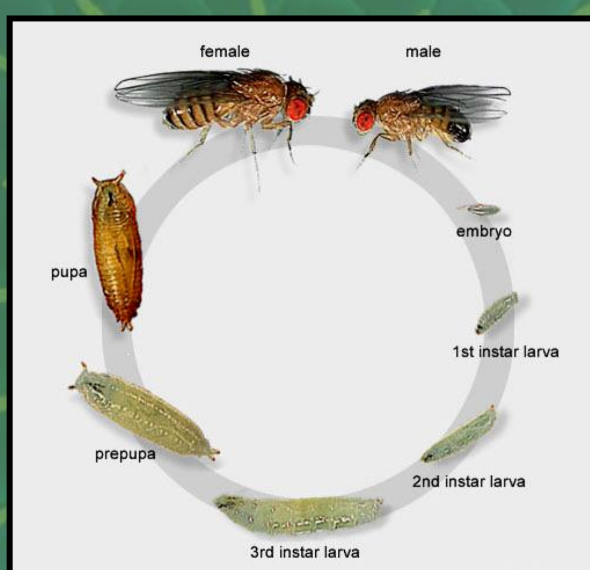
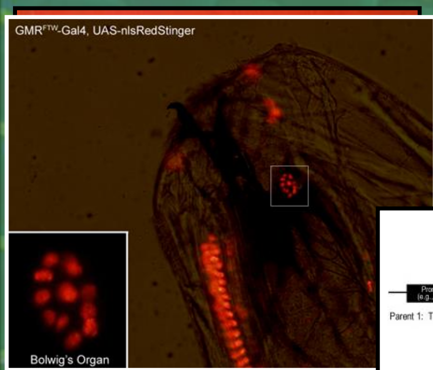
Why is it worth making eyes at Drosophila?

Fejlődés- és molekuláris genetika 2021

Life cycle of *Drosophila melanogaster*



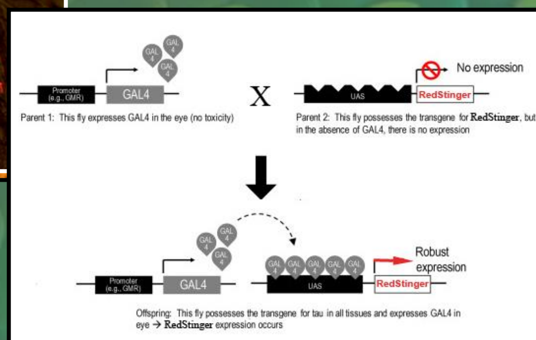
Bolwig's organ



Eucephalic Dipterans

150 Mya

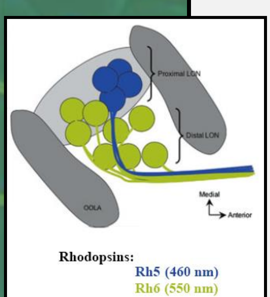
Acephalic larva

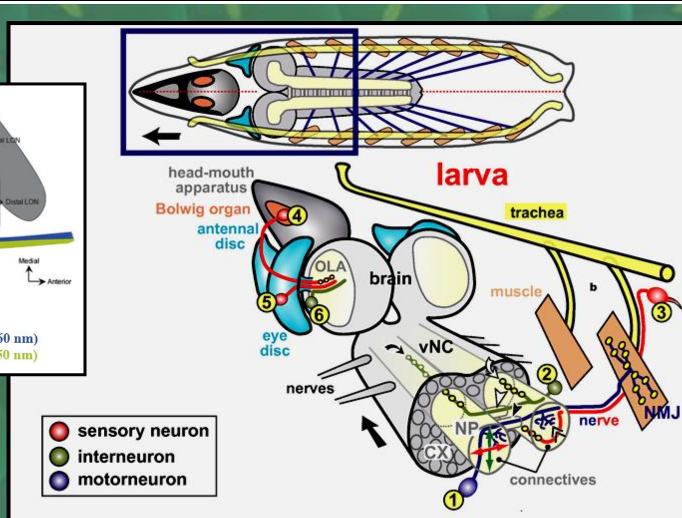


Niels Bolwig (1911 – 2004)

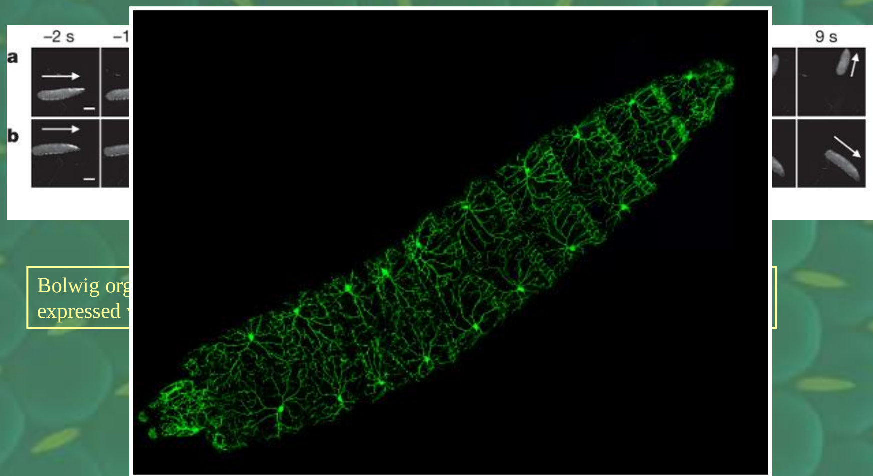
GMR: glass multiple reporter promoter elements, has been commonly utilized to express target transgenes, specifically in the developing eye

Bolwig's organ in the larva





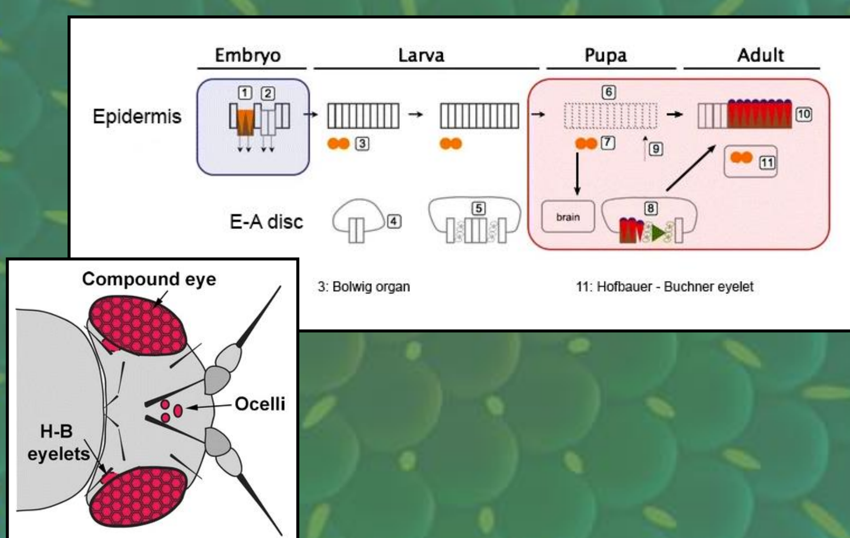
Maggots are all eyes



Bolwig org
expressed v

Xiang et al,, Nature 2010

Hofbauer – Buchner eyelets

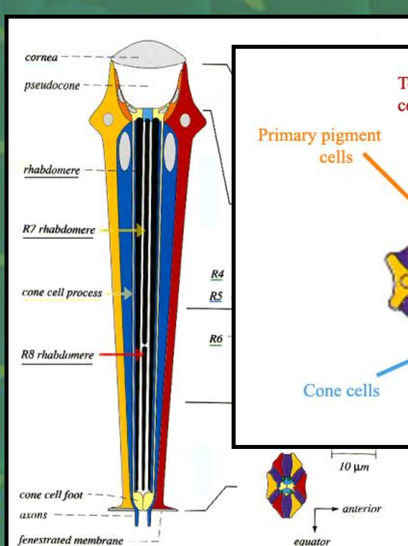


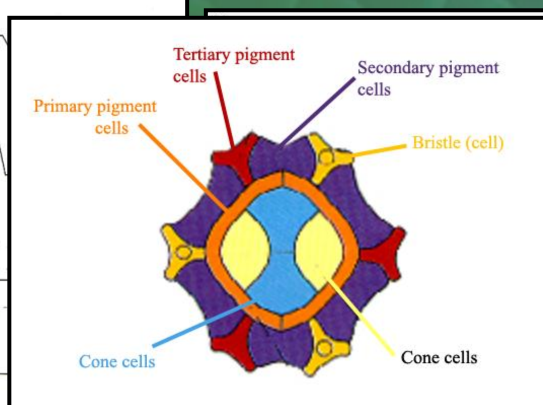
Let's see the compound eye!

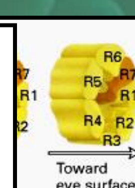
Drosophila: 750-800 ommatidium

Dragonfly: 10 000 ommatidium

An ommatidium of Drosophila eye

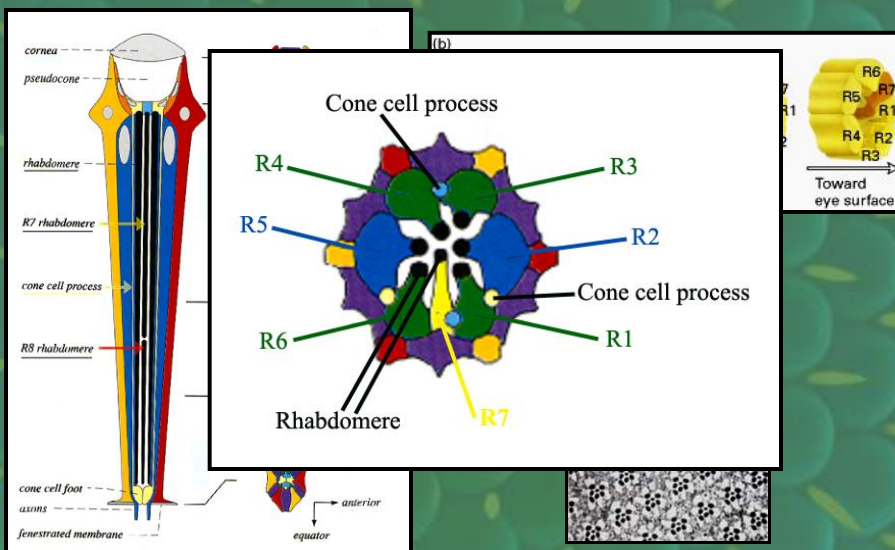




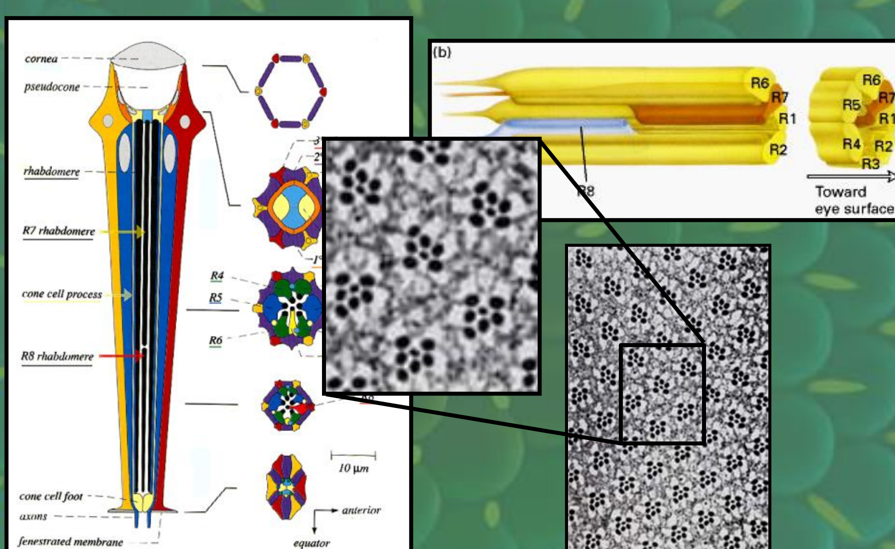


- 2 bristle cells

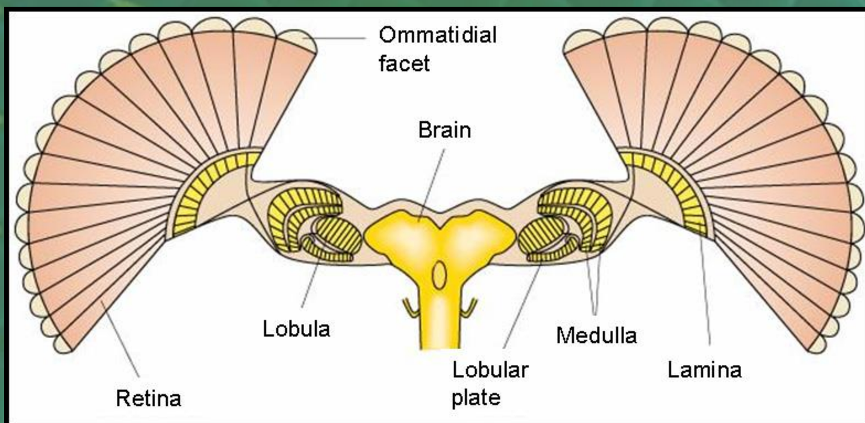
An ommatidium of Drosophila eye



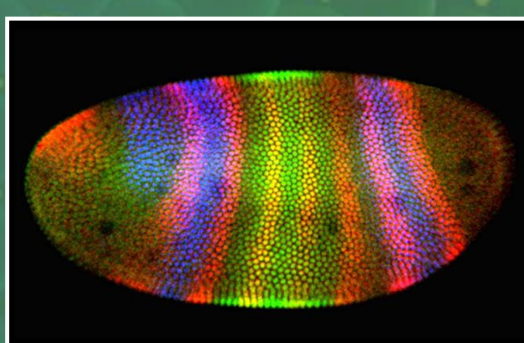
An ommatidium of Drosophila eye

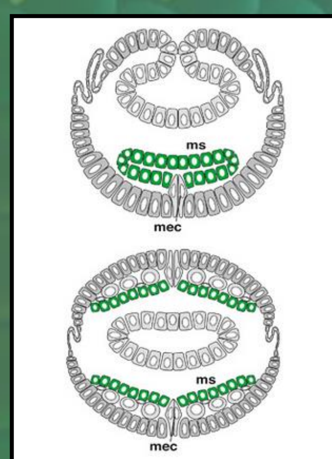


Cross-section of eyes and brain

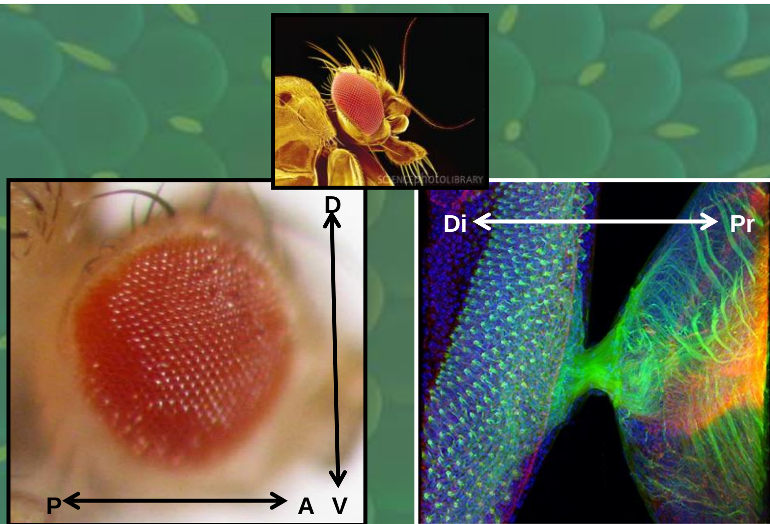


Axis polarity: in 2D

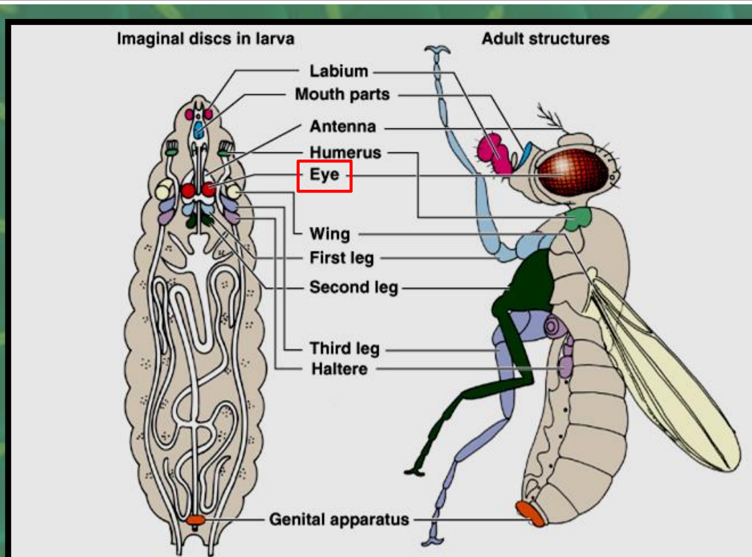




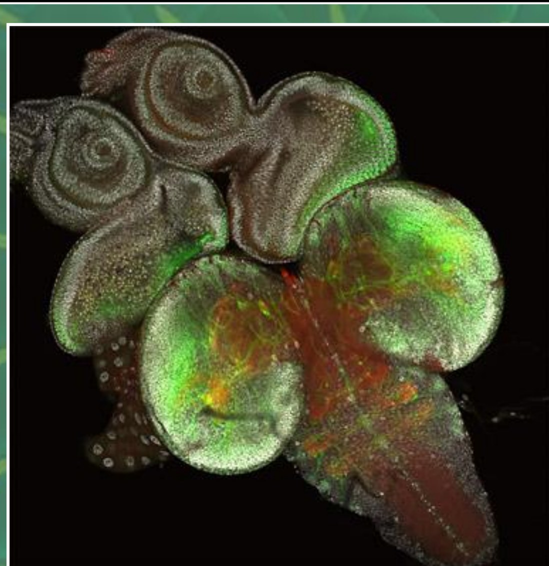
Organization of eye in 3D



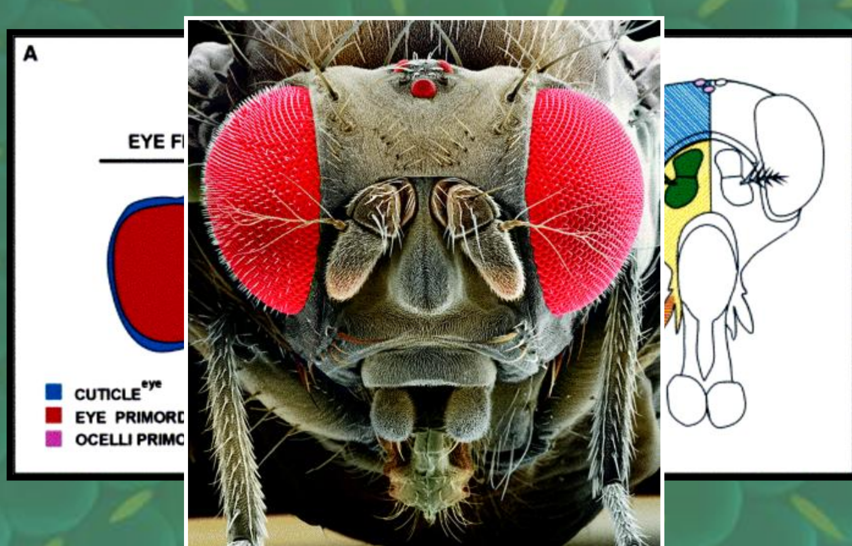
Imaginal discs



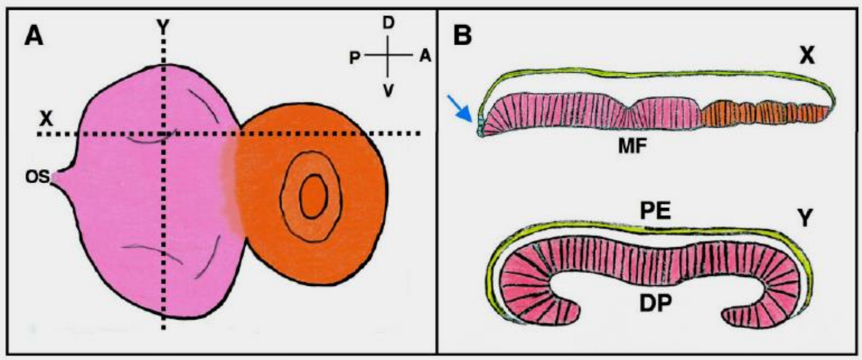
The eye-antenna discs and the brain



Derivates of E-A disc



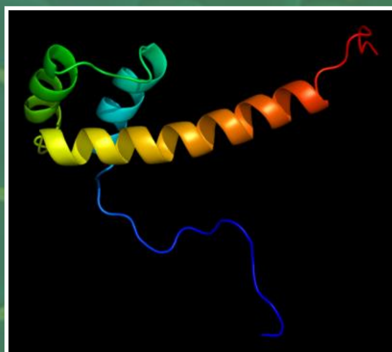
Sections of eye-antenna disc



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*



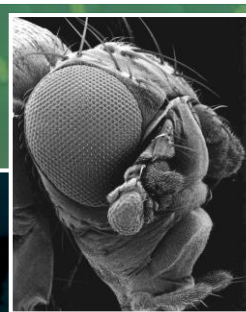
PAX 6

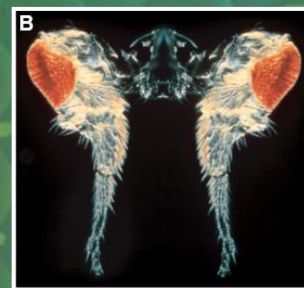
Four *Drosophila* Pax6 orthologues:
eyeless (ey)
twin of eyeless (toy)
eyegone (eyg)
twin of eyegone (toe)

Eyeless (ey)

Mildred Hoge, 1915

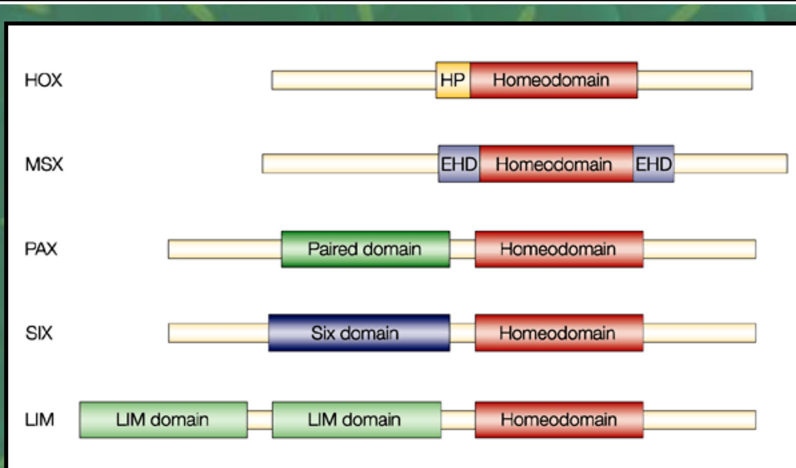
Ectopic overexpression of *ey*





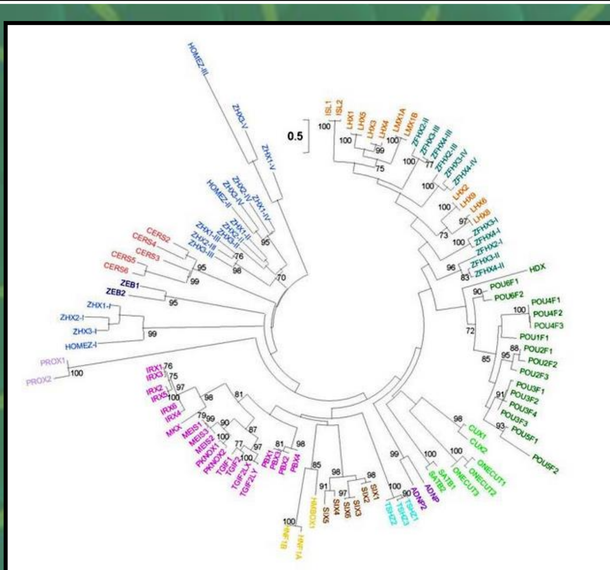
Ey: paired box (pax) gene family, tissue specific transcriptional factors

Homeobox genes and homeodomains

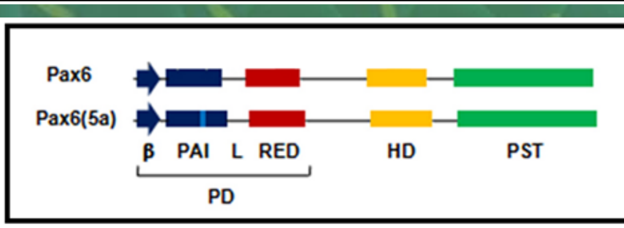


Homeobox: 180 bp, hox domain: 60 amino acids ⟶ DNA binding (enhancers)
Paired box: 384 bp, prd domain: 128 amino acids

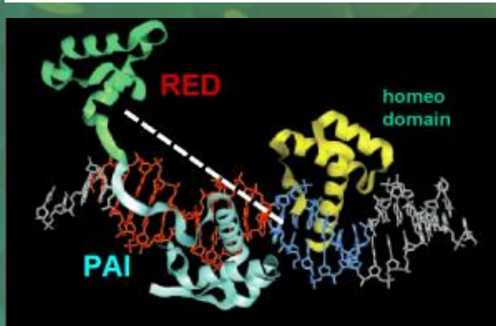
Homeobox genes and homeodomains



Pax6 protein from a distance



Pax6
Pax6(5a)
Pax6(ΔPD)



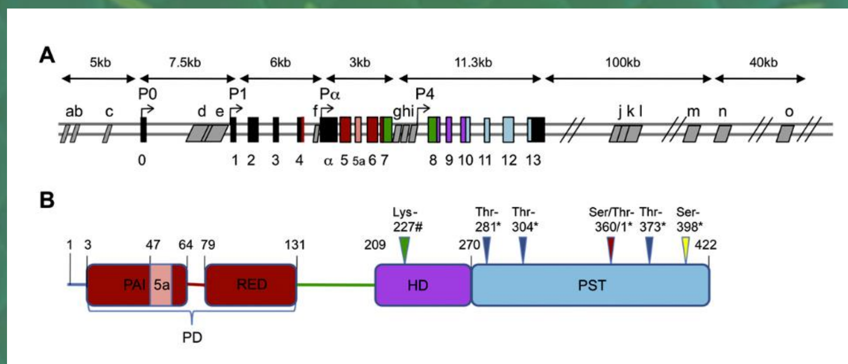
Hanson, Ped. Res., 2003

PAI and RED: two helical parts of paired domain
L: linker
HD: homeodomain
PST: proline, serine, threonine-rich domain (transactivation)
5a: alternative exon (14 amino a.)

3 alternative translation start sites within the Linker region
⇩
pairedless (ΔPD) proteins

Pax6 gene at close quarters

4 promoters: P0, P1, Pα and P4

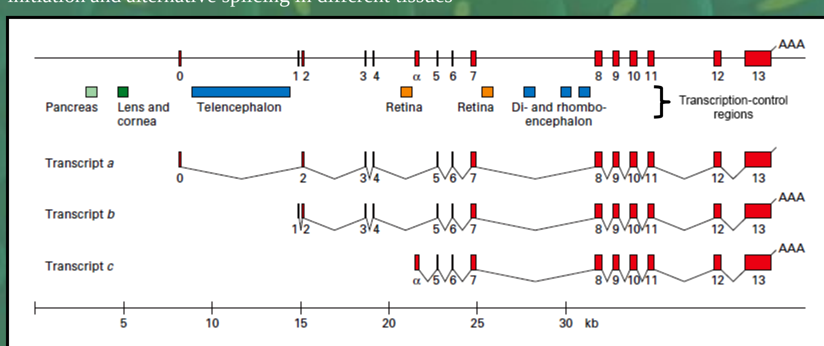


Shaham et al., Prog. Ret. Eye Res., 2012

Color indicates coding exons; they correspond to the color of domains in the protein; black indicates noncoding exons) with the numbers below them; arrows denote the transcription start sites and the names of the corresponding promoters; gray diamonds represent regulatory elements.

Pax6 gene at close quarters

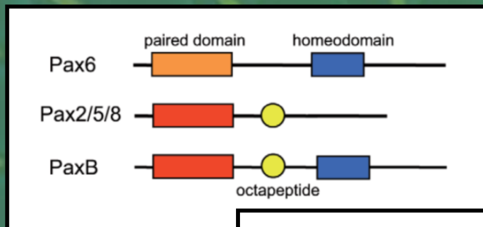
Different Pax 6 enhancers are active in different tissues: Results in different sites of transcription initiation and alternative splicing in different tissues



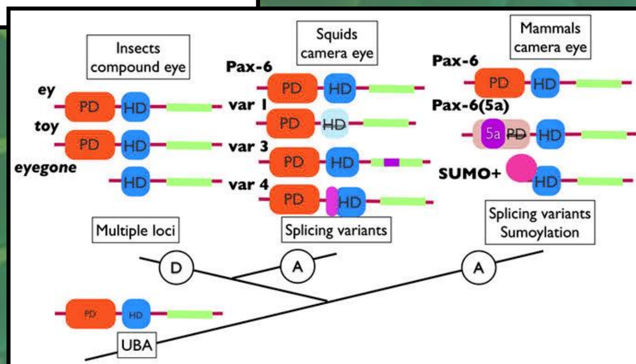
<u>Transcript *c*:</u>

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

Farewell to Pax family



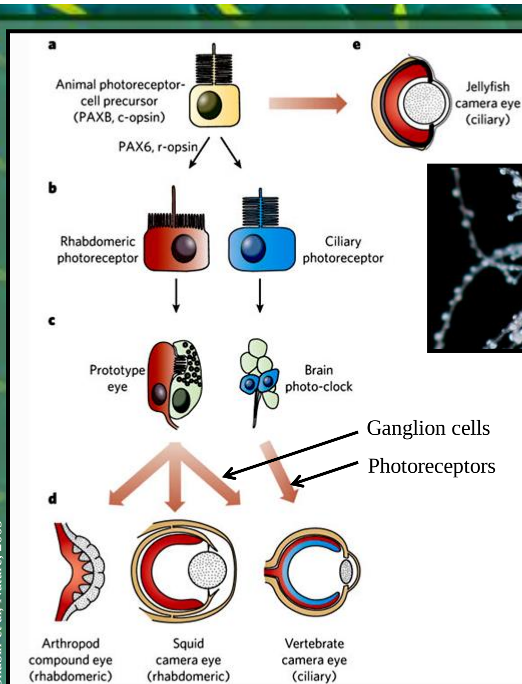
Pax6 – vertebrates' eyes and brain
Pax2, 5, 8 – vertebrates' CNS
PaxB – jellyfish' eyes

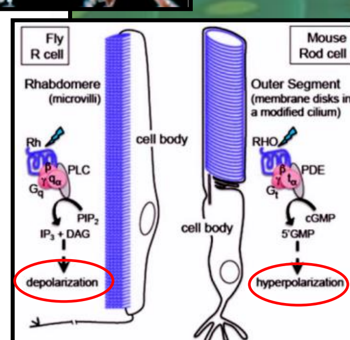


UBA:
urbilaterian ancestor

Yoshida et al., Sci. Rep. 2014

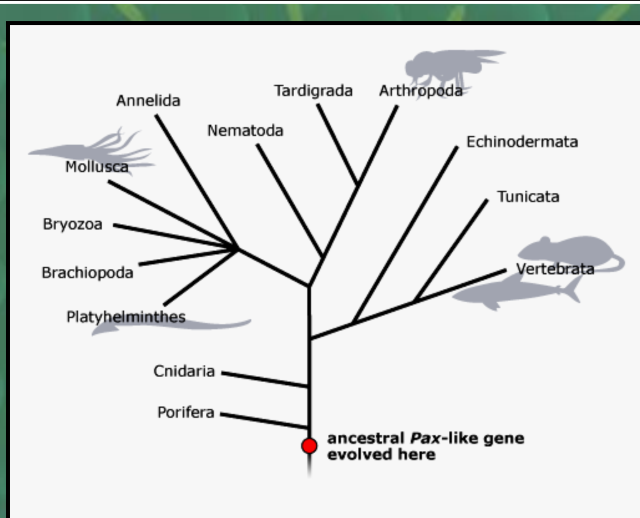
Evolution of photoreceptors



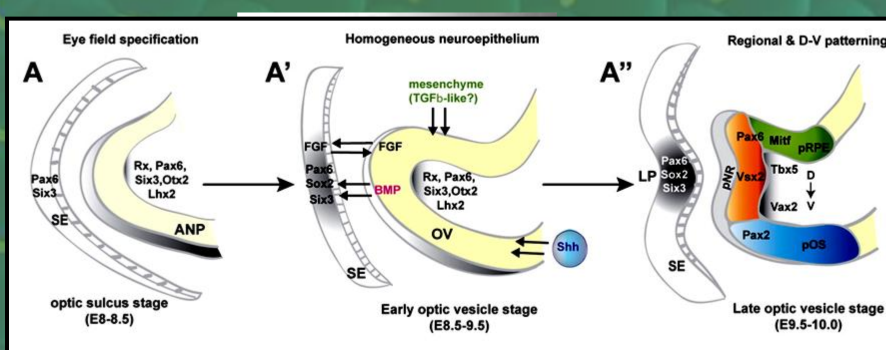


Shubin et al, Nature, 2009

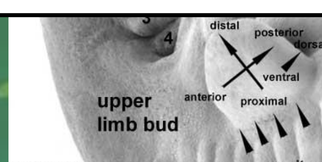
Evolution of Pax-6



Pax6 in mammalian eye development



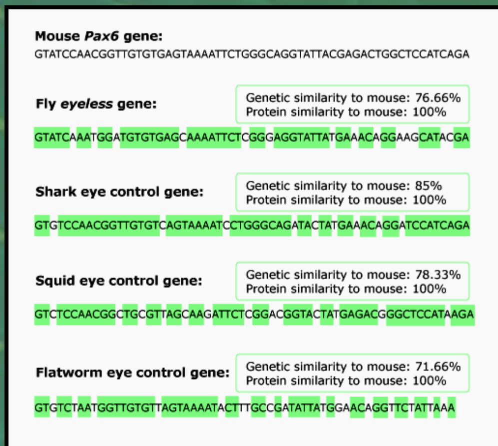
SOX: Sry-related HMG box
Tbx: T-box transcription factor
Vax: ventral anterior homeobox



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.

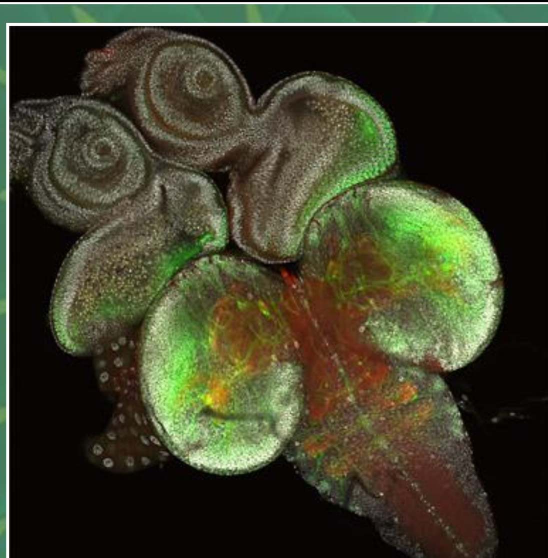
Yun et al., Development, 2009

Pax-6 othologs



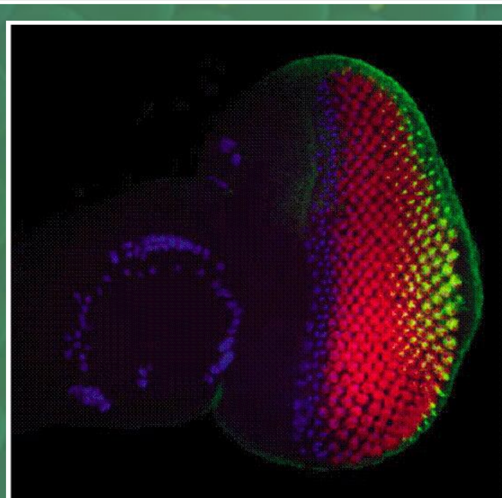
pax6^{-}/$^{+}$: aniridia, pax6^{-}/$^{-}$: anophthalmia

The eye-antenna discs and the brain



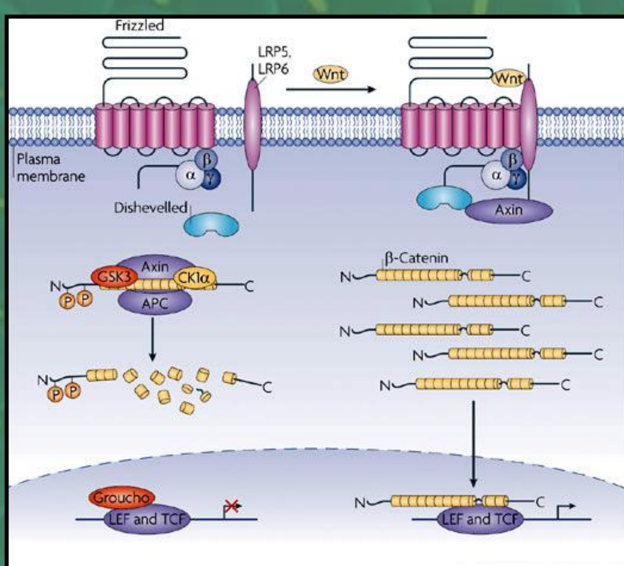
Cell determinations in the eye-antennal disc

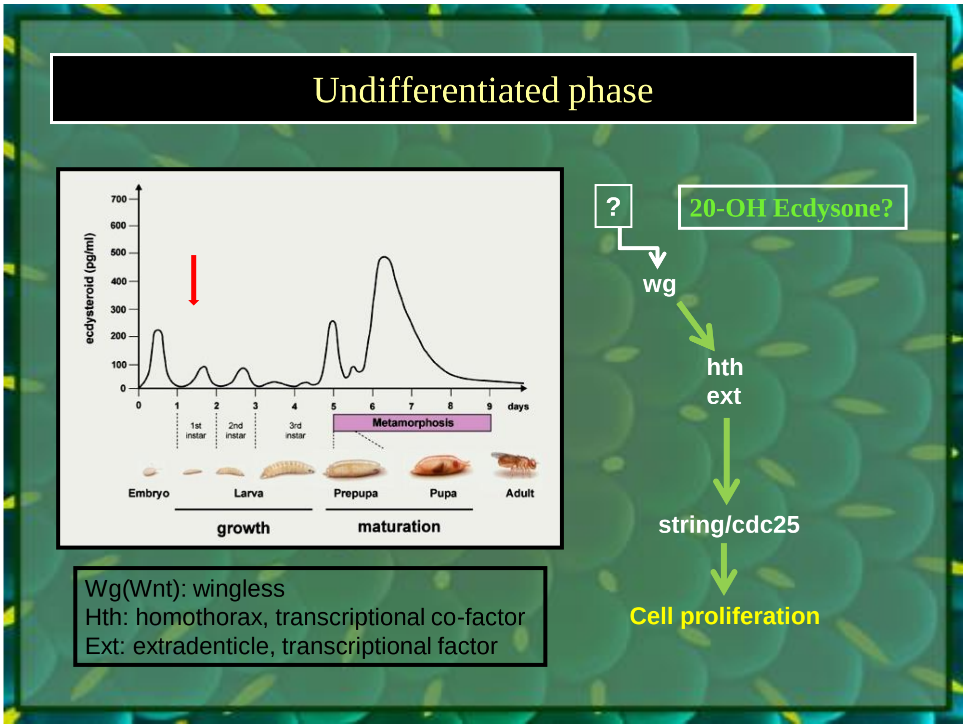
anterior

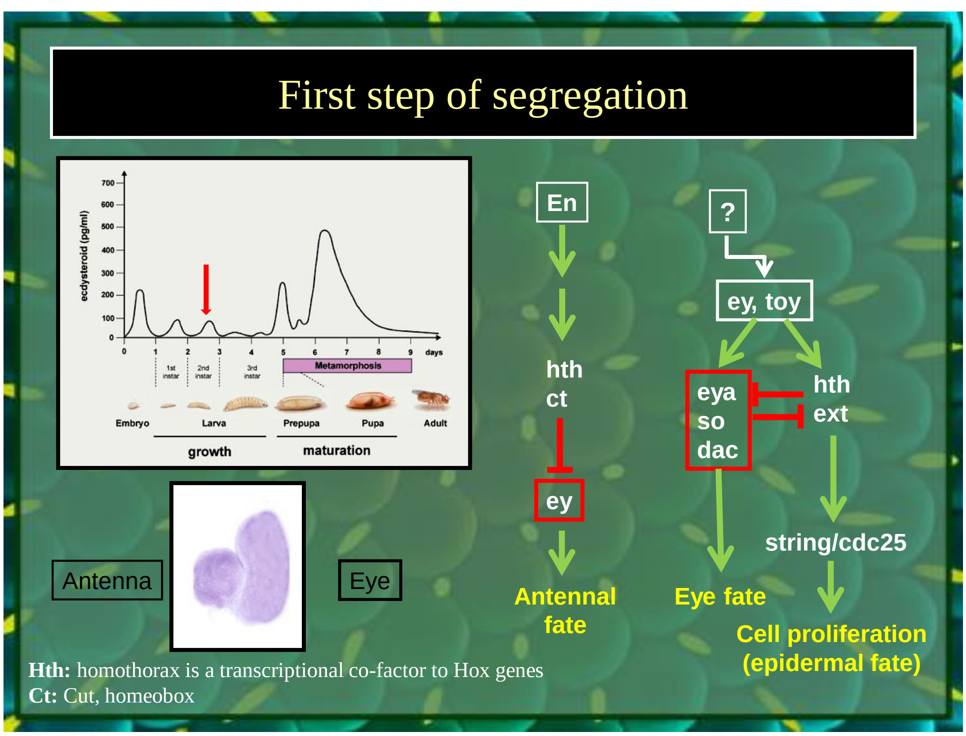


posterior

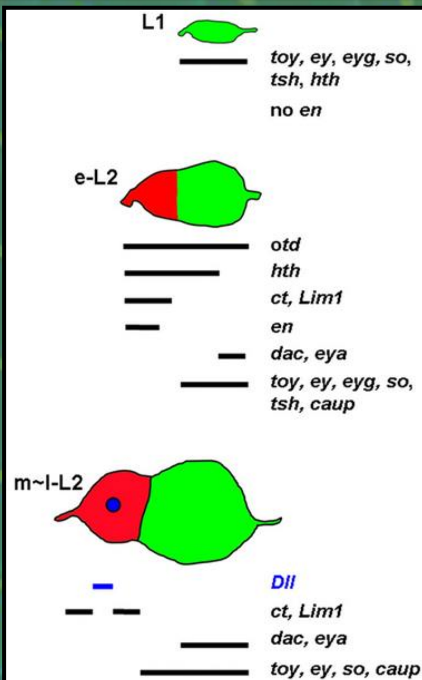
Wingless pathway

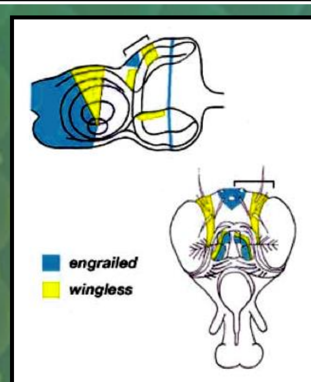



Undifferentiated phase
ecdysteroid (pg/ml)
700
600
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 days
1st instar
2nd instar
3rd instar
Metamorphosis
Embryo
Larva
Prepupa
Pupa
Adult
growth
maturation
Wg(Wnt): wingless
Hth: homothorax, transcriptional co-factor
Ext: extradenticle, transcriptional factor
?
20-OH Ecdysone?
wg
hth
ext
string/cdc25
Cell proliferation


First step of segregation
ecdysteroid (pg/ml)
700
600
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 days
1st instar
2nd instar
3rd instar
Metamorphosis
Embryo
Larva
Prepupa
Pupa
Adult
growth
maturation
Antenna
Eye
En
hth
ct
ey
Antennal fate
?
ey, toy
eya
so
dac
hth
ext
string/cdc25
Eye fate
Cell proliferation
(epidermal fate)
Hth: homothorax is a transcriptional co-factor to Hox genes
Ct: Cut, homeobox

Segregation of the eye-antennal disc

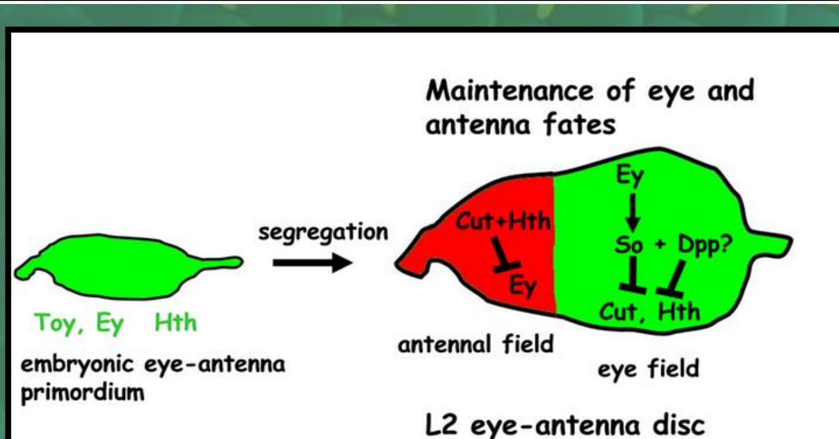




Wang and Sun, Development, 2012

Otd: ortodenticle, homeobox
Caup: caupolican, homeobox
Dll: distalles, homeobox
Lim1: homeobox
Tsh: teashirt, Zn-finger transcription factor, negative regulartion

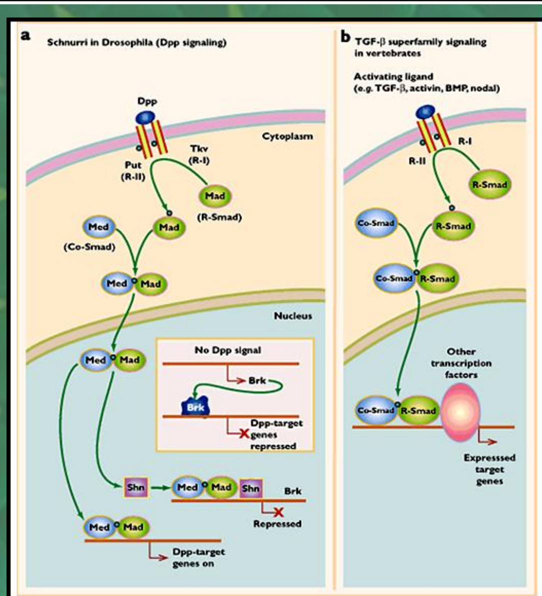
Current model of A-E segregation



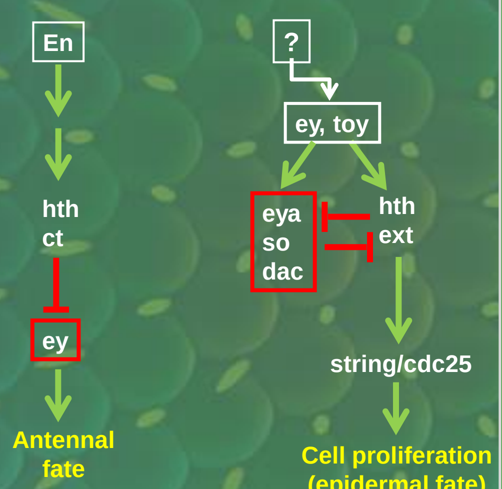
Dpp: decapentaplegic – TGFβ-like
Dac: dachshund – DNA binding

Wang and Sun, Development, 2012

Dpp signaling



Final step of segregation

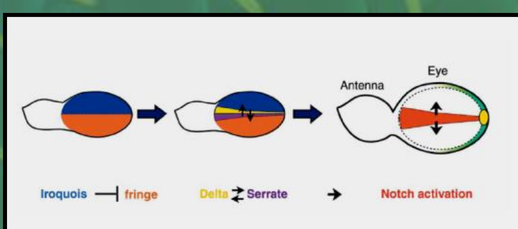


Final step of segregation

En
↓
↓
hth
ct
⊣
ey
↓
Antennal fate

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

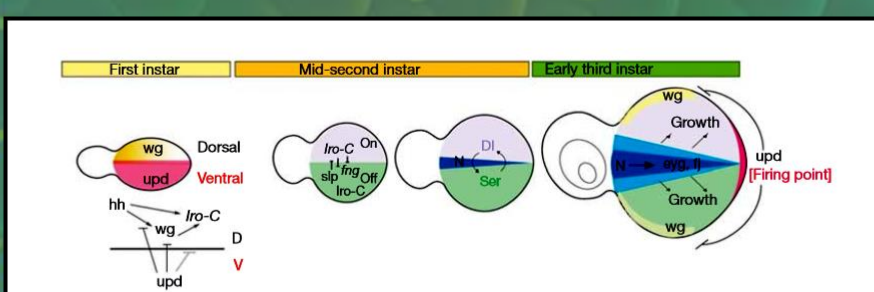
Dorsoventral polarity of the eye



First planar axis

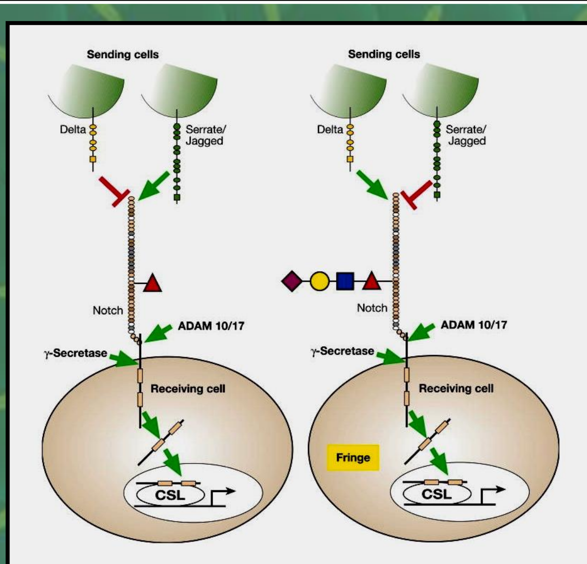
Iroquois: homeodomain transcription factor
Fringe: *N*-acetylglucosaminyltransferase

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

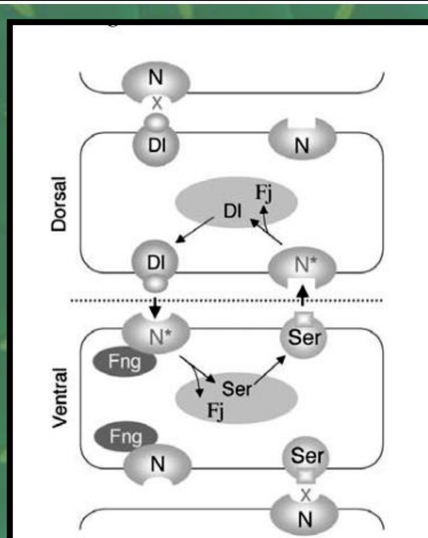


Avino et al., EMBO Rep., 2009

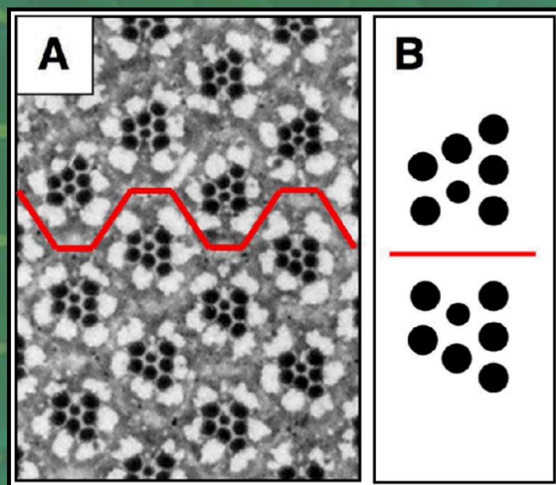
Variations on a theme :Notch/DSL signaling



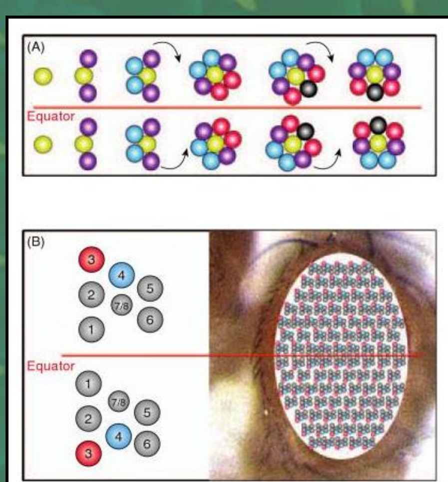
Notch at the edge



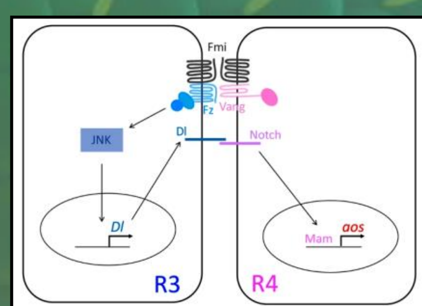
Dorsal and ventral ommatidia



Rotation of clusters



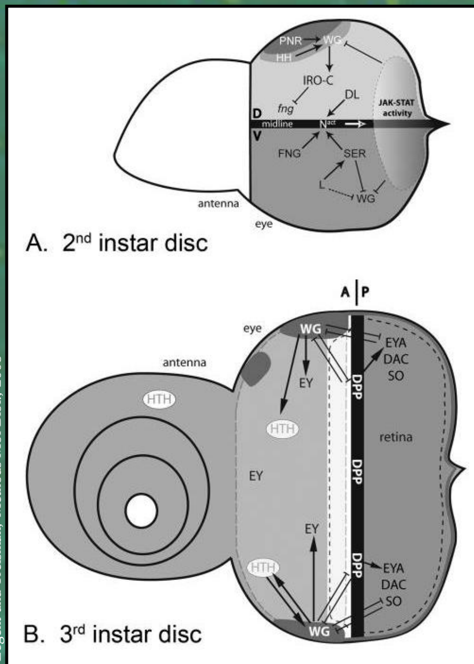
Nemo-like kinase – planar polarity



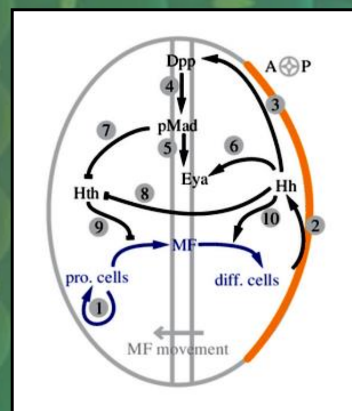
Koca et al., Nat. Sci. Rep., 2019

Fmi: flamingo, a seven-pass transmembrane cadherin
Fz: Frizzled receptor
Vang: a four-pass transmembrane protein
Dl:delta
Mam: Mastermind, a transcriptional coactivator
Aos: argos, antagonist for the Egfr receptor

Second axis (P-A) formed by Dpp signaling



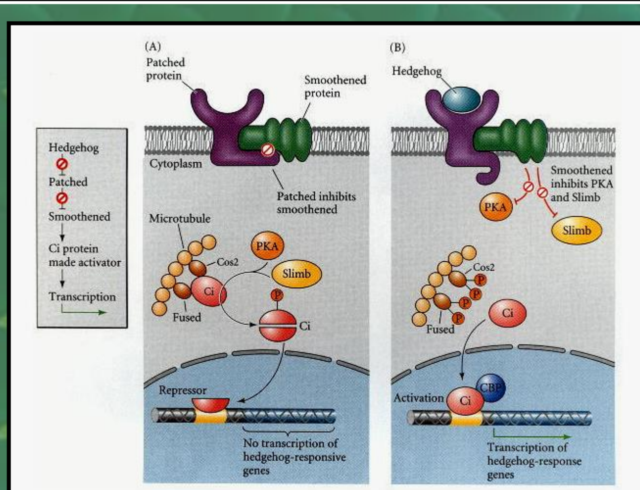
Legent and Treisman, Methods Mol Biol., 2008

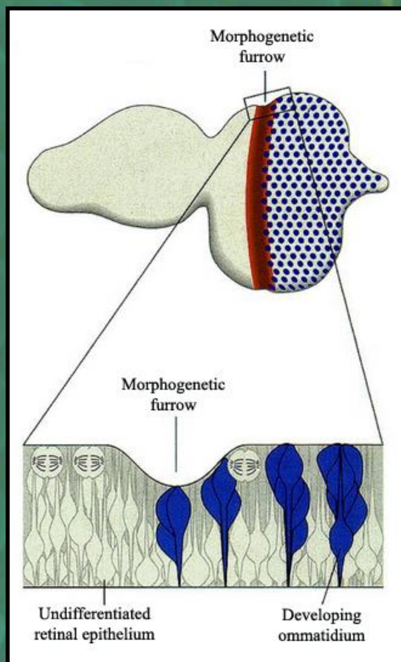


Wollmer et al., Open Biol., 2017

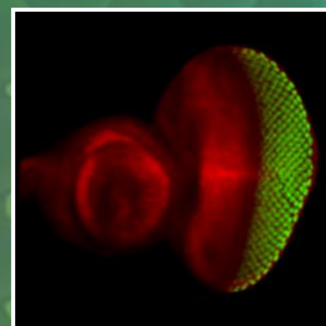
Hedgehog signaling

Hedgehog signaling antagonizes Notch: Mitotic cycles are instead of endoreduplications

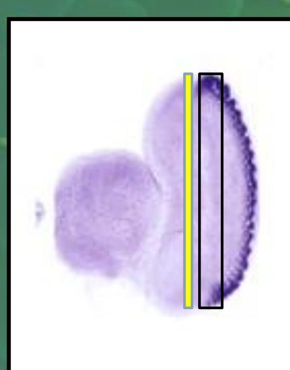


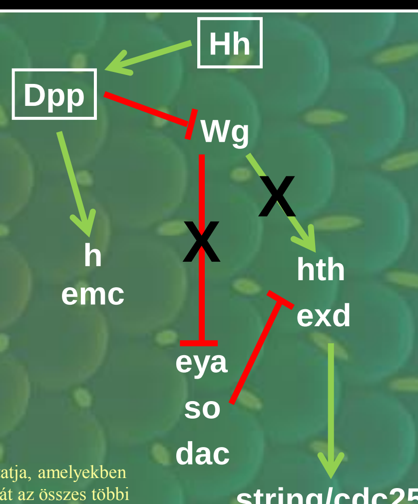


Morphogenetic furrow

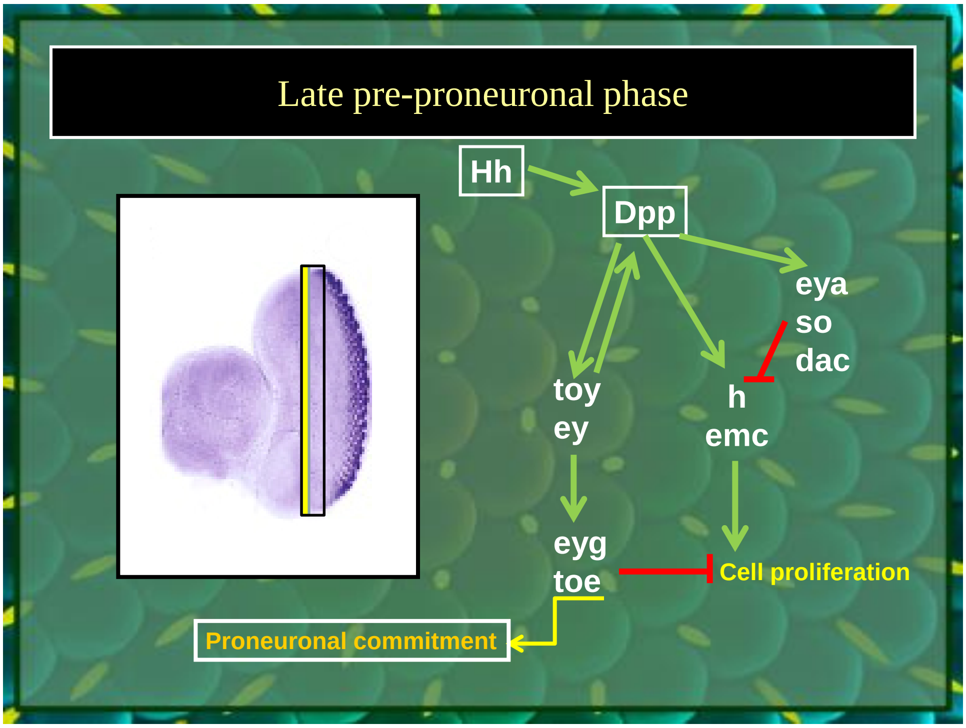


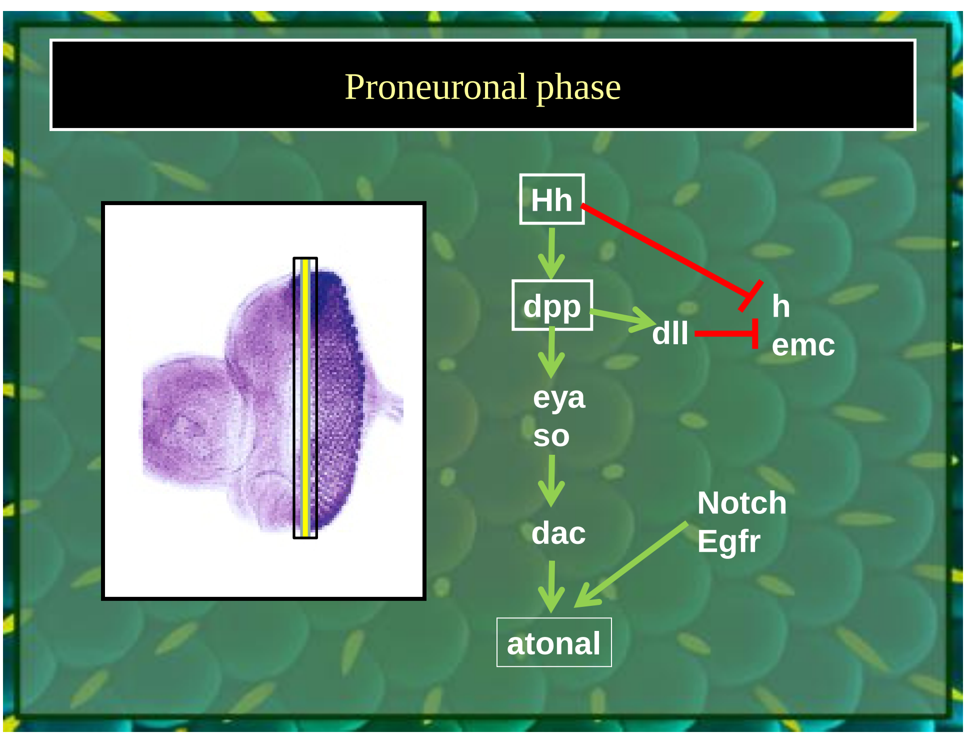
Early pre-proneuronal phase





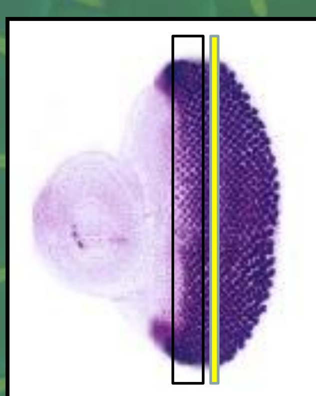
A képen a sárga vonal azoknak a sejteknek a helyét mutatja, amelyekben éppen a jobb oldali folyamatok zajlanak. Ez a vonal tehát az összes többi ábrán azonos helyen áll, míg a MF-t jelző keret lassan halad jobbról balra (posterior végtől az anterior vég felé). Így az általa elhagyott sejtek egyre idősebbek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

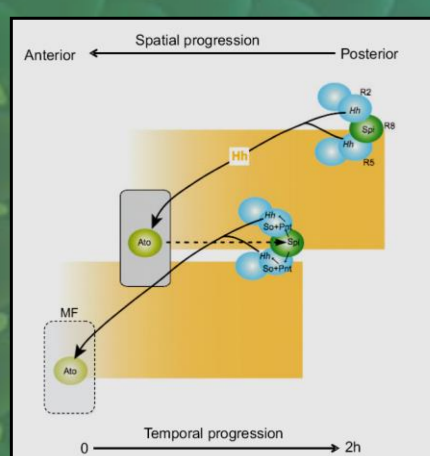

Late pre-proneuronal phase
Hh
Dpp
eya
so
dac
toy
ey
h
emc
eyg
toe
Cell proliferation
Proneuronal commitment


Proneuronal phase
Hh
dpp
dll
h
emc
eya
so
dac
Notch
Egfr
atonal

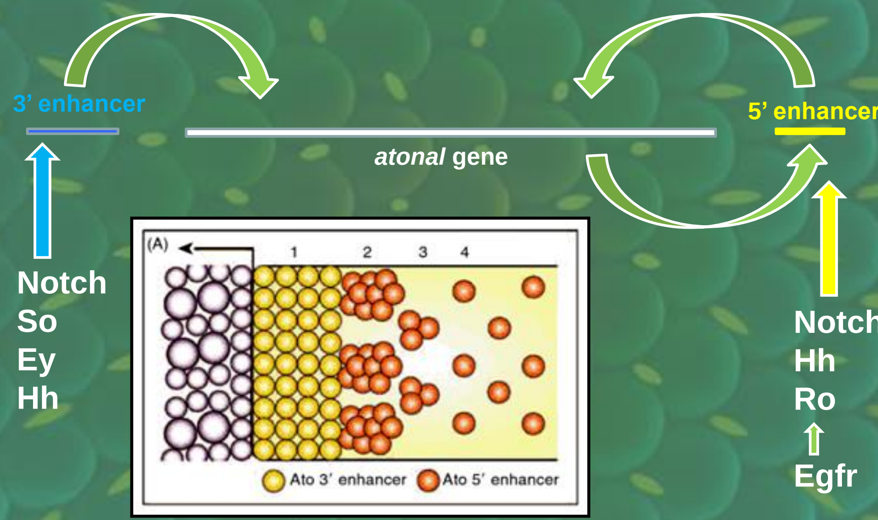
Expression of *atonal*

Neuronal development

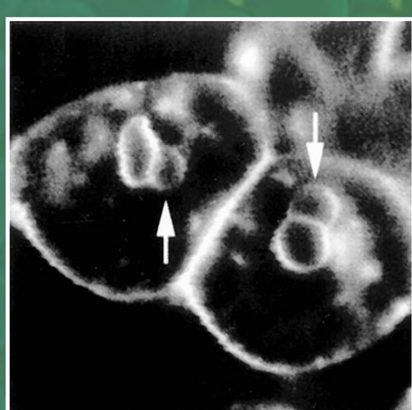


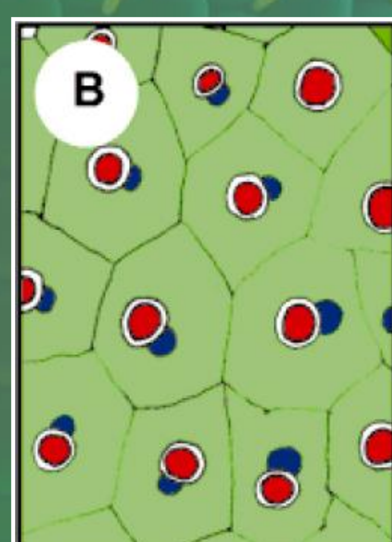


Enhancers of atonal



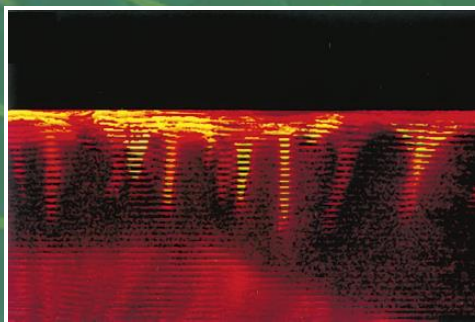
Perinuclear lobes

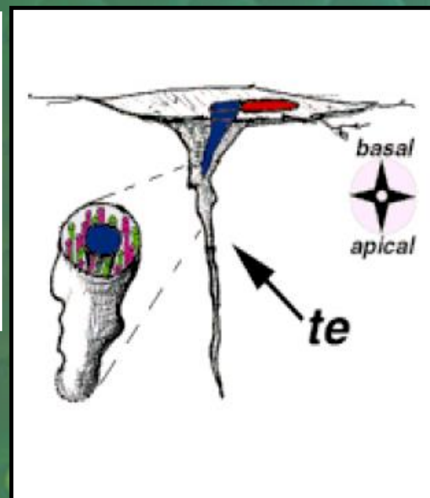




Gibson and Schubiger, 2000, Cell

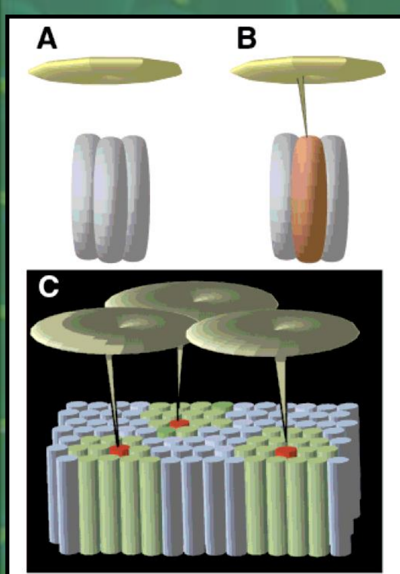
Translumenal extensions

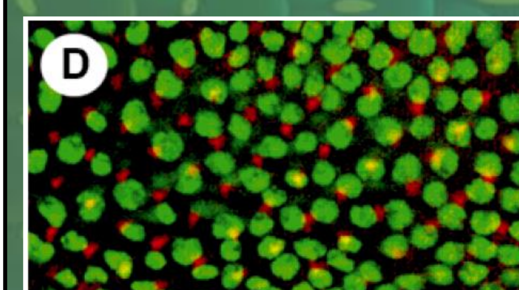




Gibson and Schubiger, 2000, Cell

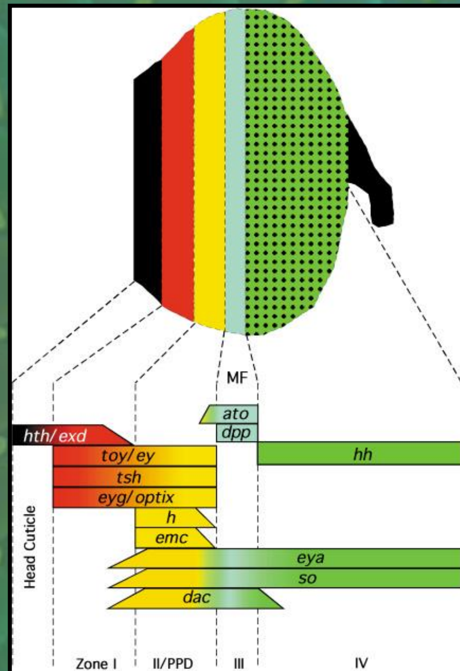
Designation of R8 founder cells



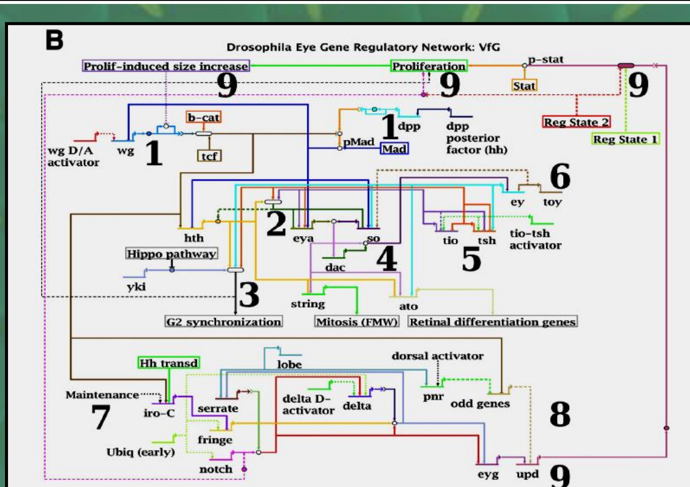


Gibson and Schubiger, 2000, Cell

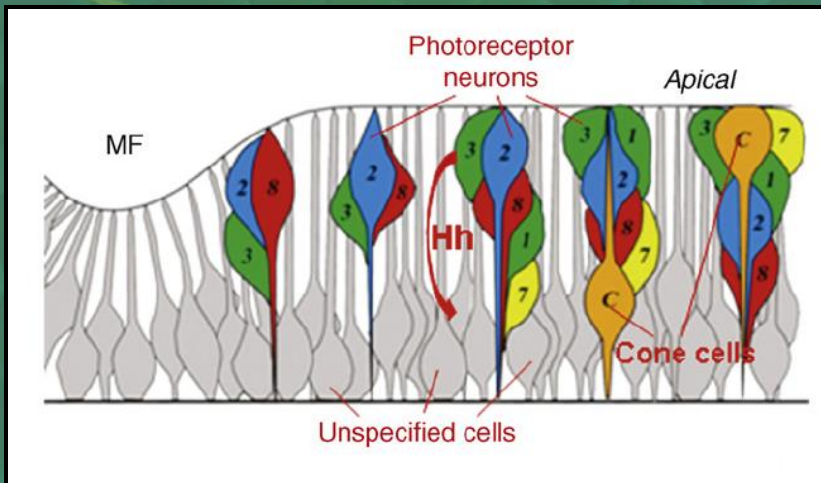
Pattern of the active genes in the developing eye



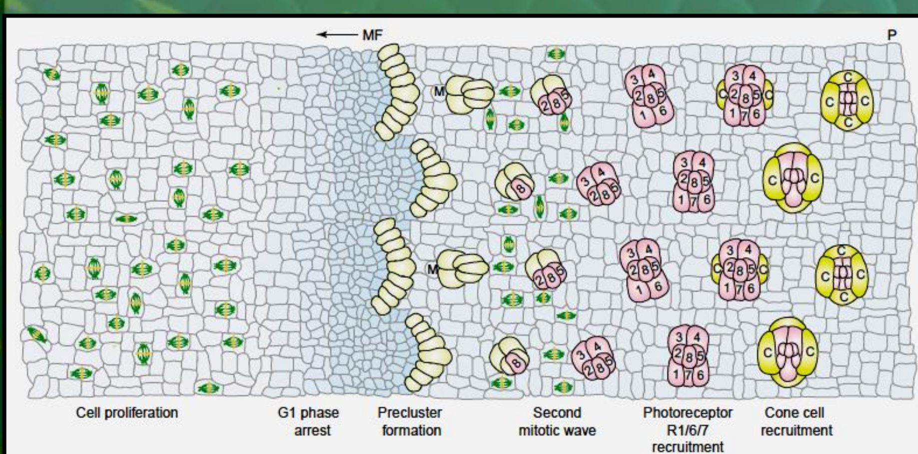
Eye gene network



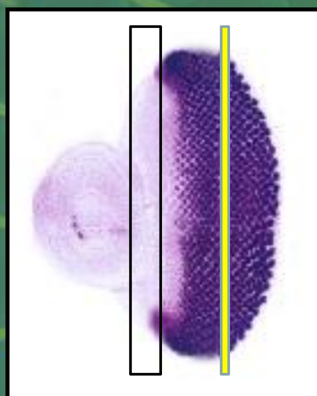
Third axis: proximal-distal by apical – basal interactions

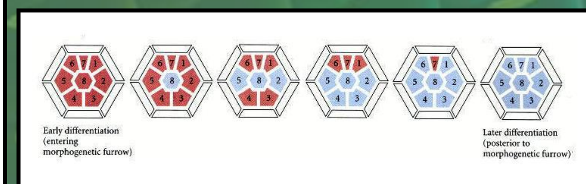


Formation of the clusters

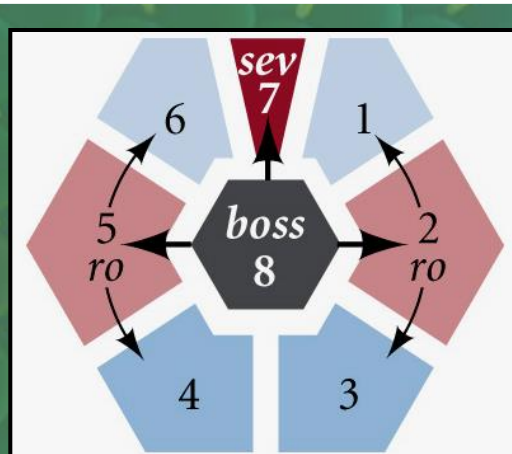


Differentiation



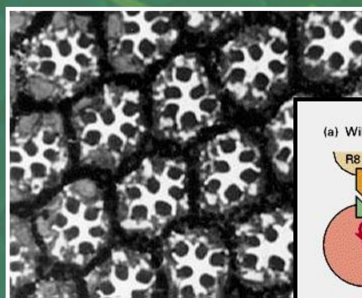


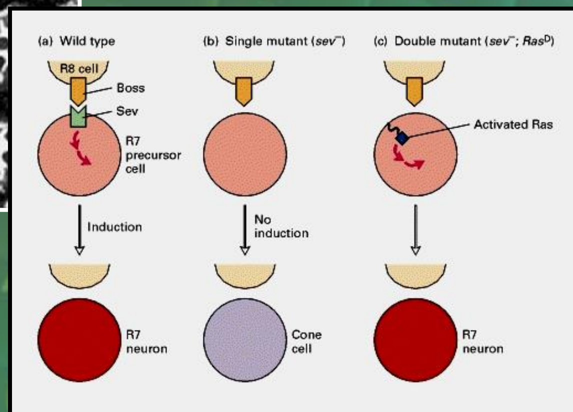
Signaling of the proneural clusters



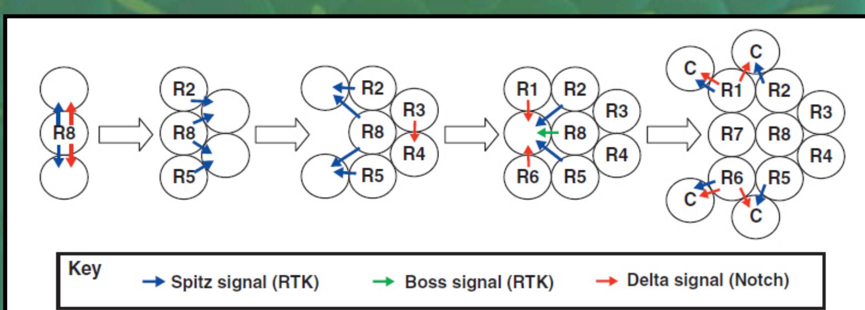
Sev: sevenless (receptor)
boss: bridge of sevenless (ligand)
ro: rough – homeobox domain

R7 determination - sevenless

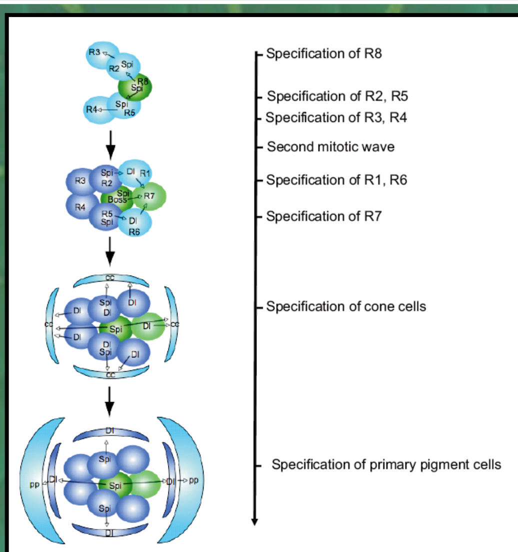




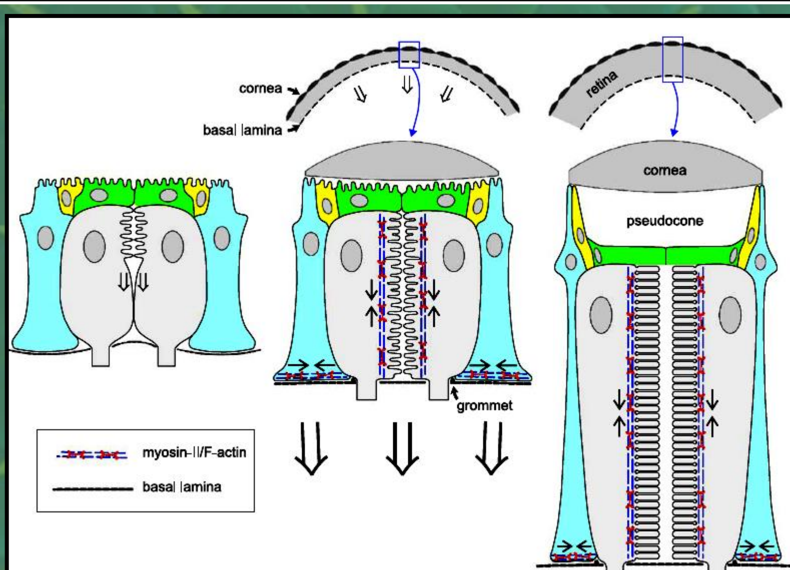
Main signalings



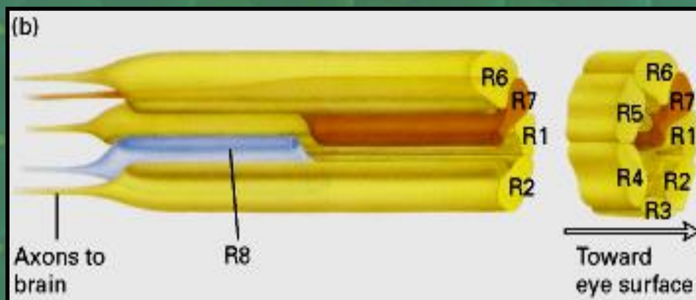
Summary of the specification of cell in an ommatidium



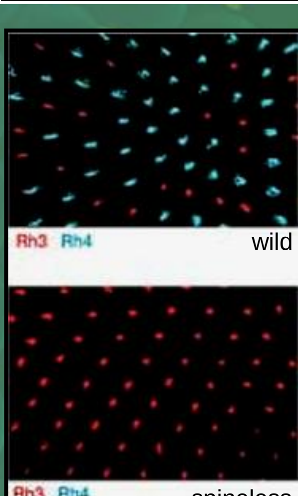
Elongation of cells



Final arrengement of retinula cells



Expression of opsins in R cells

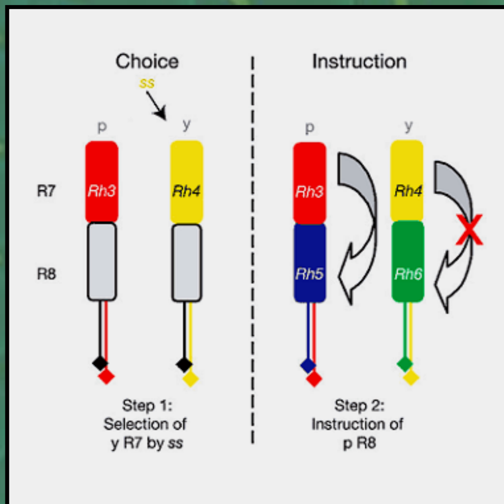


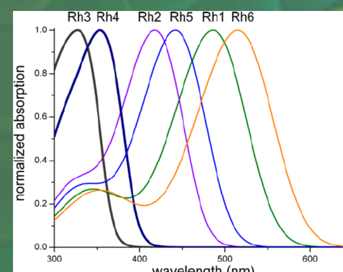
R1 – R6 rhabdomeres express:
rhodopsin1 (blue/light)

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.

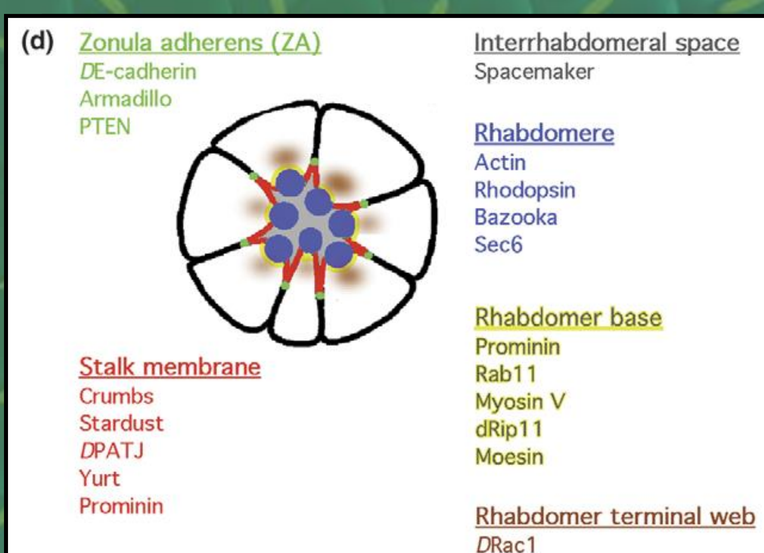
Effects of the spineless



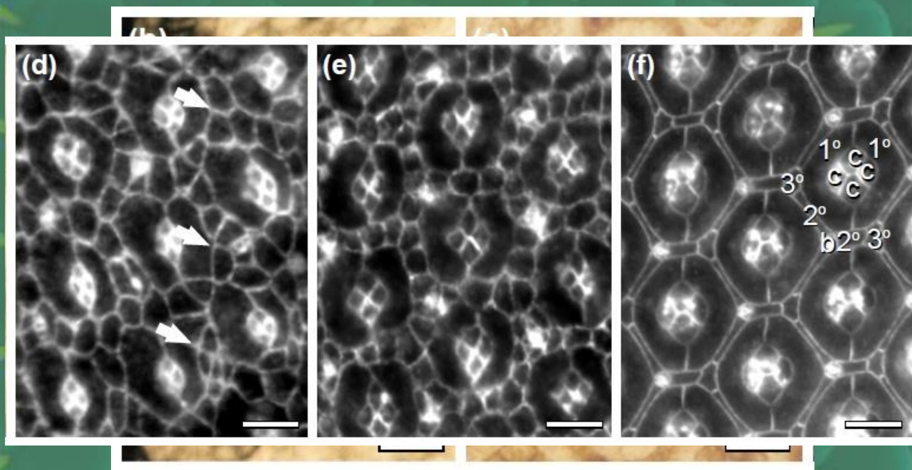


Formation of the rhabdomer

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