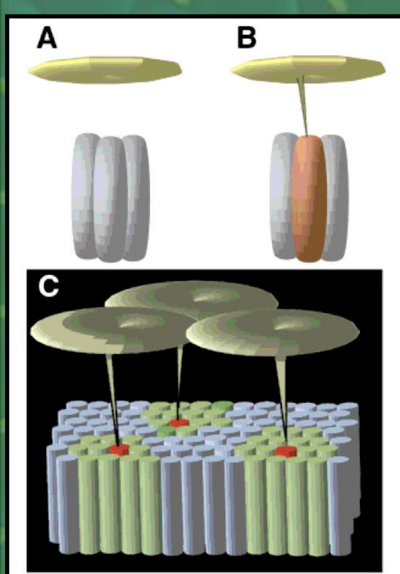
Translumenal extensions

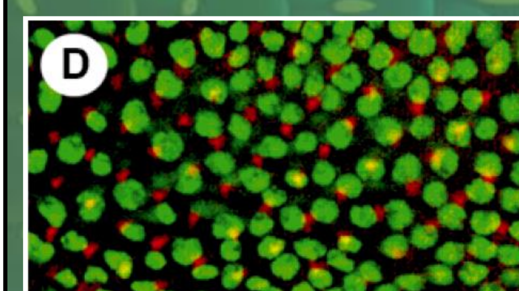




Gibson and Schubiger, 2000, Cell

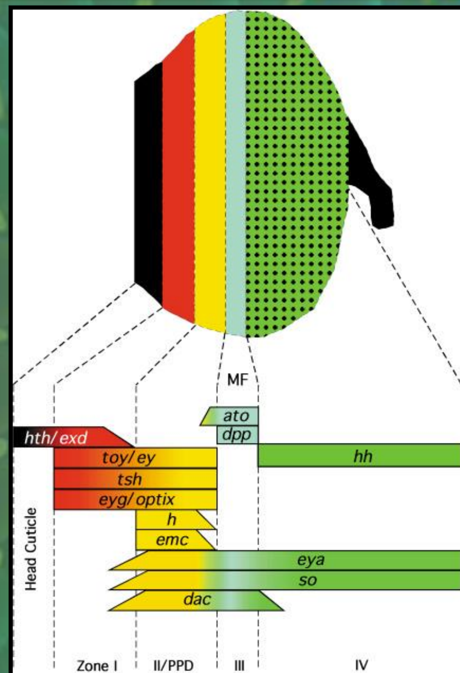
Designation of R8 founder cells



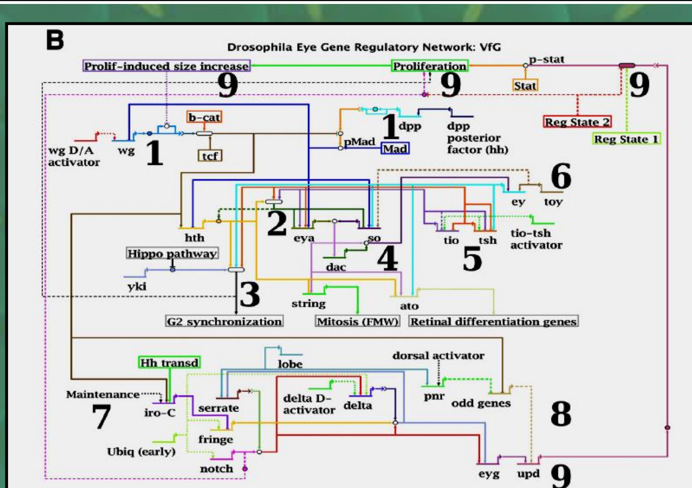


Gibson and Schubiger, 2000, Cell

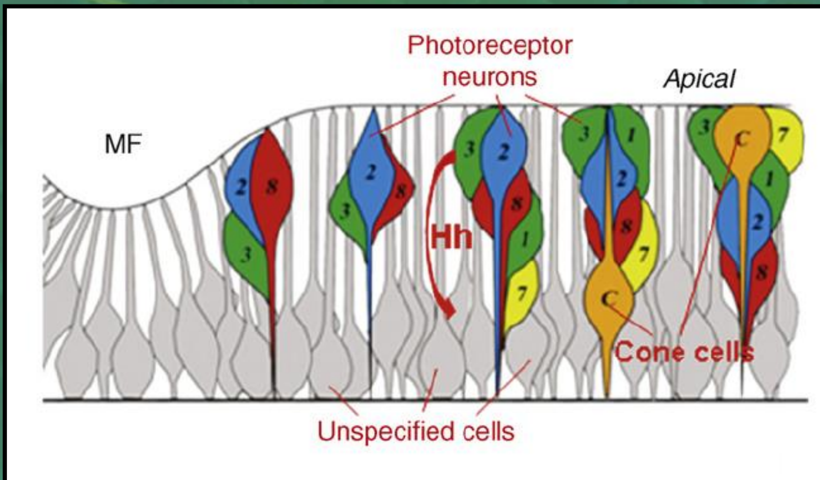
Pattern of the active genes in the developing eye



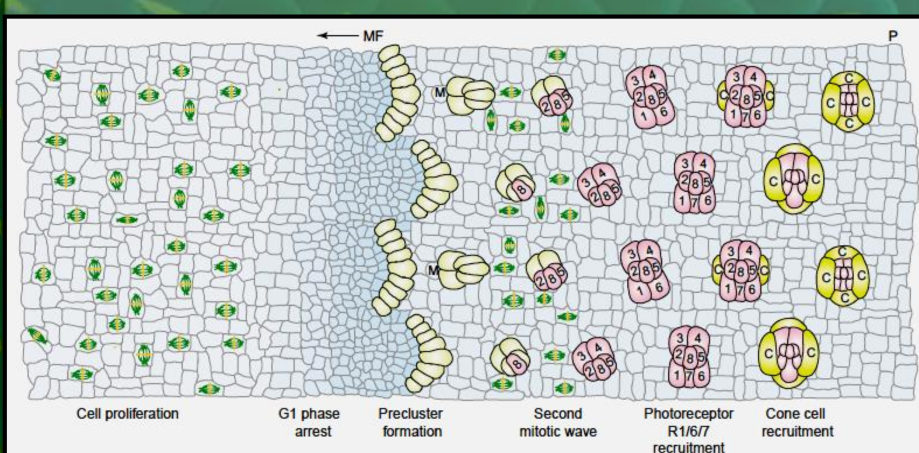
Eye gene network



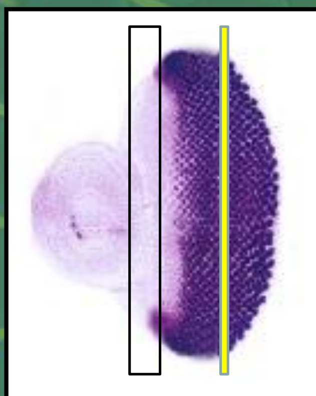
Third axis: proximal-distal by apical – basal interactions

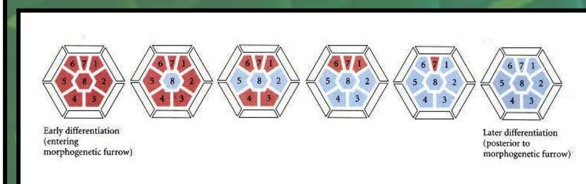


Formation of the clusters

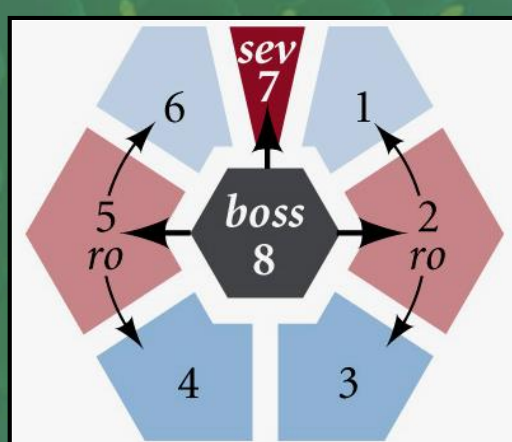


Differentiation

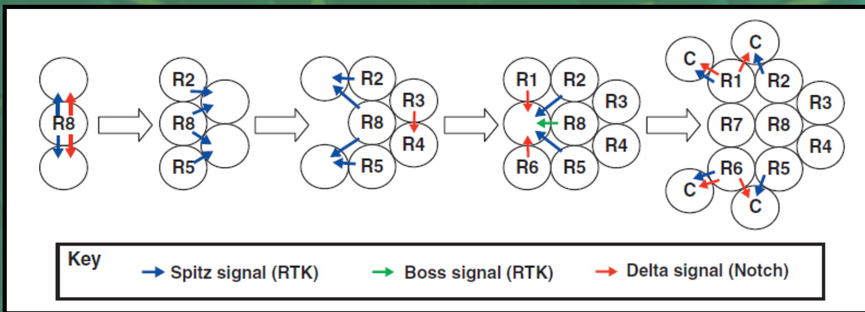




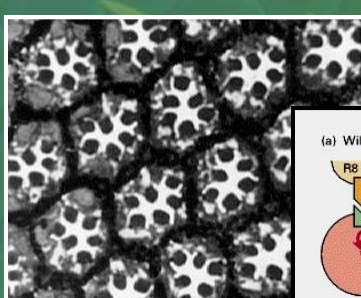
Signaling of the proneural clusters

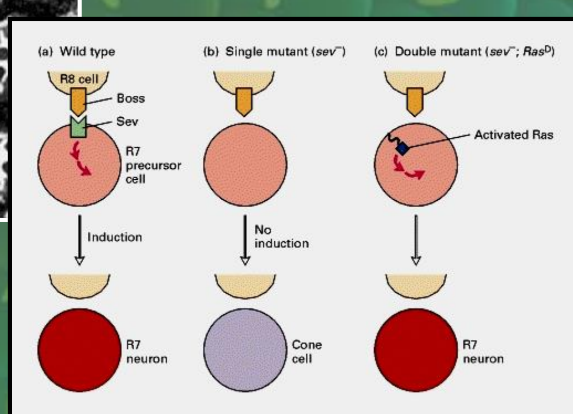


Main signalings

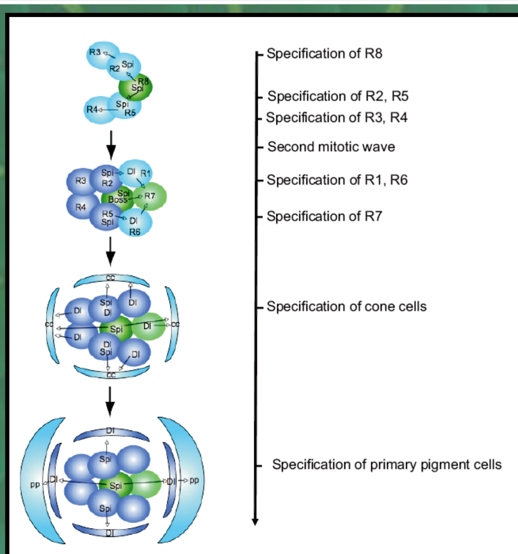


R7 determination - sevenless

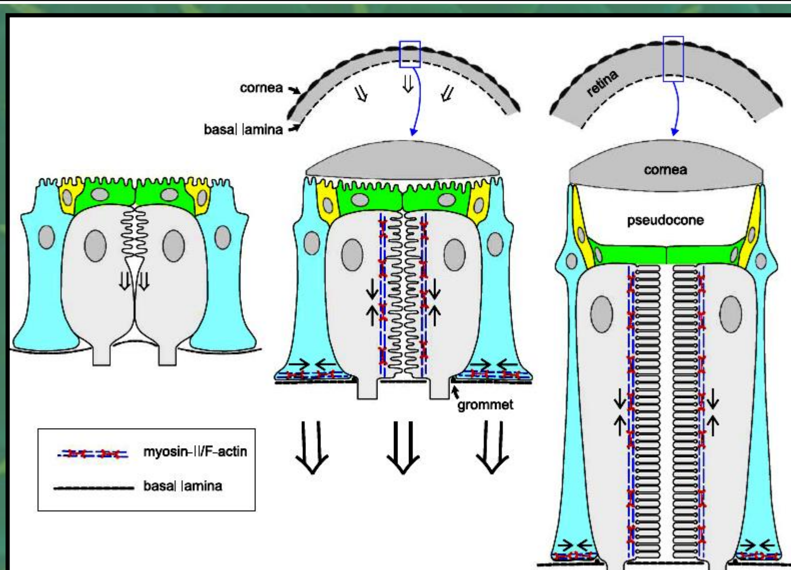




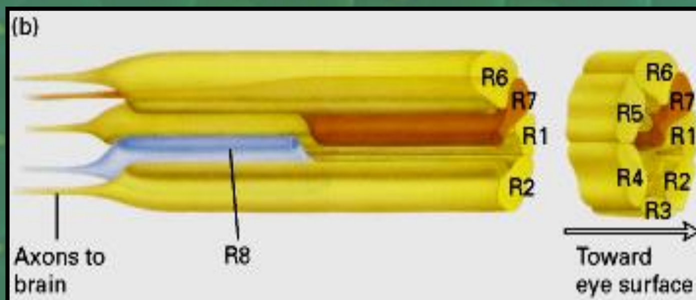
Summary of the specification of cell in an ommatidium



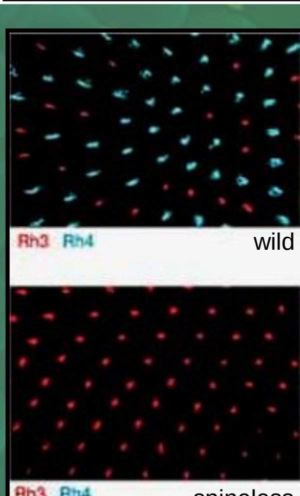
Elongation of cells



Final arrengement of retinula cells



Expression of opsins in R cells

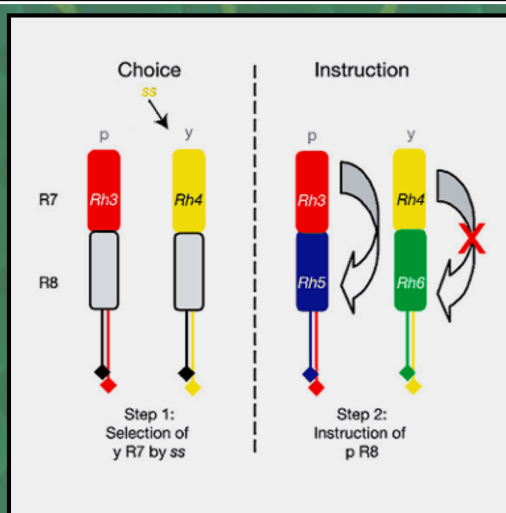


R1 – R6 rhabdomeres express:
rhodopsin1 (blue/light)

R7 and R8 rhabdomeres express a combination of two rhodopsins:
(rhodopsin3 *or* rhodopsin4) (UV)
and
(rhodopsin5 *or* rhodopsin6) (yellow)

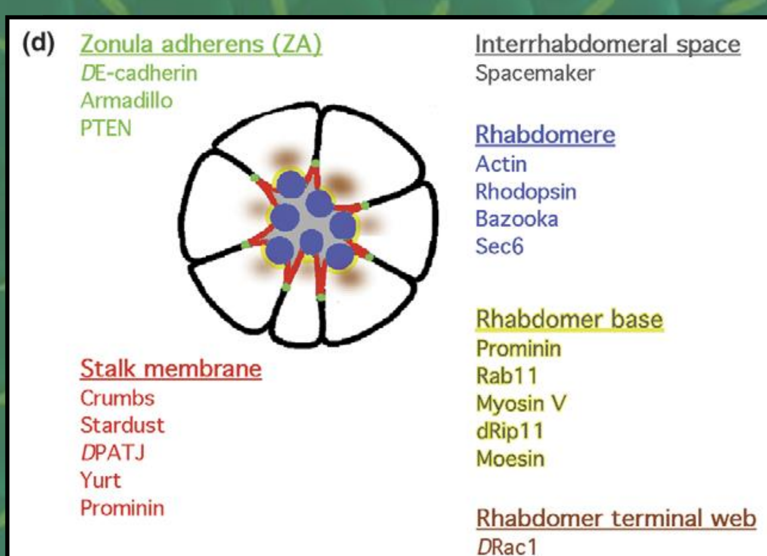
The default fate for an R7 receptor is to express the *Rh3* opsin, but the presence of the *spineless* protein above a certain level is sufficient to induce it to express *Rh4* instead. In a mutant in which no *spineless* is present, the R7 cells all make the *Rh3* protein.

Effects of the spineless

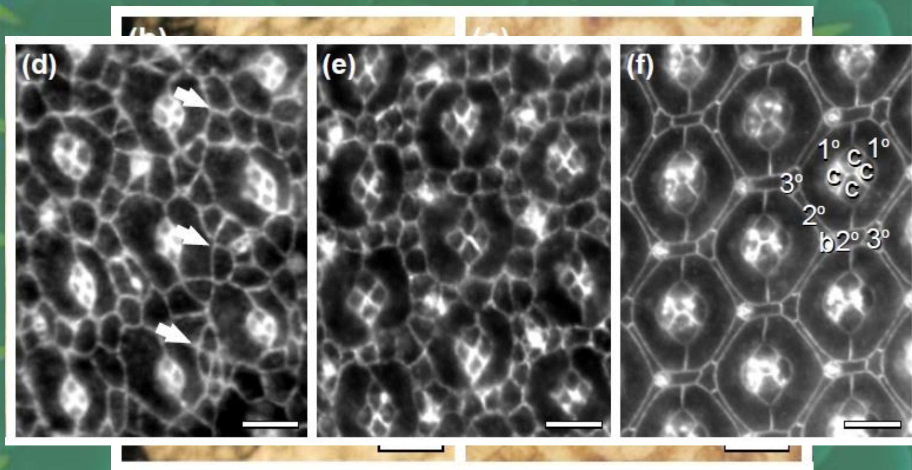


Formation of the rhabdomer

Crumbs
↓
PTEN
↓
PInst3P
↓
Akt1
↓
Apical Membrane lack



The obsolete cells are removed by apoptosis



Initiator caspases - inhibitor of apoptosis

Is it worth making eyes at Drosophila?

Oh, yes!


The End