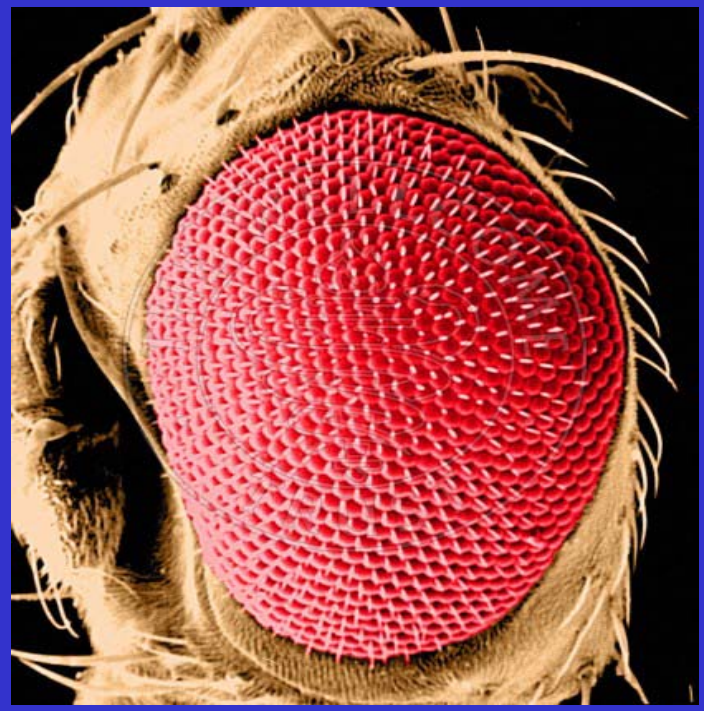
PY4302 Developmental Neuroscience

Eye Development



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Vertebrate eye development

Development of the retina

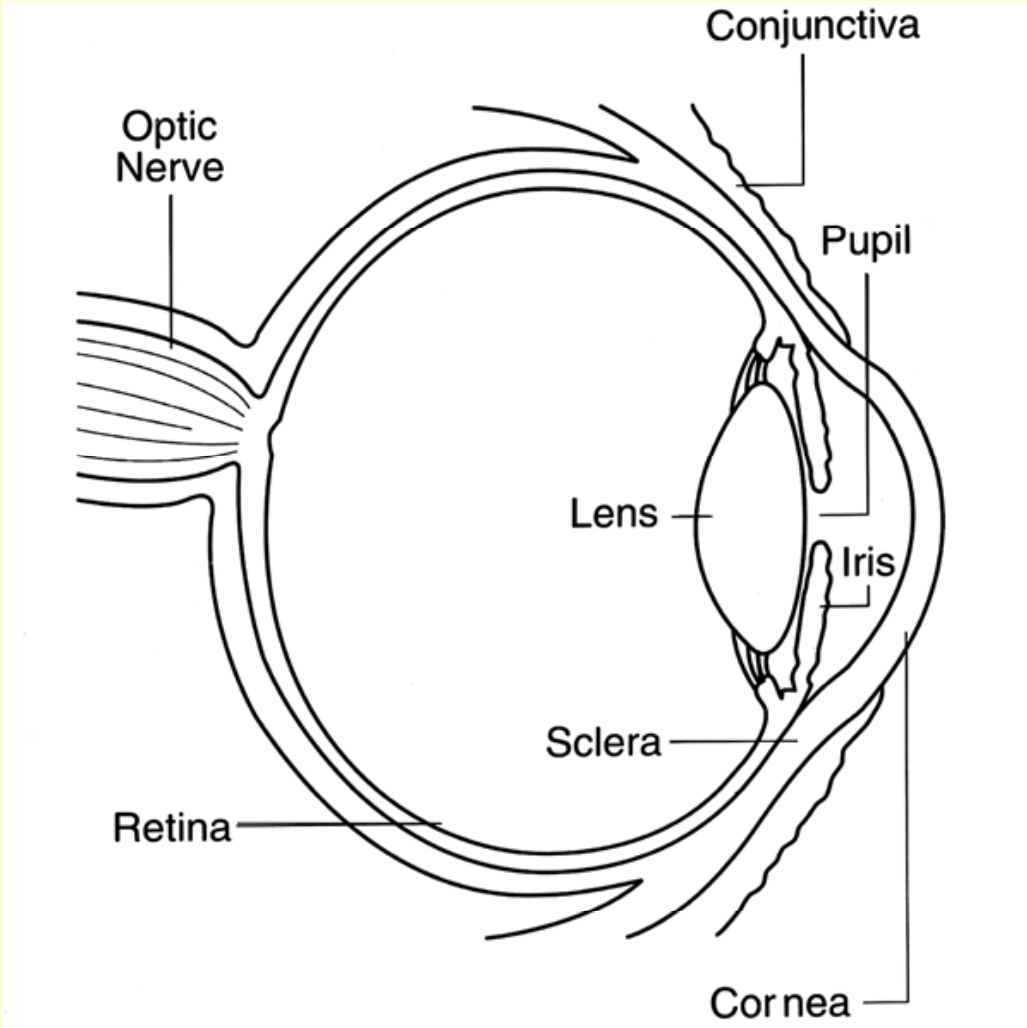
2D patterning. Specification of different cells.

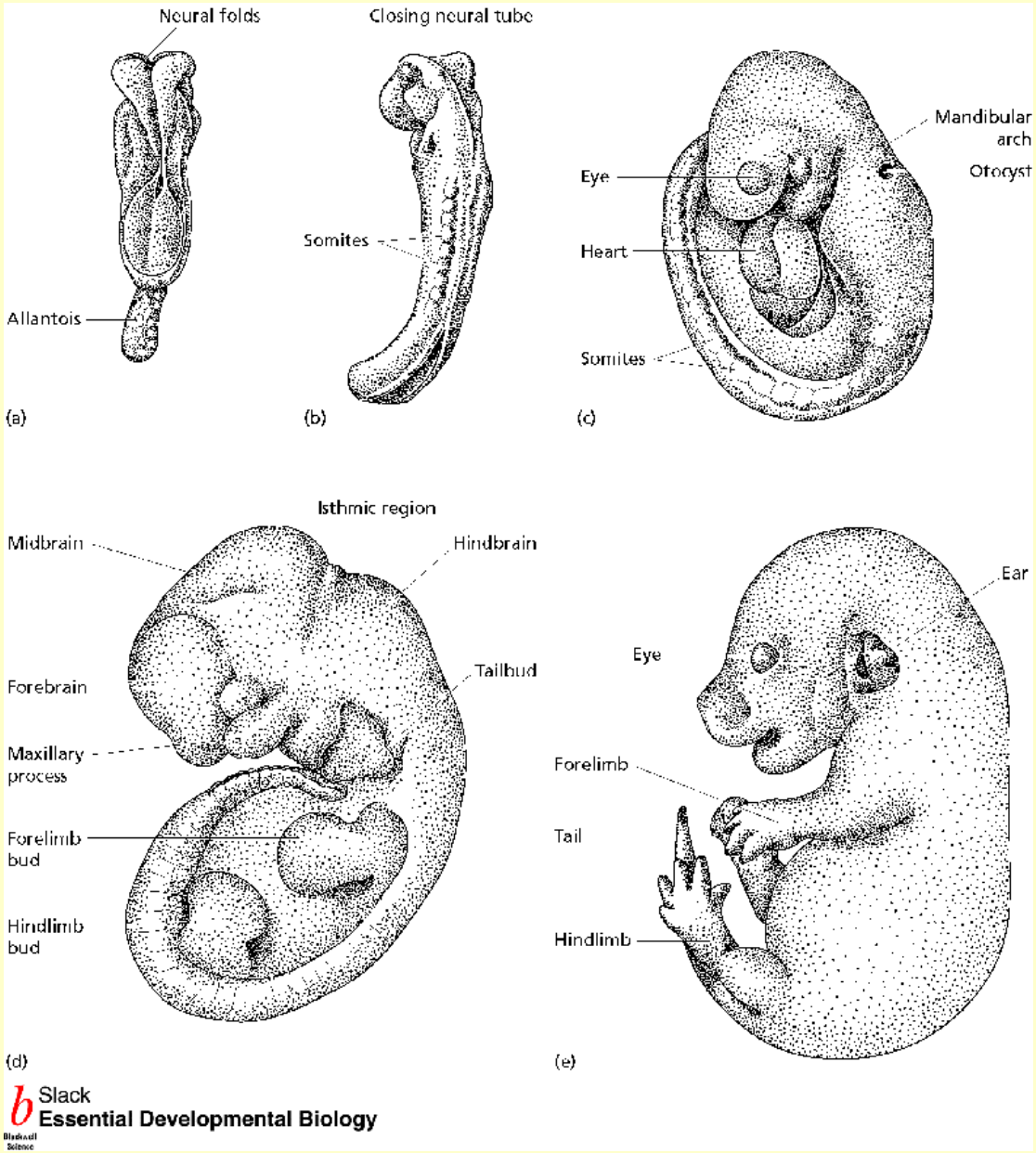
How does an apparently uniform sheet of neural precursor cells differentiate into a functional neural network with many different neuronal cell types.

Conservation of genetic pathways controlling eye development.

Are vertebrate and invertebrate eyes as different as they look?

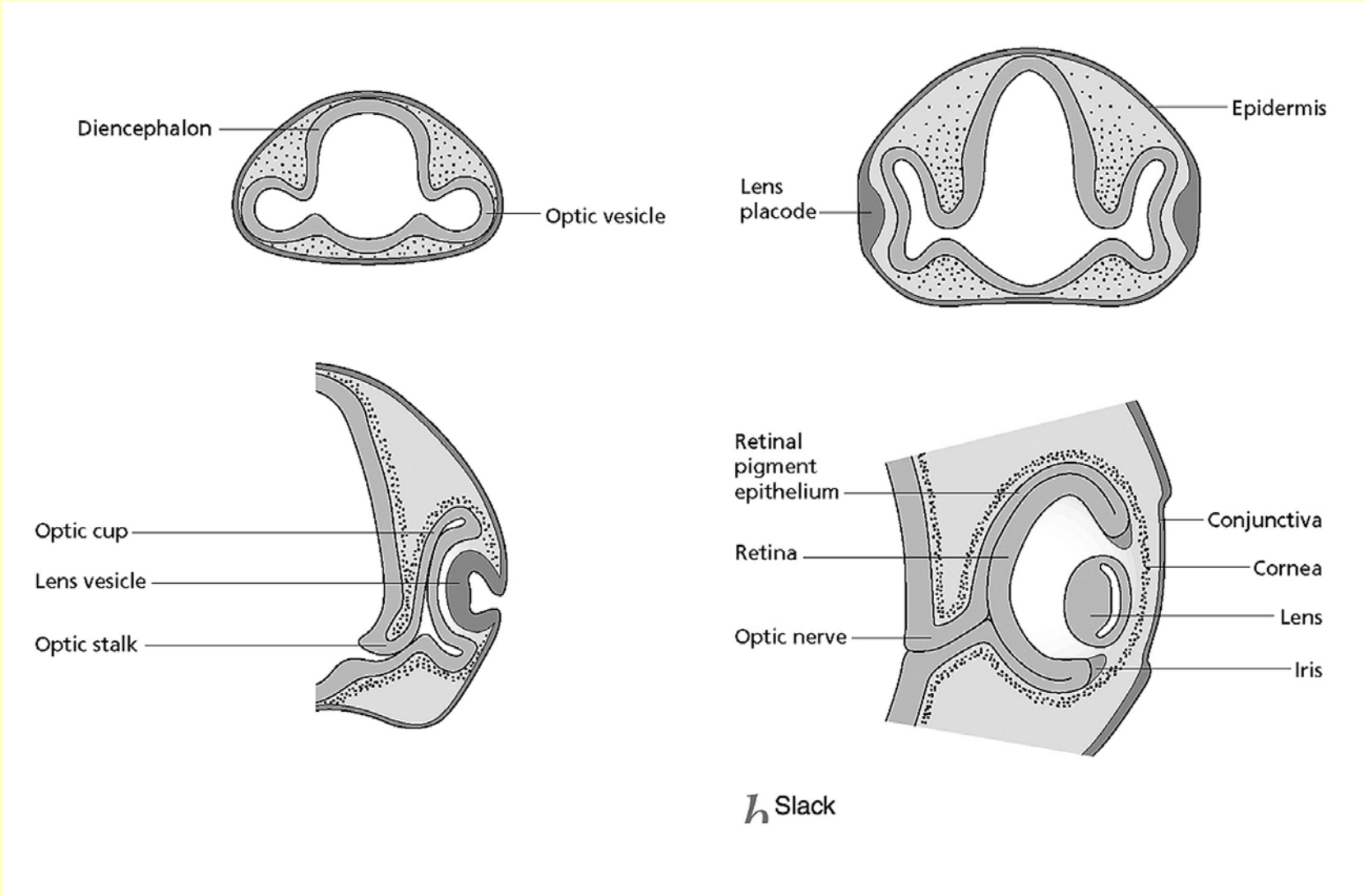
THE VERTEBRATE EYE




Neural folds
Allantois
(a)
Closing neural tube
Somites
(b)
Mandibular arch
Otocyst
Eye
Heart
Somites
(c)
Isthmic region
Midbrain
Hindbrain
Forebrain
Tailbud
Maxillary process
Forelimb bud
Hindlimb bud
(d)
Ear
Eye
Forelimb
Tail
Hindlimb
(e)
b Slack
Essential Developmental Biology
Blackwell Science

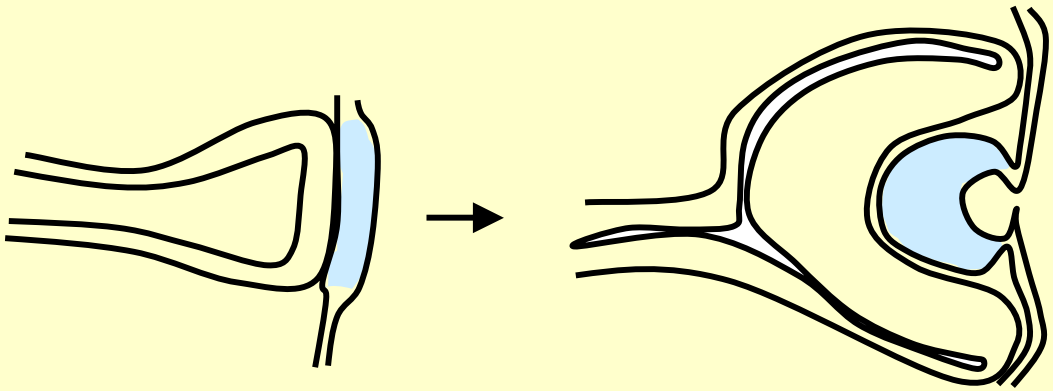
Eye Development

Tissue sections through the head of a vertebrate embryo



Inductive interactions: development of the lens.

The lens develops from and epithelial thickening (placode) that forms in the surface of the head after contact from underneath by the optic vesicle.



Experiments by Spemann (1900-20s) and Lewis (1904) suggested that contact by the optic vesicle induced the formation of a lens placode and was sufficient to force lens placode formation in epithelia that would not normally form the lens.

THIS IS **INDUCTION** - CELLS THAT WOULD BECOME SKIN HAVE BEEN TURNED INTO LENS AS A RESULT OF CONTACT WITH OPTIC VESICLE

http://zygote.swarthmore.edu/regul2.html

Inductive interactions leading to lens development:

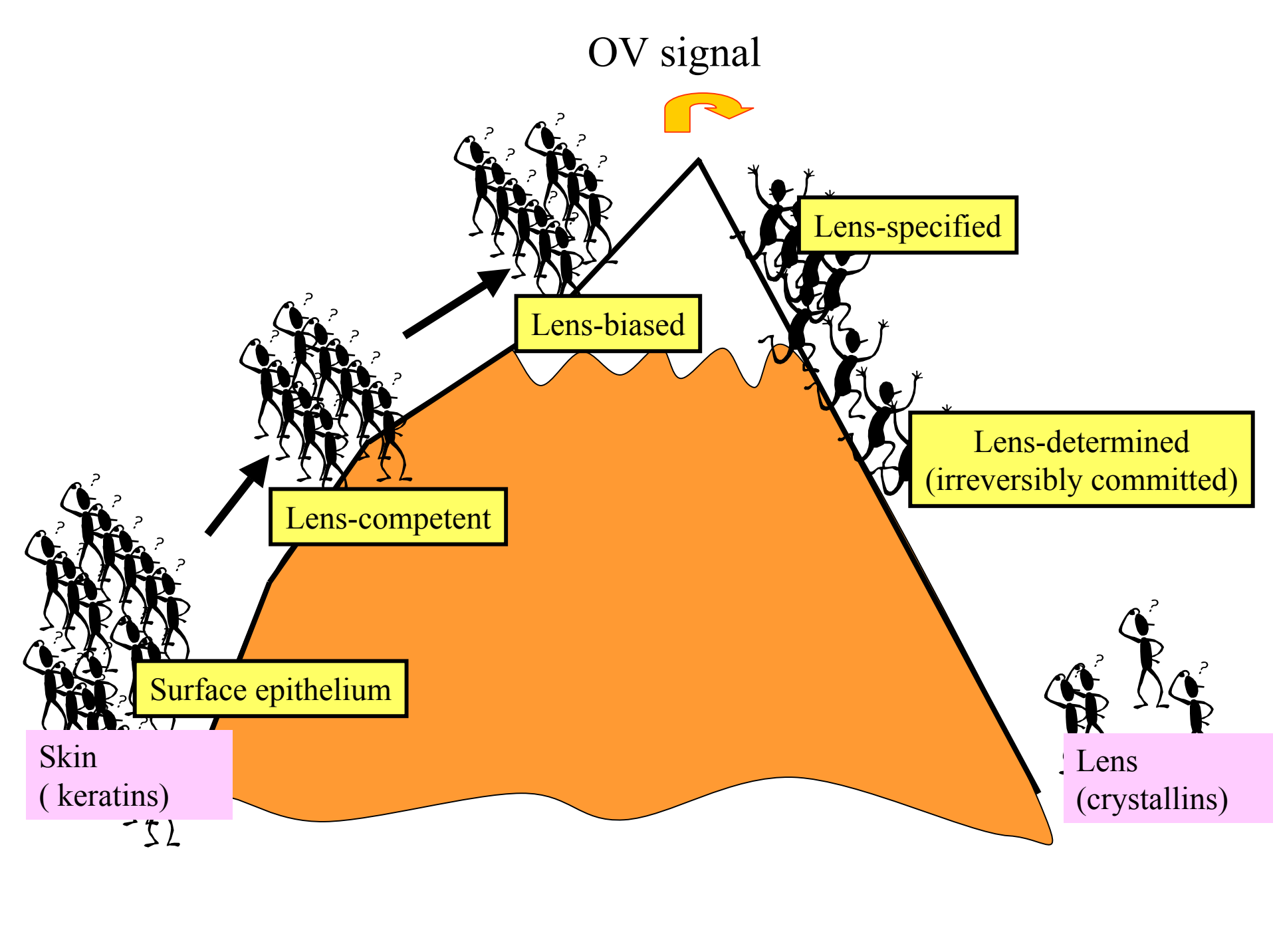
Best data from Xenopus, may not be same in other vertebrates (even amphibia).

Signals from dorsal mesoderm (muscle etc.) and from the gut creates zone of **lens competence** in to prospective head ectoderm.

Signals from developing neurectoderm further push lens-competent epithelium to be **lens-biased**.

Signal from optic vesicle causes formation of **lens-determined** placode. Once tissue is determined it can **only** form lens. The epithelial tissue is said to be **lens-specified** if it will differentiate into lens-like bodies if taken into culture (I.e needs no other signals to complete full differentiation process).

See Grainger R. M. (1992). Embryonic lens induction: shedding light on vertebrate tissue determination. *Trends Genet*. 8, 349-355.

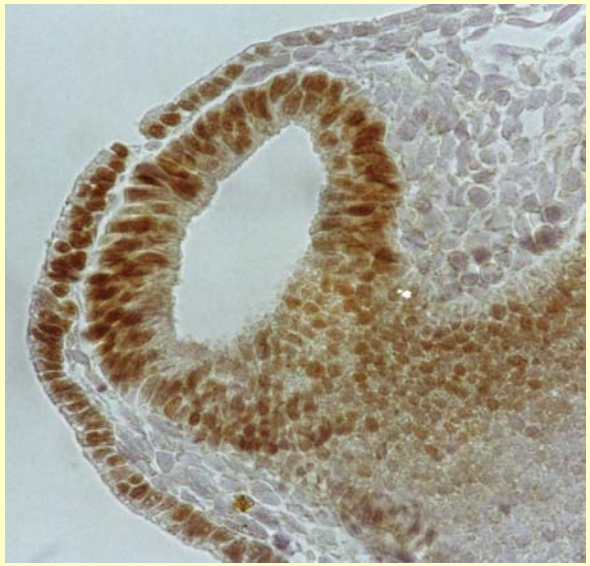

OV signal
Lens-specified
Lens-biased
Lens-determined
(irreversibly committed)
Lens-competent
Surface epithelium
Skin
(keratins)
Lens
(crystallins)

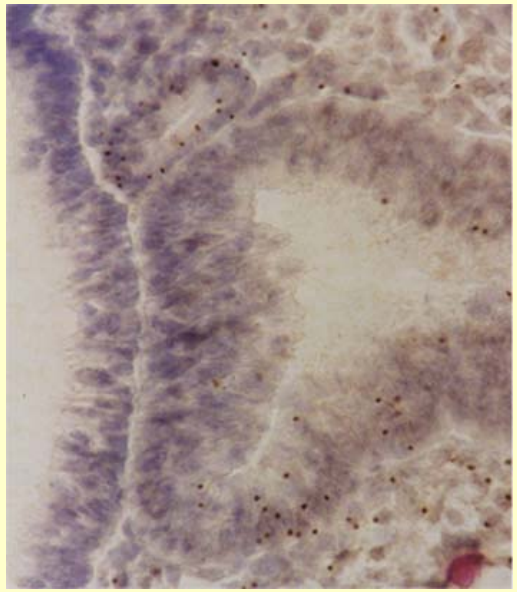
Molecular mediation of lens development 1: lens competence

The transcription factor Pax6 is possibly required for maintenance of lens competence in mice.

Expressed from early stages in head ectoderm in a region which overlaps, but is wider than the area fated to form the lens.

In chimeric mice that were a mixture of *Pax6*$^{+/+}$ and *Pax6*$^{-/-}$ cells, the mutant cells were excluded from this wider area of the head ectoderm prior to lens placode formation. I.e. need *Pax6* to be able to contribute to area of lens competence.



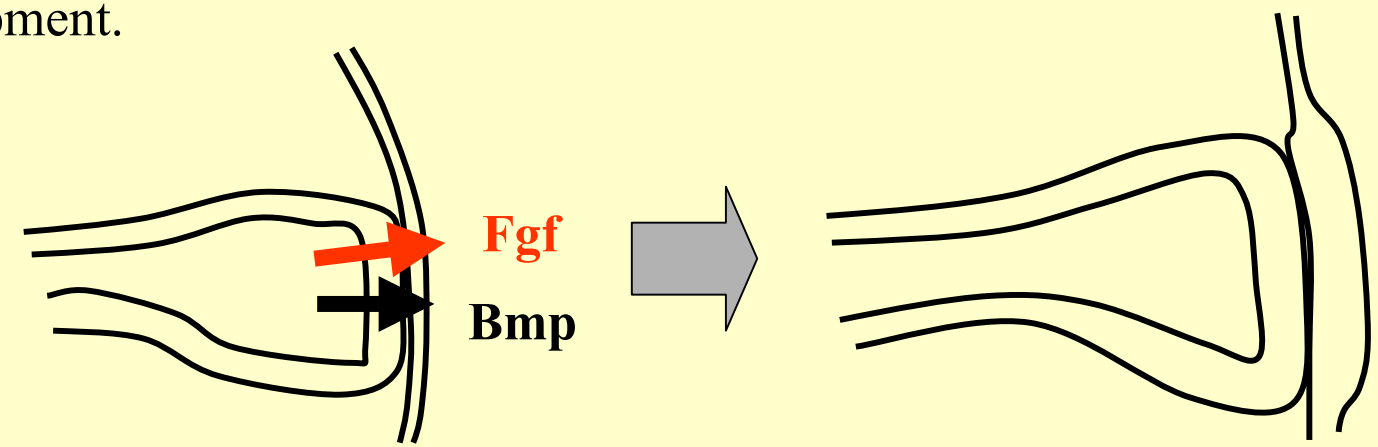


Molecular mediation of lens development 2: lens induction by the optic vesicle.

Molecules secreted by optic vesicle function to induce lens in **competent** facial epithelium.

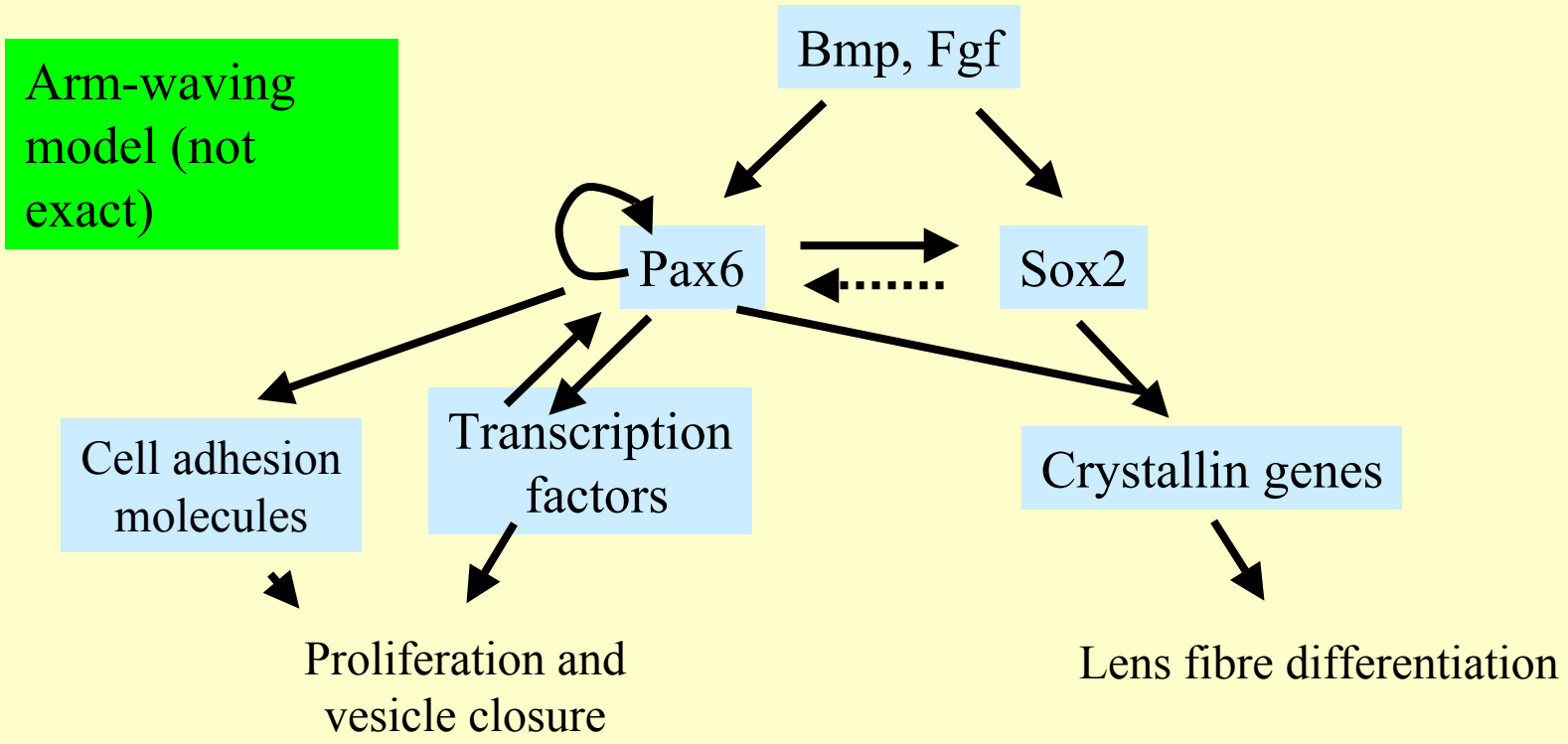
Bone morphogenetic proteins 4 and 7 - *Bmp4*, *Bmp7*. (Evidence from KOs).
Fibroblast growth factors (?*Fgf15*) (Evidence from dnFGFRs in lens placode).
Something else? (Bmps and Fgfs not enough in culture).

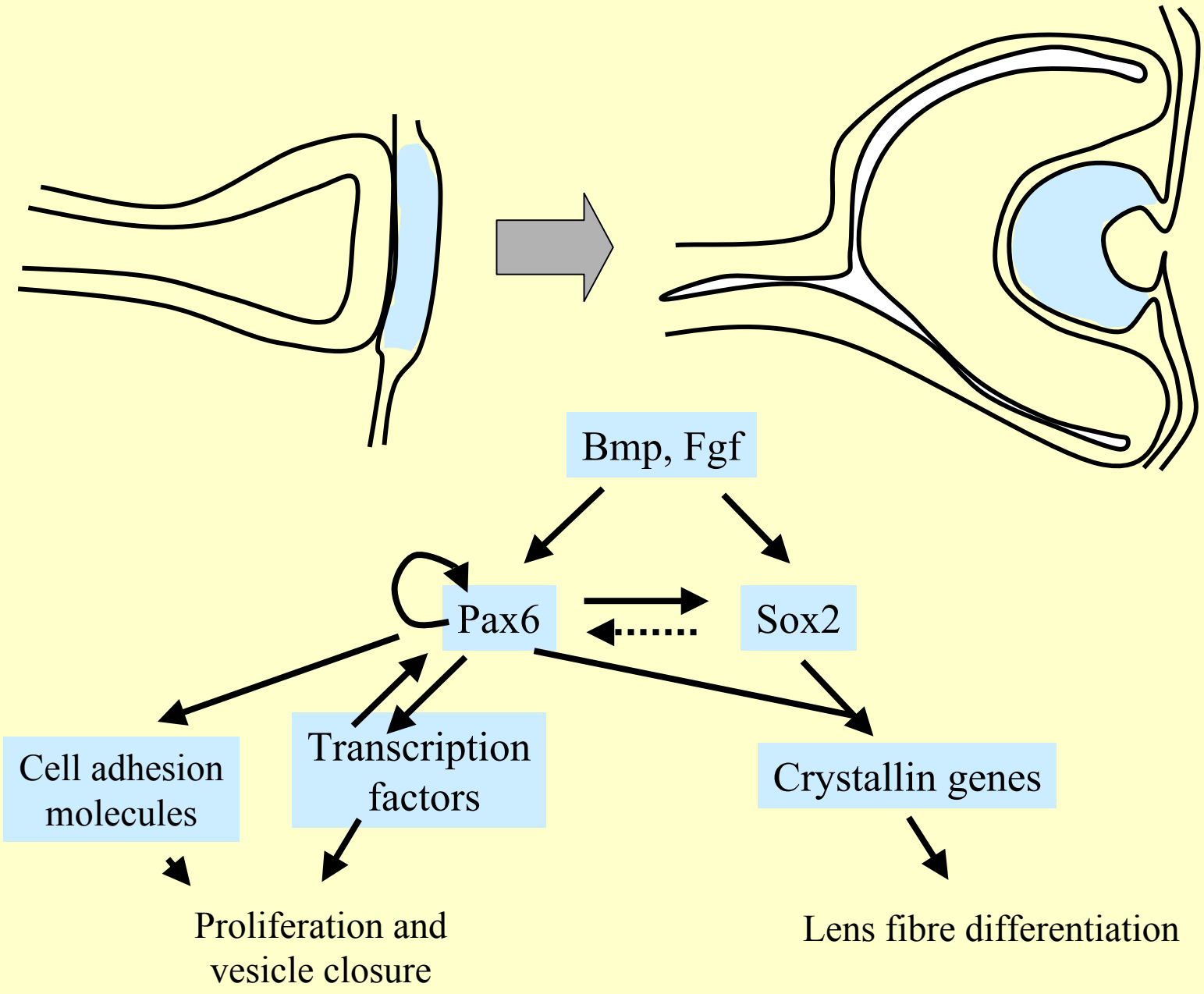
Their receptors are transmembrane receptor tyrosine kinases or serine-threonine kinases that set off secondary messenger pathways in the prospective lens. These kick off the genetic pathways that lead to lens development.



Molecular mediation of lens development 3: lens invagination and differentiation.

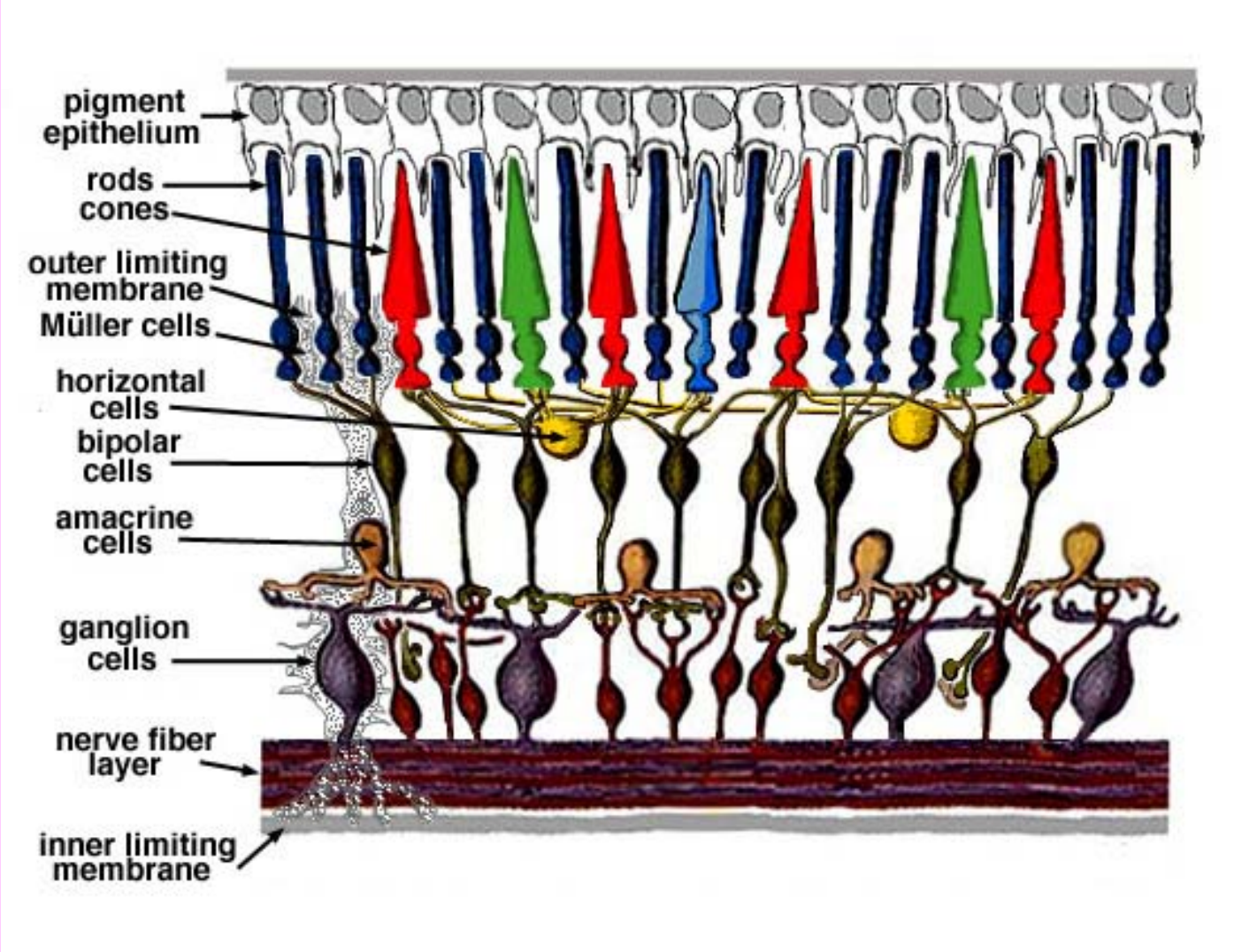
After induction of the lens placode, a genetic pathway starts in the lens, marked by **strong, autoregulated expression of *Pax6***, and expression of another transcription factor, *Sox2*. **Sox2 complexes with Pax6** to control the expression of other genes important for lens development.

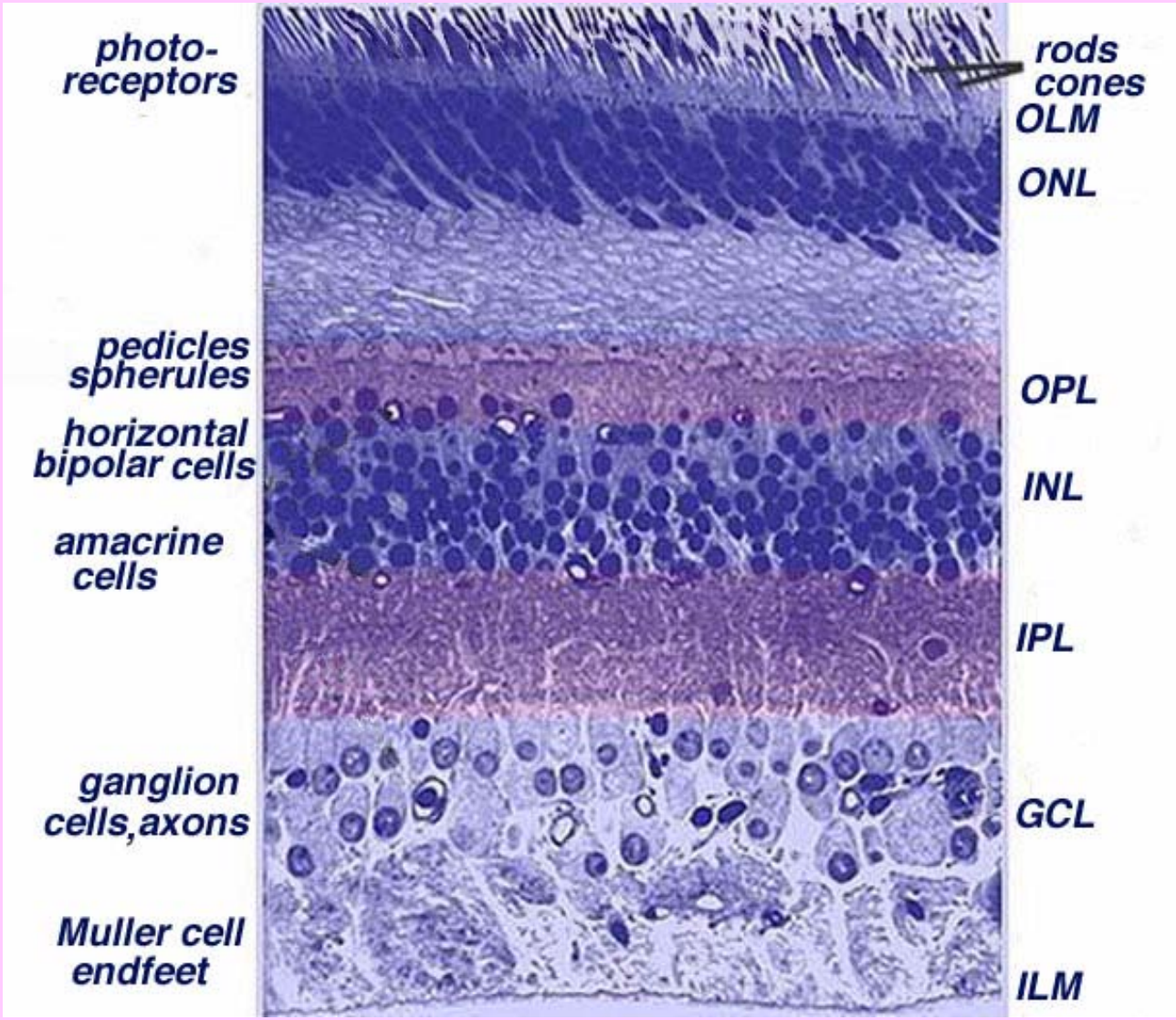



Bmp, Fgf
Pax6
Sox2
Cell adhesion molecules
Transcription factors
Crystallin genes
Proliferation and vesicle closure
Lens fibre differentiation

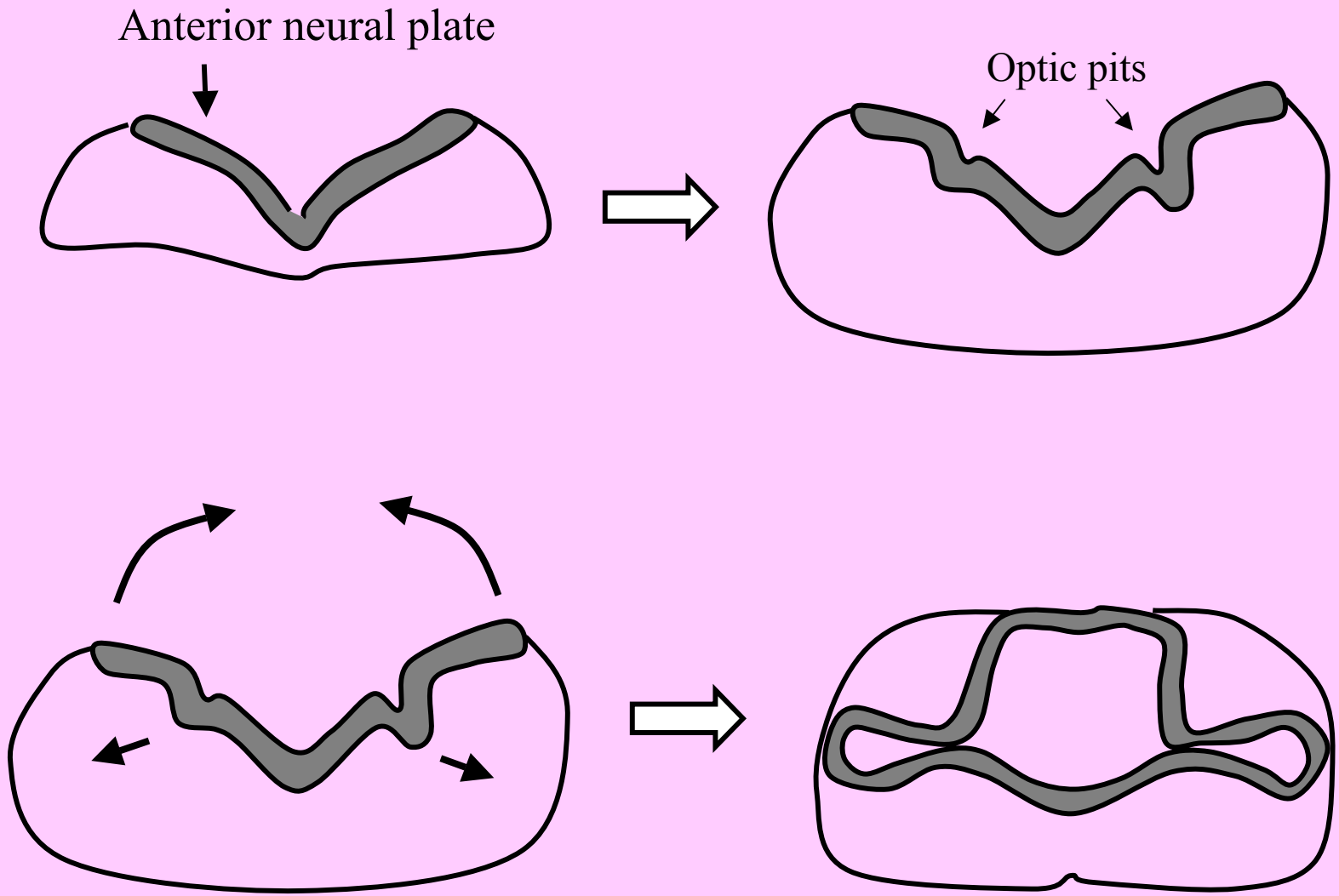
DEVELOPMENT OF THE RETINA

The adult retina in section





Complex, but develops from a uniform sheet of brain epithelium, early in development



Genetic patterning of the anterior normal plate precedes the first signs of morphological differentiation (the optic pits).

WHY DO WE HAVE TWO EYES?

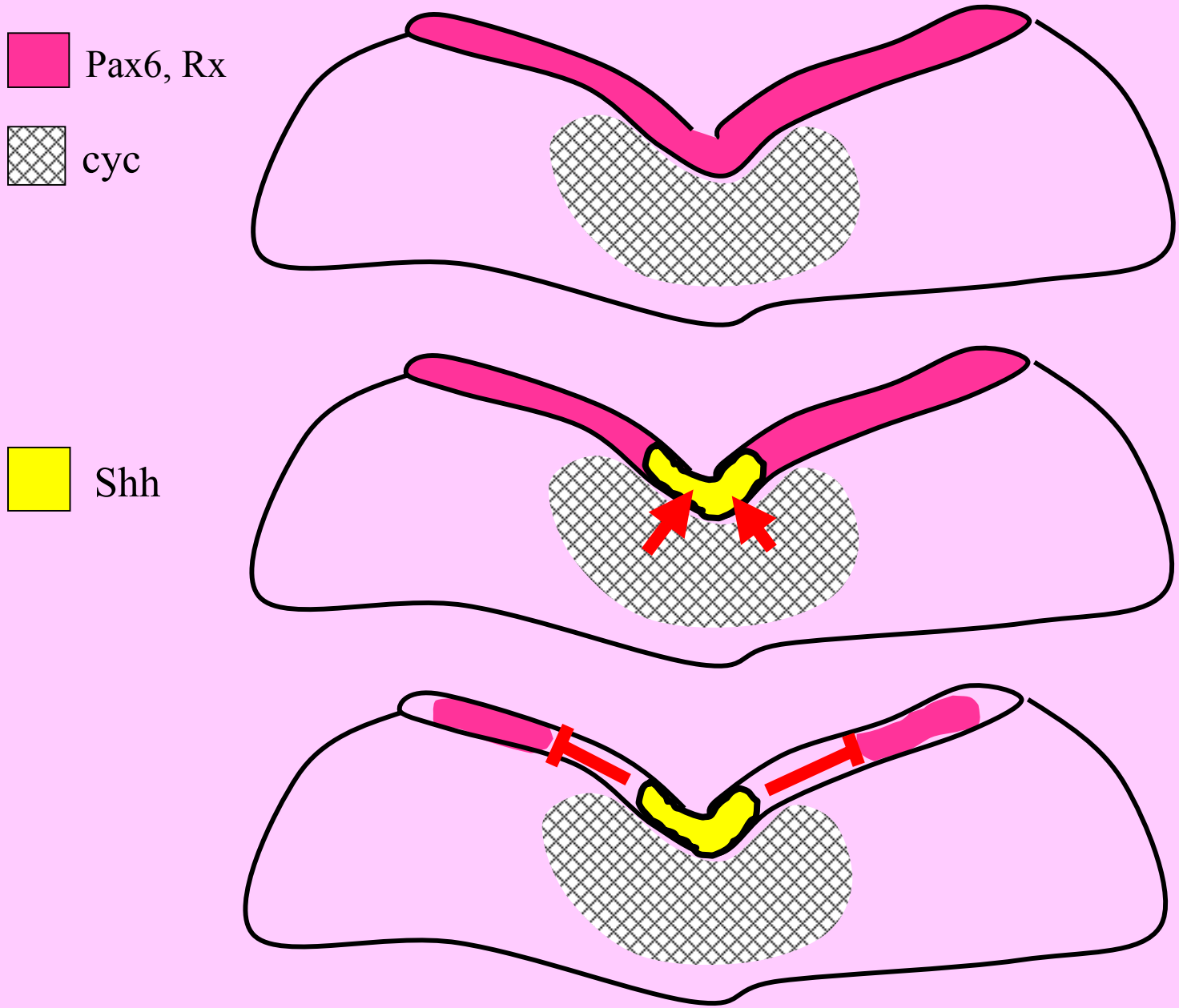
Many of the genes that will be used in retinal development are already expressed across the whole forebrain at earliest stages.

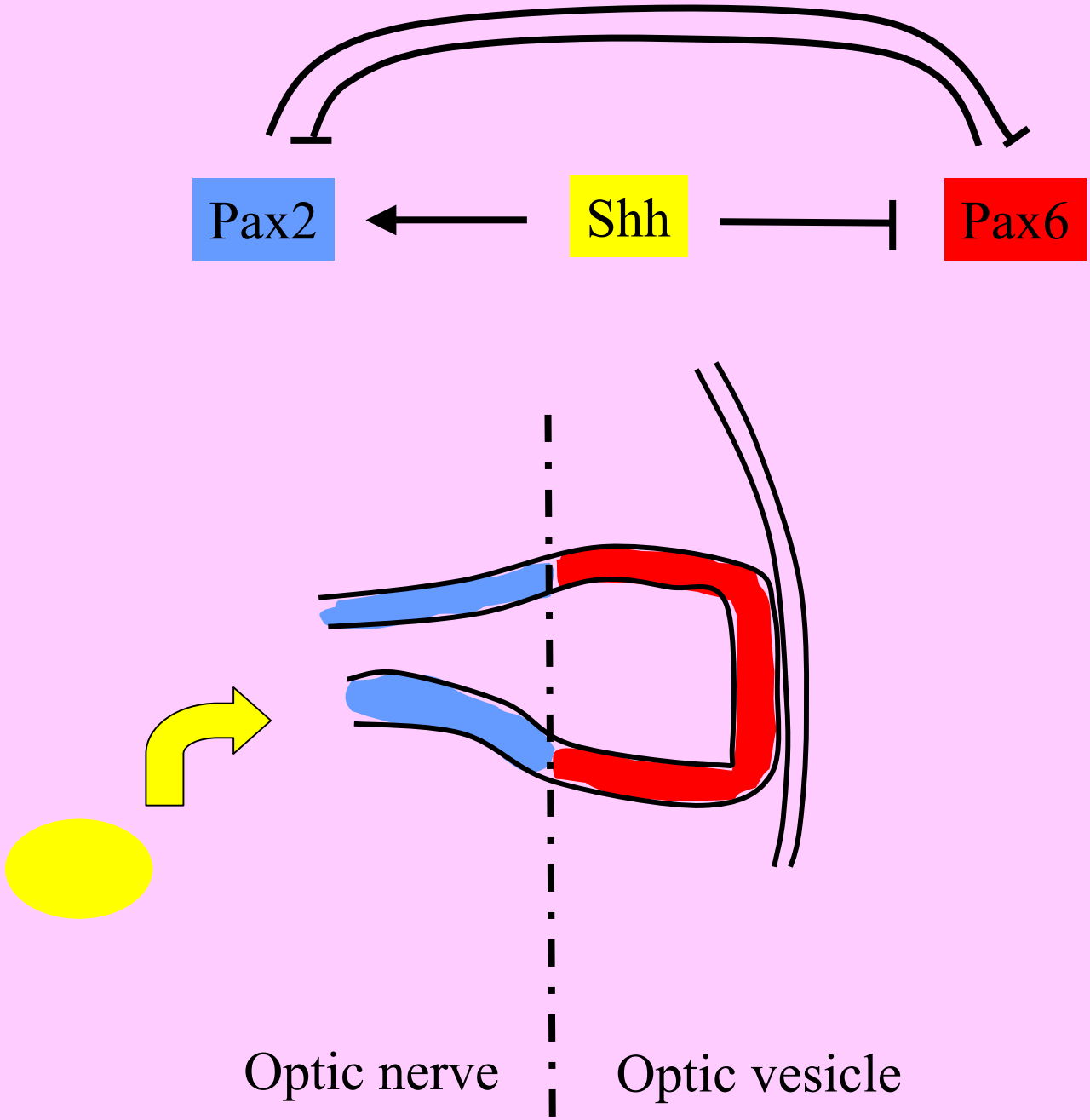
Includes *Pax6*, *Rx*.

These genes may be turned on by signalling factors (Wnts) prior to and during gastrulation.

Uniform early expression of *Pax6* and *Rx* across the anterior normal plate is split by the action of a TGF-β type molecule called *cyclops* (experiments in zebrafish), and a signalling molecule, *Sonic hedgehog,* that split the eye field, activate expression of genes required to make optic stalk (*Pax2*) and repress genes which are required for retina (*Pax6* and *Rx*).

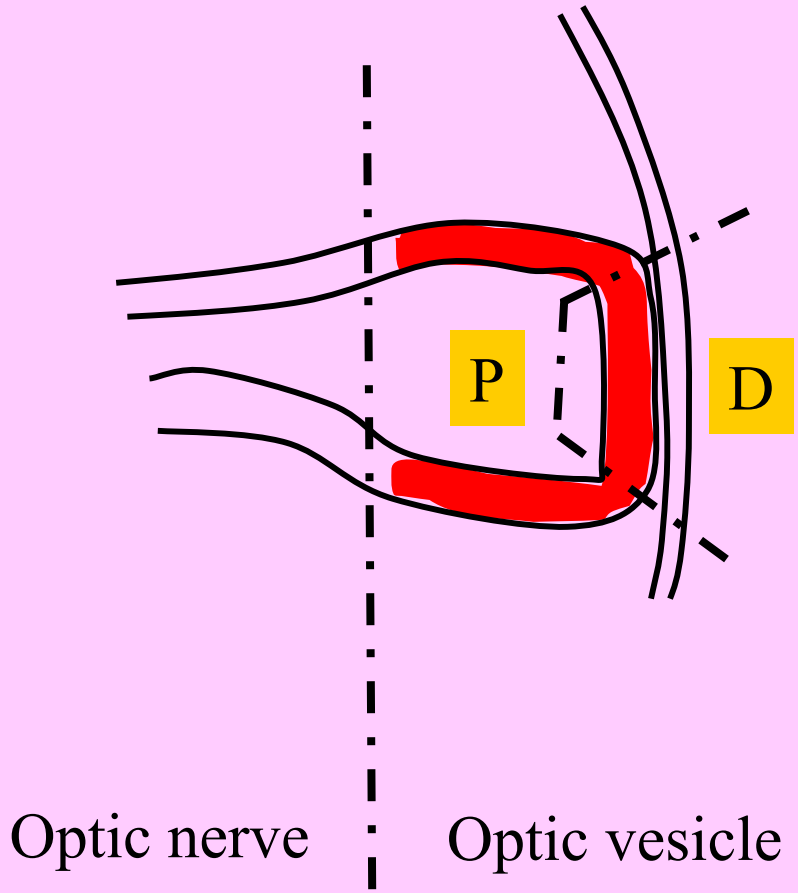
cyc$^{-/-}$ or *Shh*$^{-/-}$ give cyclopic embryos. Overexpression of *Shh* reduces the eye field.


Pax6, Rx
cyc
Shh


Pax2
Shh
Pax6
Optic nerve
Optic vesicle

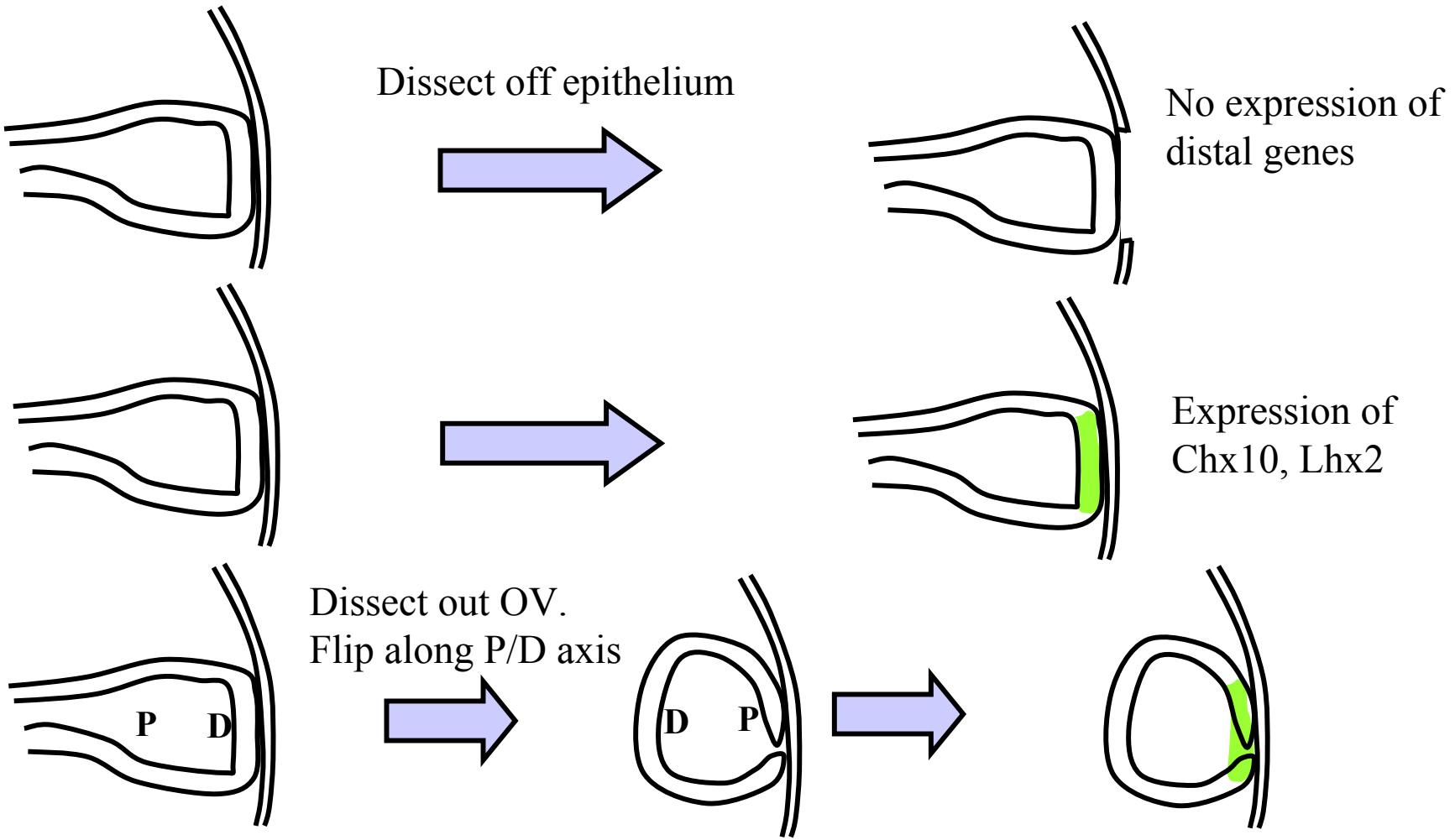
Proximo-distal specification of the optic vesicle

The part of the optic vesicle in contact with the head surface epithelium is termed distal and will form the neural retina. Those proximal regions become retinal pigment epithelium (RPE). How?



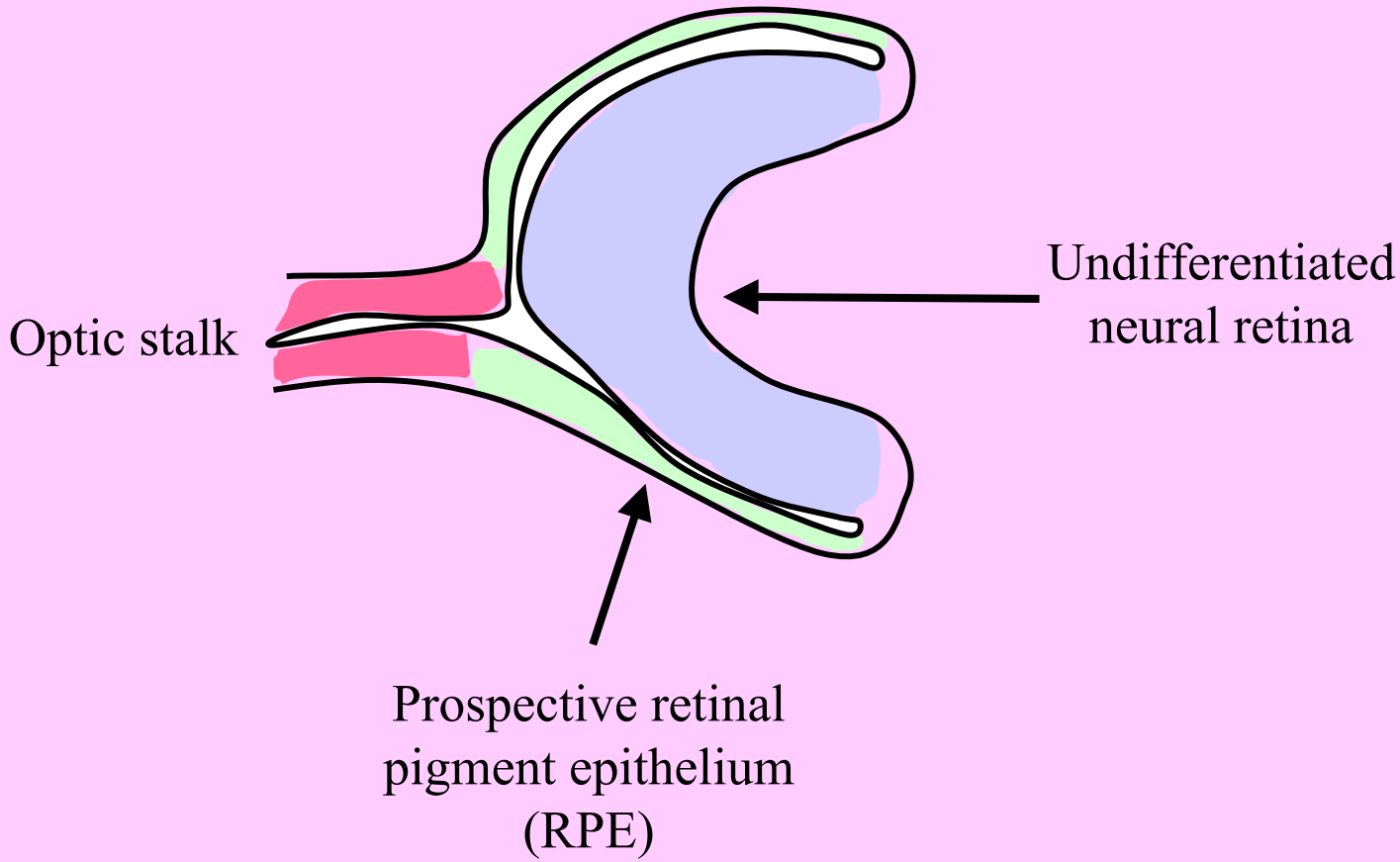
Proximo-distal specification of the optic cup

Extrinsic signals from the overlying (lens placode) epithelium turn on genes such as *Chx10* and *Lhx2* distally which are important for development of neural retina.



Morphological and cellular differentiation of the retina

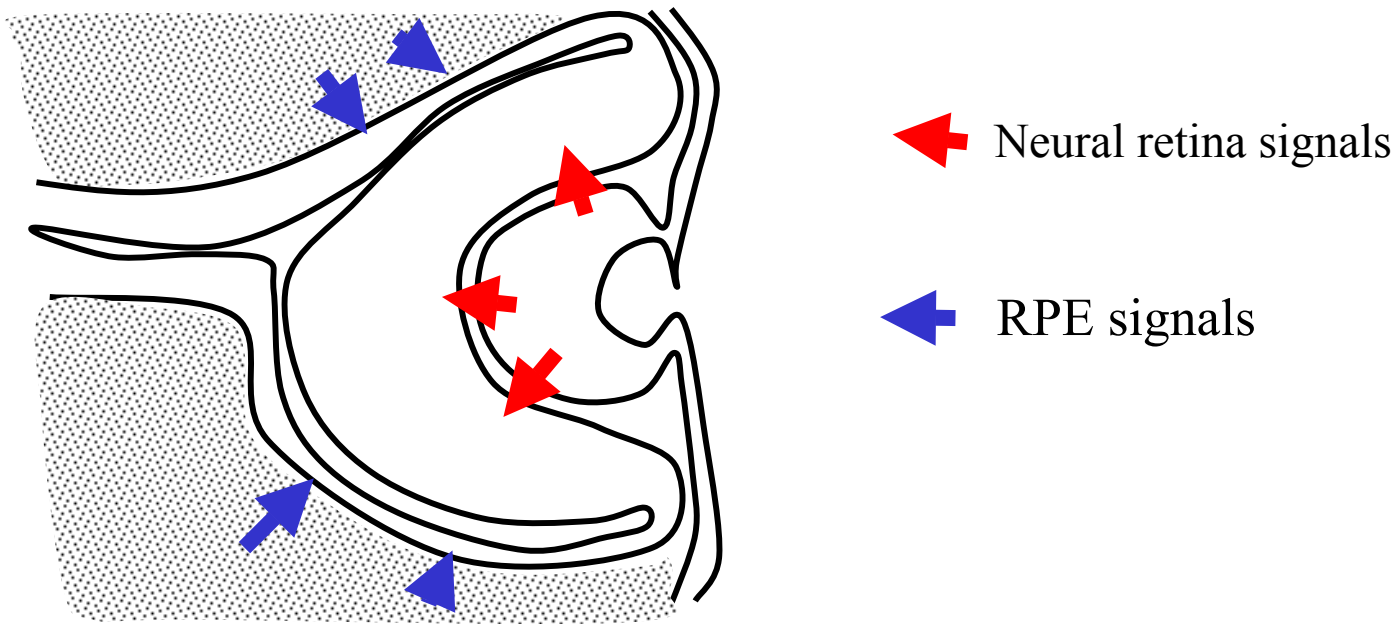
Mouse E10.5 optic cup



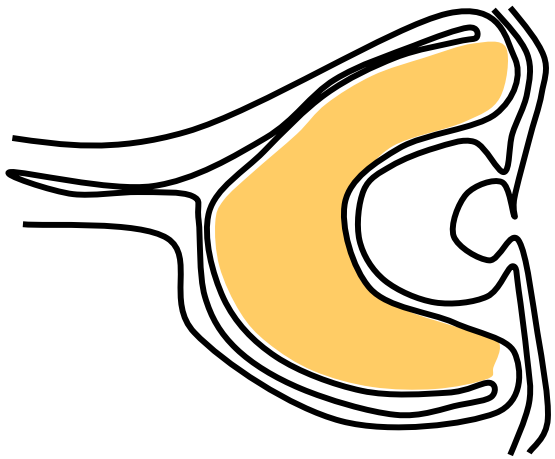
Proximo-distal specification of the optic cup

Gene knockouts (*in vivo*), and application of growth factors *in vitro* all suggest that fibroblast growth factors, FGFs, upregulate neural retina genes and downregulate RPE genes.

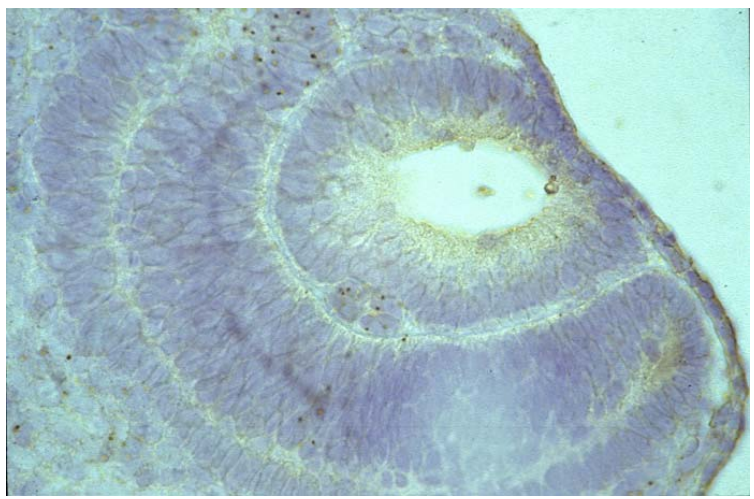
Extra-ocular mesenchyme (the cells surrounding the optic cup), upregulates genes specific for the RPE.

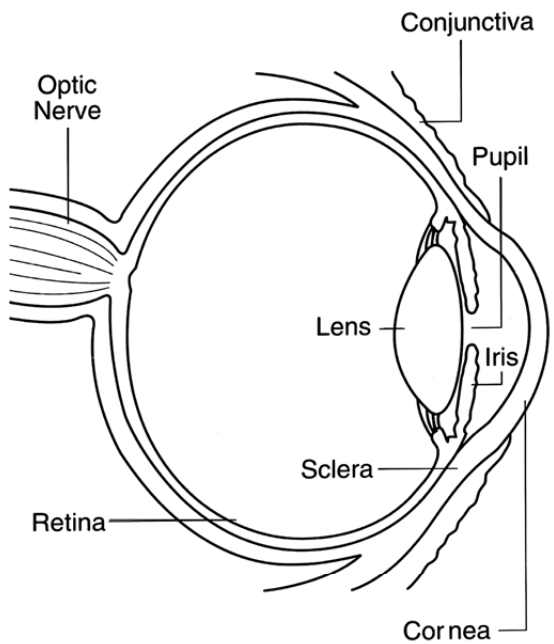


Differentiation of retinal cells



E10.5 mouse embryo - neural retina is composed of a field of undifferentiated retinal progenitor cells (RPCs).
All RPCs express a common suite of transcription factors.
Pax6, Rx1, Six3, Six6, Lhx2, Hes1.
They are **multipotent** and can differentiate into ganglion cells, bipolar, amacrine, horizontal cells, photoreceptors and Müller glia.



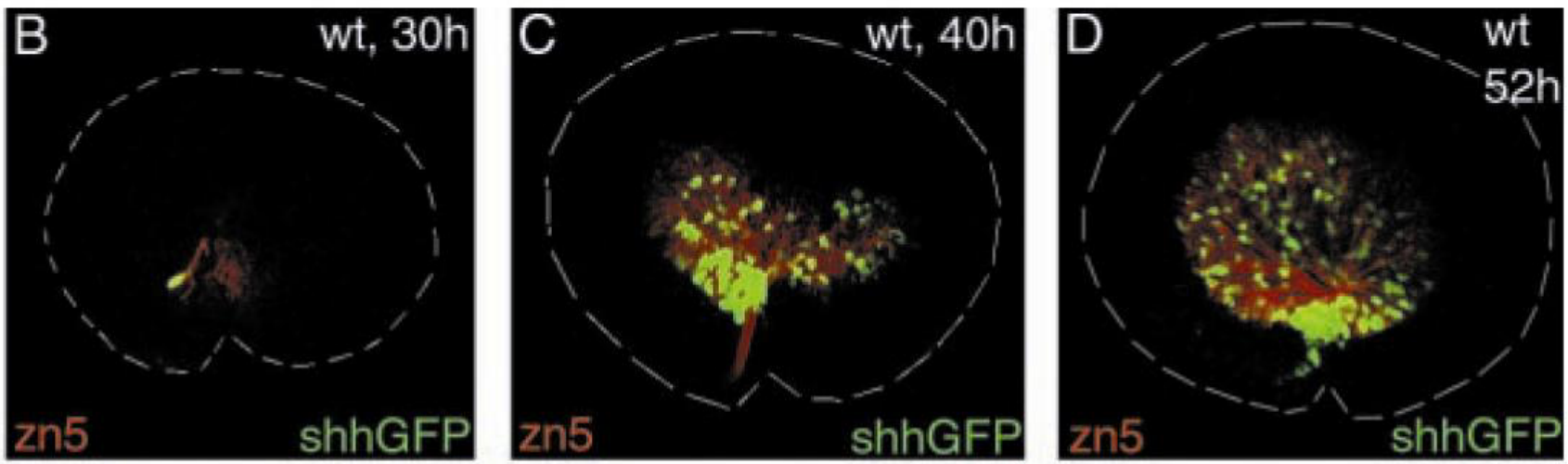


The process of neuronal differentiation begins.

A wave of Sonic hedgehog (Shh) expression sweeps through the retina.

1) Propagates itself (Shh signals to cells ahead to express more Shh).
2) Signals differentiation of retinal ganglion cells (first retinal cells to differentiate).

Works via upregulation of a transcription factor, Math5, in RGCs



Neumann & Neusslein-Volhard. 2000. *Science* 289, 2137-2139

Retinal cells differentiate in waves

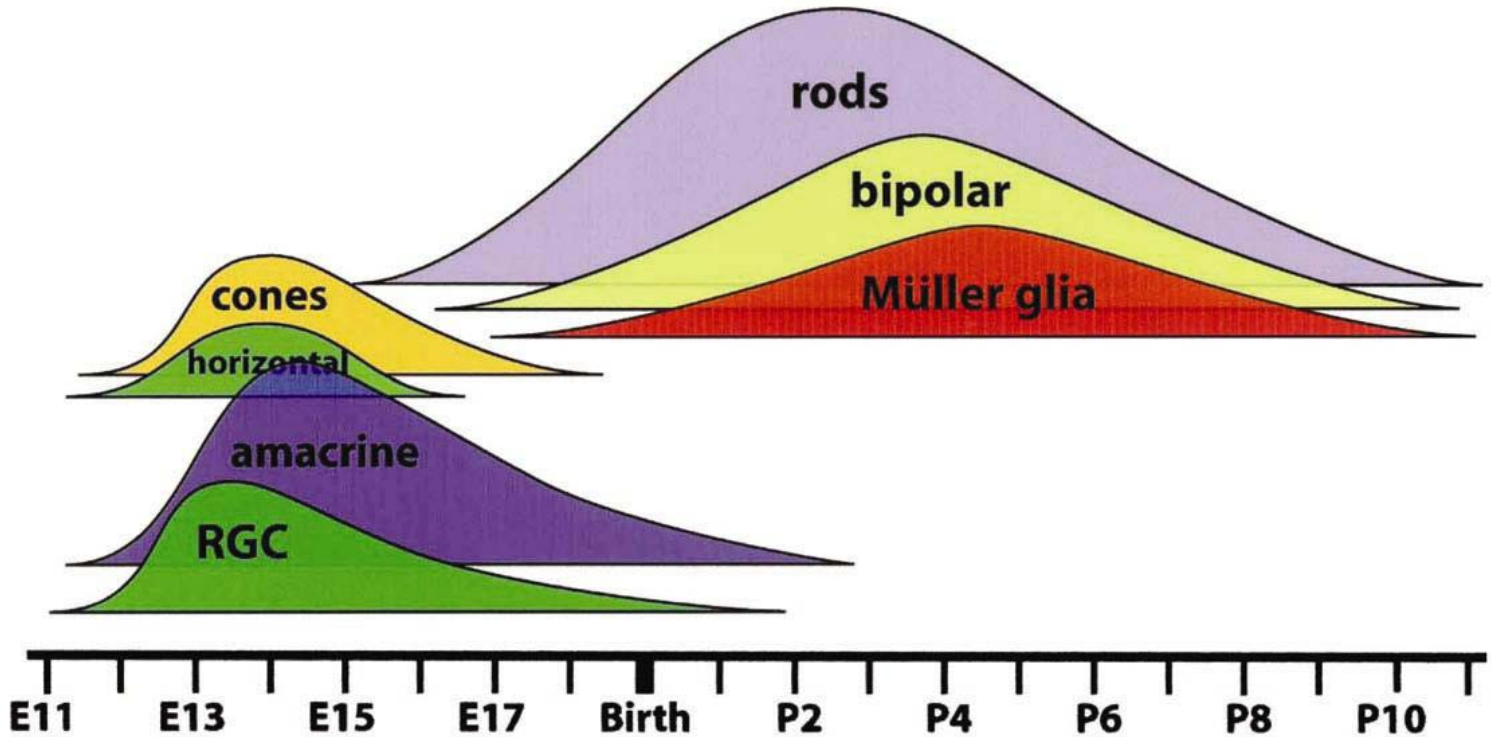


Fig. 1. Two waves of retinal cell type differentiation. The curves depict the relative number of retinal progenitors that exit the cell cycle and commit to a specific fate over time. RGCs appear at E11 and peak at E13.5 as do amacrine cells, horizontal cells, and cone photoreceptor cells. The other cell types, bipolar cells, Müller glia, and rod photoreceptor cells, appear later as indicated. Modified from [2,42].

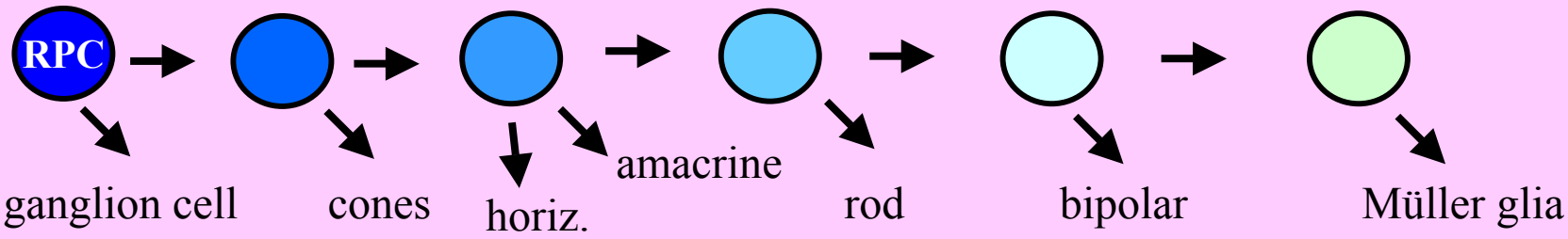
Wang et al., 2002. Methods 28, 448-456.

The undifferentiated neural retina consists of **multipotent** retinal progenitor cells

Different retinal cell types are generated from these progenitors in fixed chronological sequence during late embryogenesis and early postnatal life.

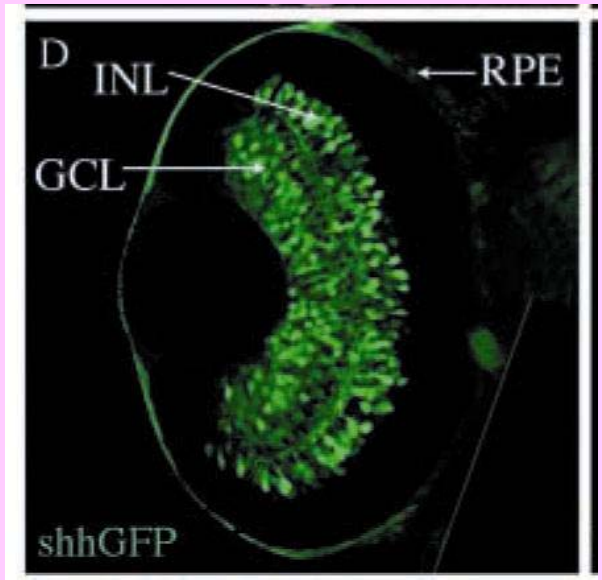
Retinal ganglion cells and horizontal cells are generated first, followed by cones, amacrine cells, rods, bipolar cells and Müller glia. (There is some overlap).

'Arm-waving' model



The schedule of differentiation of retinal progenitor cells (RPCs) is controlled by both intrinsic genetic programs (not all RPCs are equal) and extrinsic cues from their environment.
Cepko, C. L. (1999). The role of intrinsic and extrinsic cues and bHLH genes in the determination of retinal cell fates. *Curr. Opin. Neurobiol*. 9, 37-46.

While Shh is sweeping through the ganglion cell layer, there is an independent wave of Shh sweeping through the inner nuclear layer, making other neuronal cell types start to differentiate. (2004. *Development* 131, 3849-3858)



Intrinsic factors

Some RPCs are *biased* to produce certain types of progeny.

E.g. some RPCs (labeled with VC1.1 antisera) tend to produce amacrine and horizontal cells.

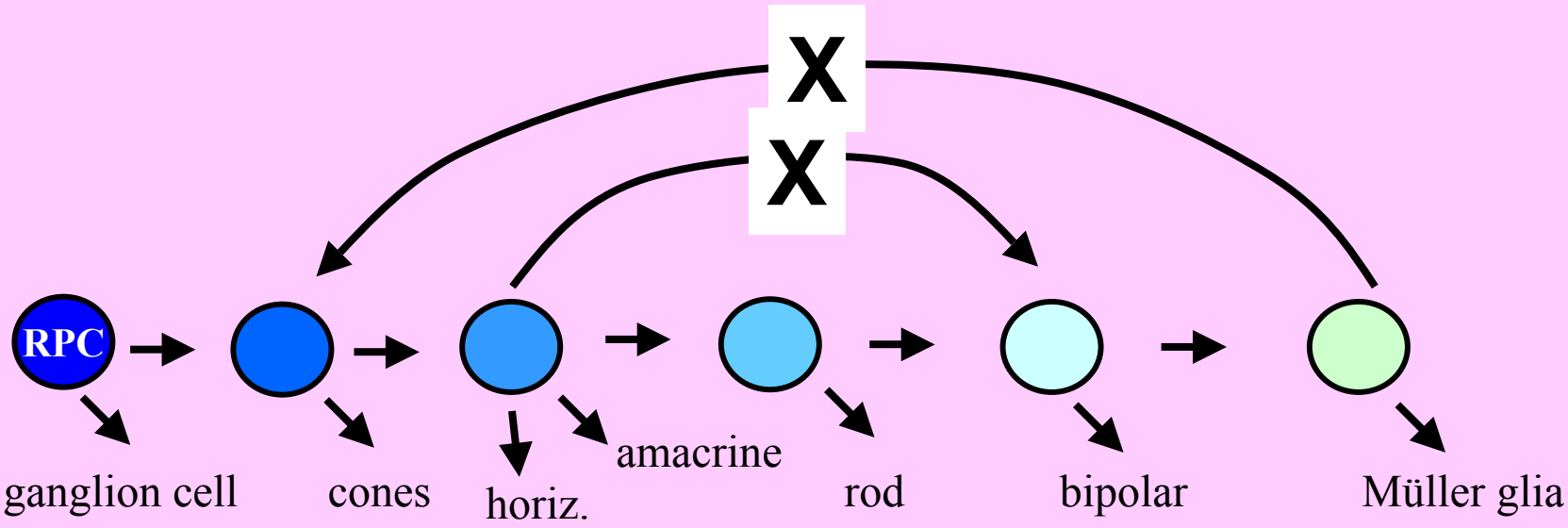
Extrinsic factors

Signals released by differentiating or differentiated cells influence the RPCs around them.

E.g. amacrine cells release a signal that inhibits further differentiation of RPCs into amacrine cells in culture. Limits numbers of amacrine cells produced. Amacrine cells secret Shh, which acts as a short range signal to direct differentiation of other neurones.

Similar results for ganglion cells in chick retina.

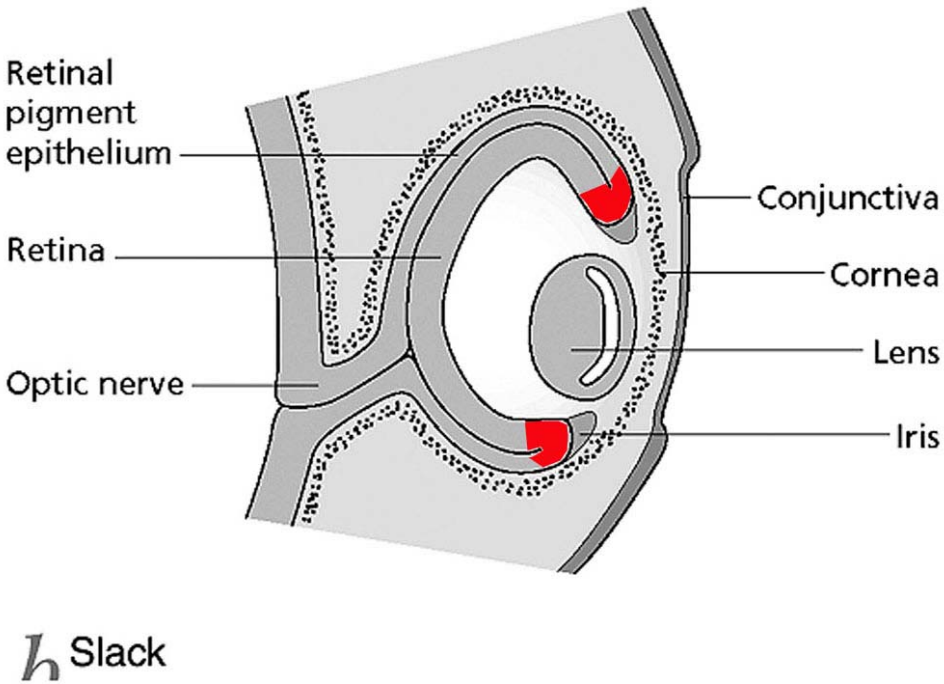
‘Arm-waving’ model



Intrinsic genetic programme is not locked - influenced by environment and stochastic process.

RPCs can be bumped about experimentally (I.e. persuaded to differentiate abnormally) but maybe can’t skip stages and can’t go back.

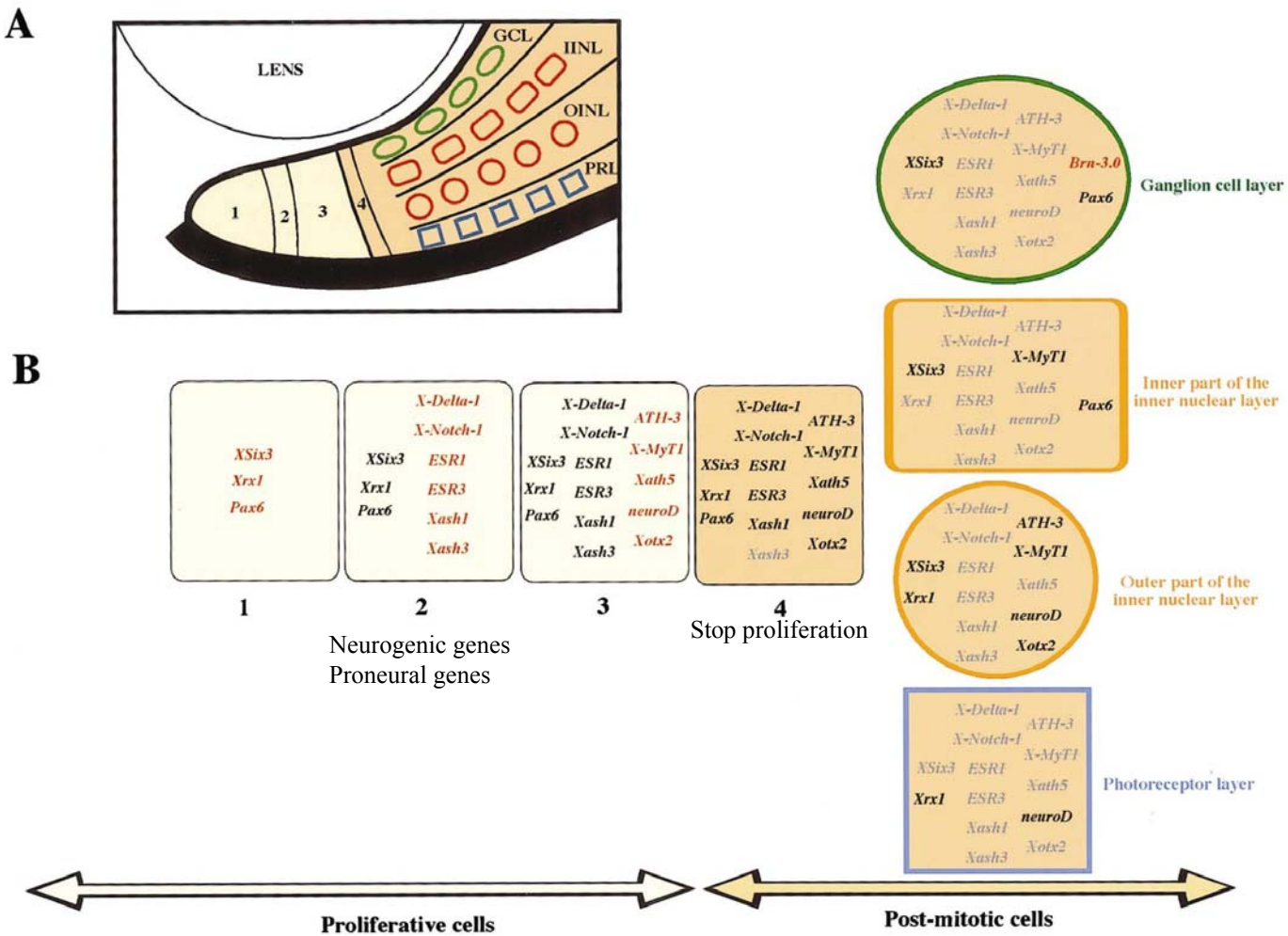
The ciliary marginal zone



Distal rim of retina (just before the iris) – last bit to differentiate. In many species, retains undifferentiated or 'stem like' properties.

Frogs have a well characterised CMZ.
CNZ is deeply asleep in mammals – not much stem cell activity found. But may be potential source of retinal regenerative potential in medicine.

Genetic control of retinal neuron development



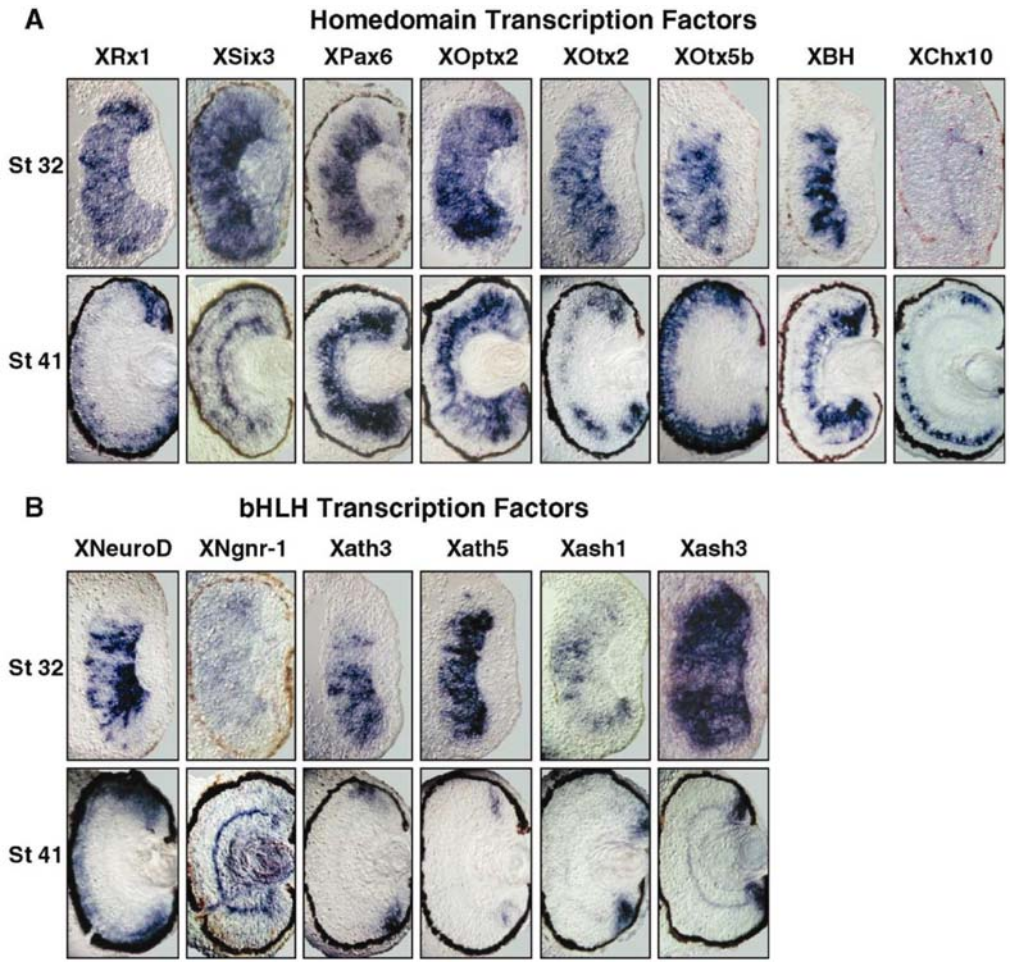
The ciliary margin zone – a domain of undifferentiated retinal cells that can persist into adulthood
Genes expressed for first time in red. Genes turned off in grey
1 = stem cells. 2, 3 = proliferating RPCs with evolving genetic programme. 4+ = non-proliferating.

Transcription factor control of retinal cell specification

Two classes of transcription factor: basic helix-loop-helix (bHLH) TFs, and homeodomain TFs.

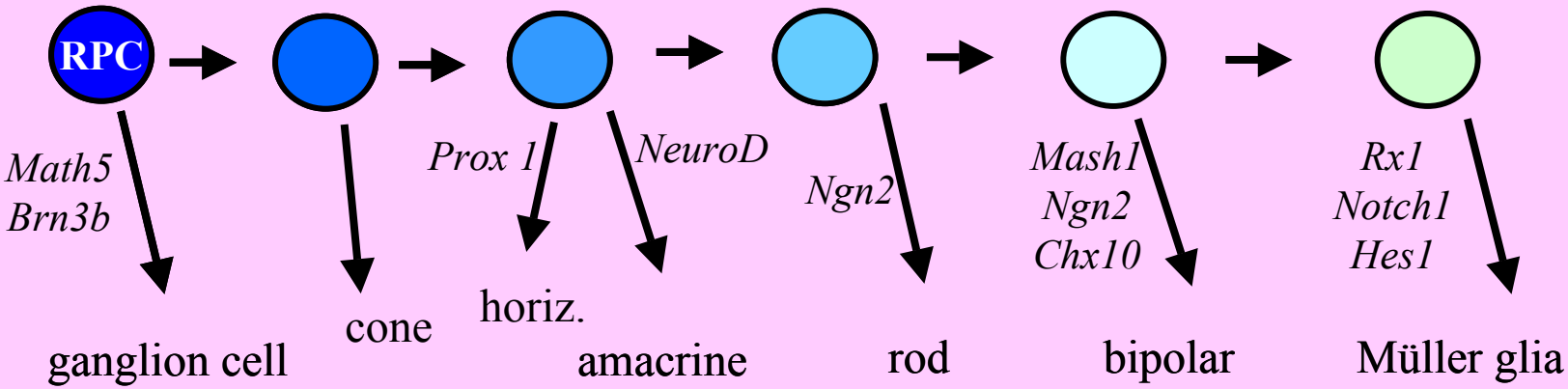
Work in combination to modulate retinal progenitor fate.

Ignore details

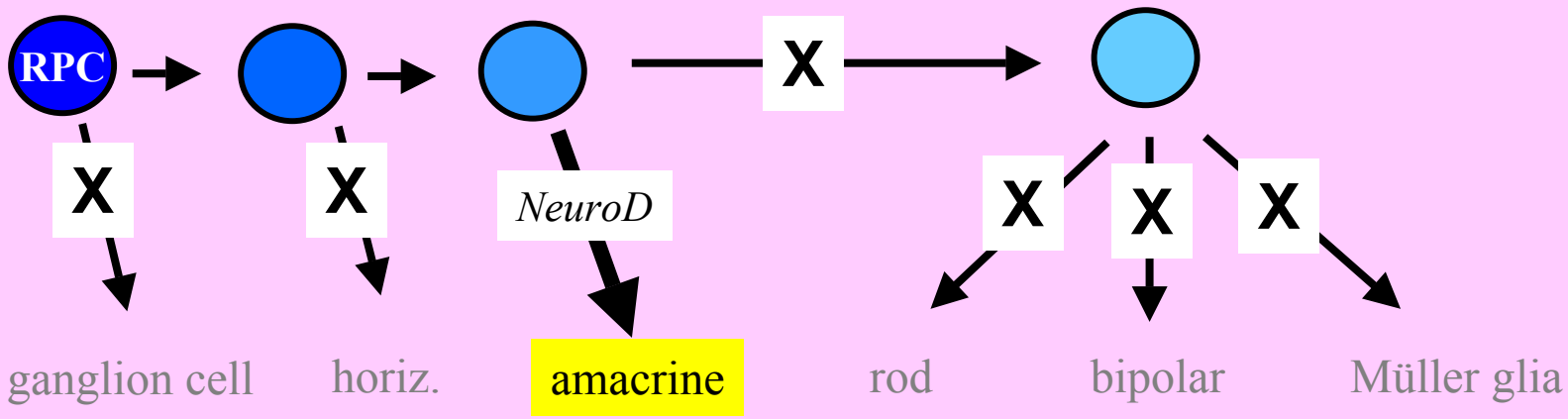


Wang et al., 2005. *Dev. Biol.* 285, 101-115.

Pax6 is required for normal retinal development

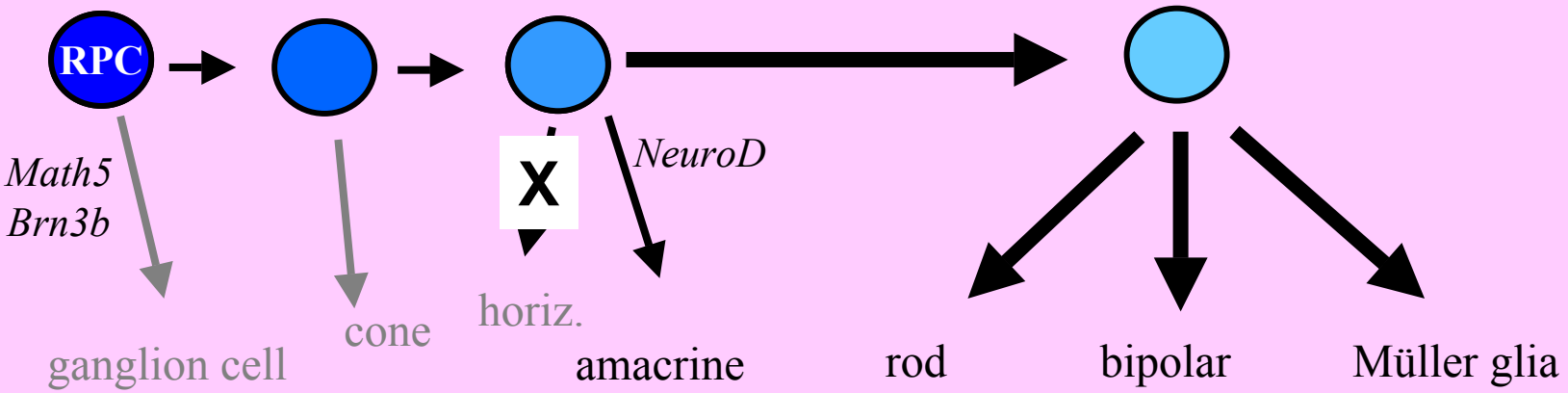


Pax6 inactivated in RPCs



Marquardt *et al*. (2001) Cell 105, 43-55.

Prox1 also controls retinal development

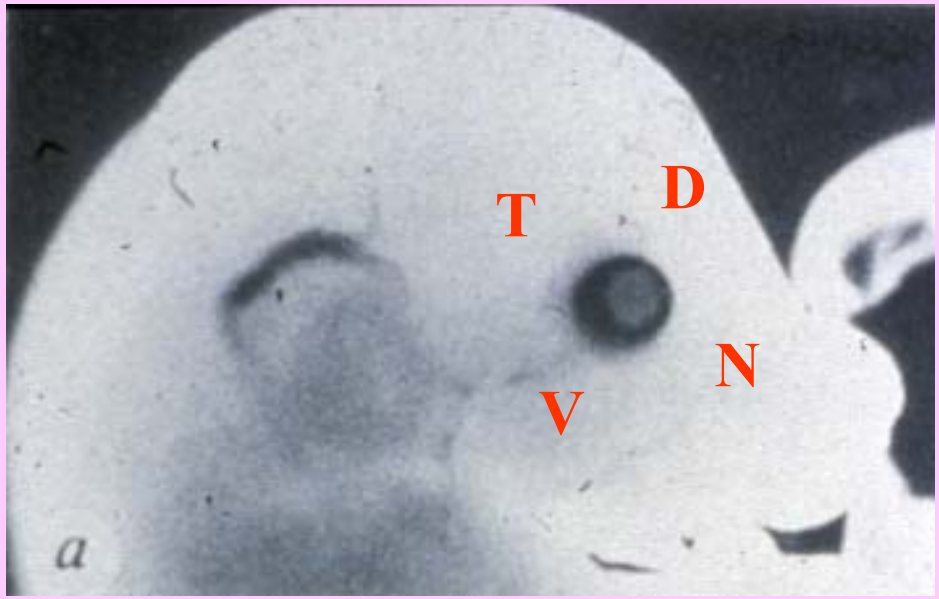


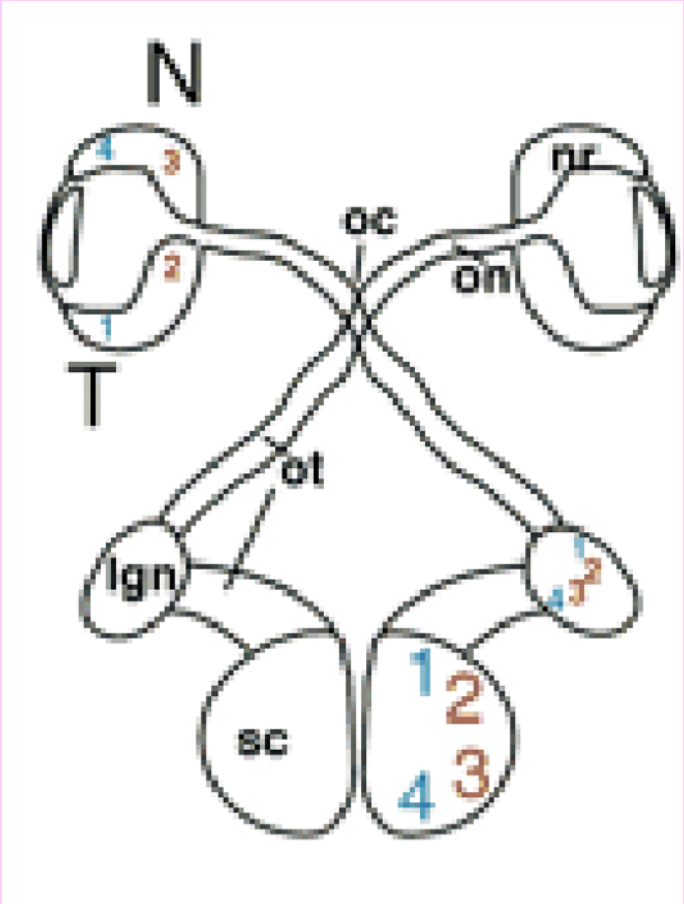
Prox1 is a transcription factor
Expression seems to induce differentiation of RPCs

Loss of Prox1 leads to loss of early-determined RPCs (espec. horizontal cells) because they don't get the Prox signal to differentiate.
RPCs continue until they get later rod/bipolar differentiation signal.

I.e. loss of Prox1 causes conversion one sort of retinal cell into others.
Remember this for later.

Dorso-ventral and naso-temporal specification of the retina





Retinal ganglion axons originating from specific points along the nasotemporal or dorsoventral axes of the retina stereotypically project to specific points within the lateral geniculate nucleus (LGN) and superior colliculus (SC). The point-to-point topography of the retina is therefore projected faithfully into the primary visual centres in the mesencephalon and diencephalon.

How is this organised?

Sperry, R. W. (1963). *Proc. Natl. Acad. Sci*. (USA) 50, 703-710.

The topographic targeting of retinal ganglion cell (RGC) axons is governed by graded distributions of molecules in the retina and the tectum (= superior colliculus) that confer positional addresses.

These are chemical gradients of a family of 14+ transmembrane receptor tyrosine kinases (**Eph-receptors**) primarily in the retina and topographically related gradients of their ligands (**ephrins**) primarily in the tectum/superior colliculus.

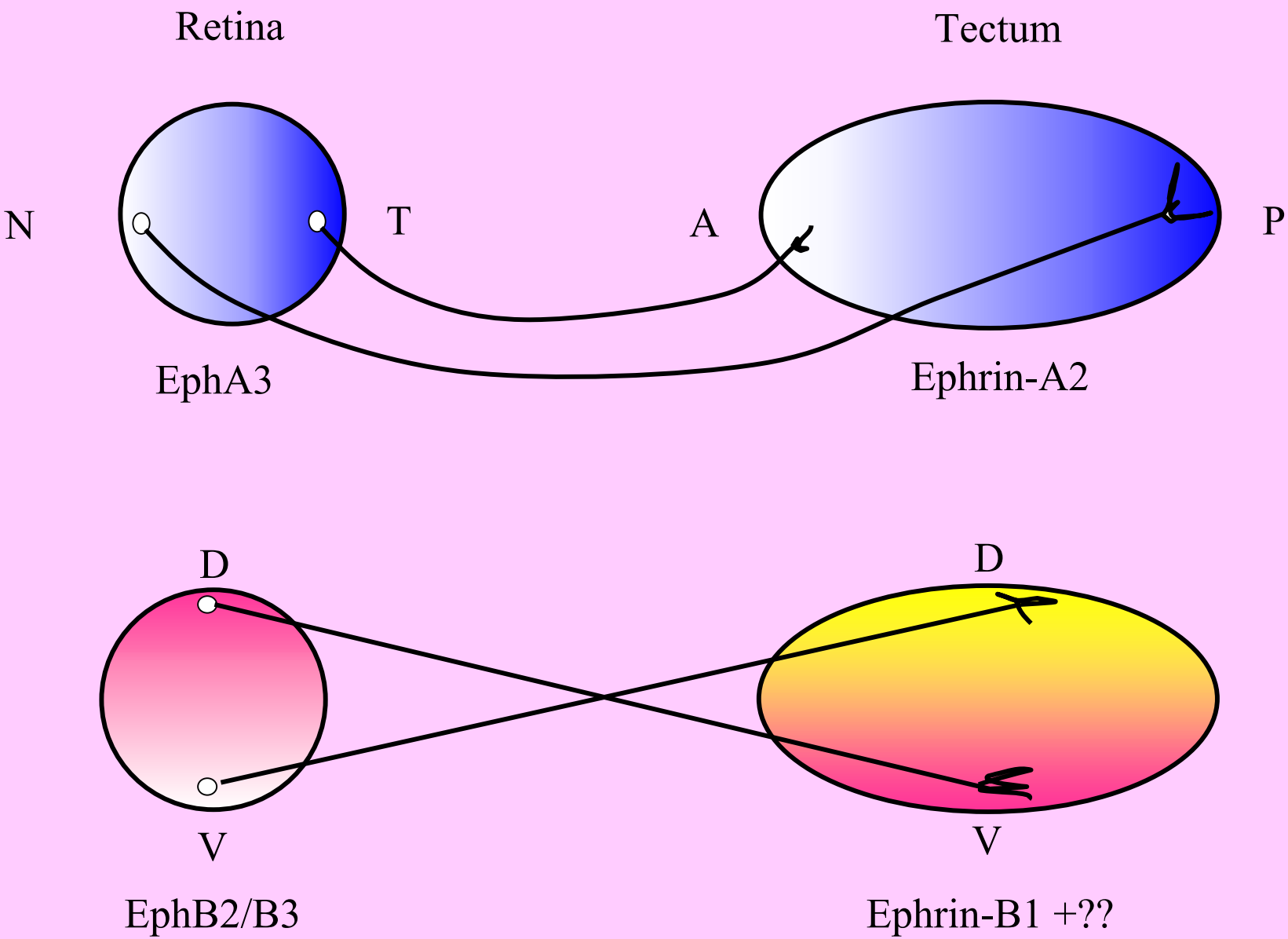
Binding of an ephrin ligand to its Eph receptor in a growth cone results in axonal repulsion. (Mainly)

In the developing retina, different Ephs are localised in dorso-ventral or naso-temporal gradients. Each retinal ganglion cell is therefore uniquely labelled ('painted') by the levels of Ephs on its cell surface (e.g. 'has x and y coordinates').

Potential targets of the RGC axons in the optic tectum or superior colliculus are similarly labelled by graded A/P and D/V gradients of different ephrins.

Axons of RGCs will therefore project to the point where they experience least repulsion (or most attraction) in a pattern that faithfully recapitulates the spatial origin of the RGCs in the retina.

Not necessarily absolutely true - lots still to be discovered
see O'Leary, D. D. M & Wilkinson, D. G. (1999) Eph receptors and ephrins in neural development. *Curr. Opin. Neurobiol.* 9, 65-73.

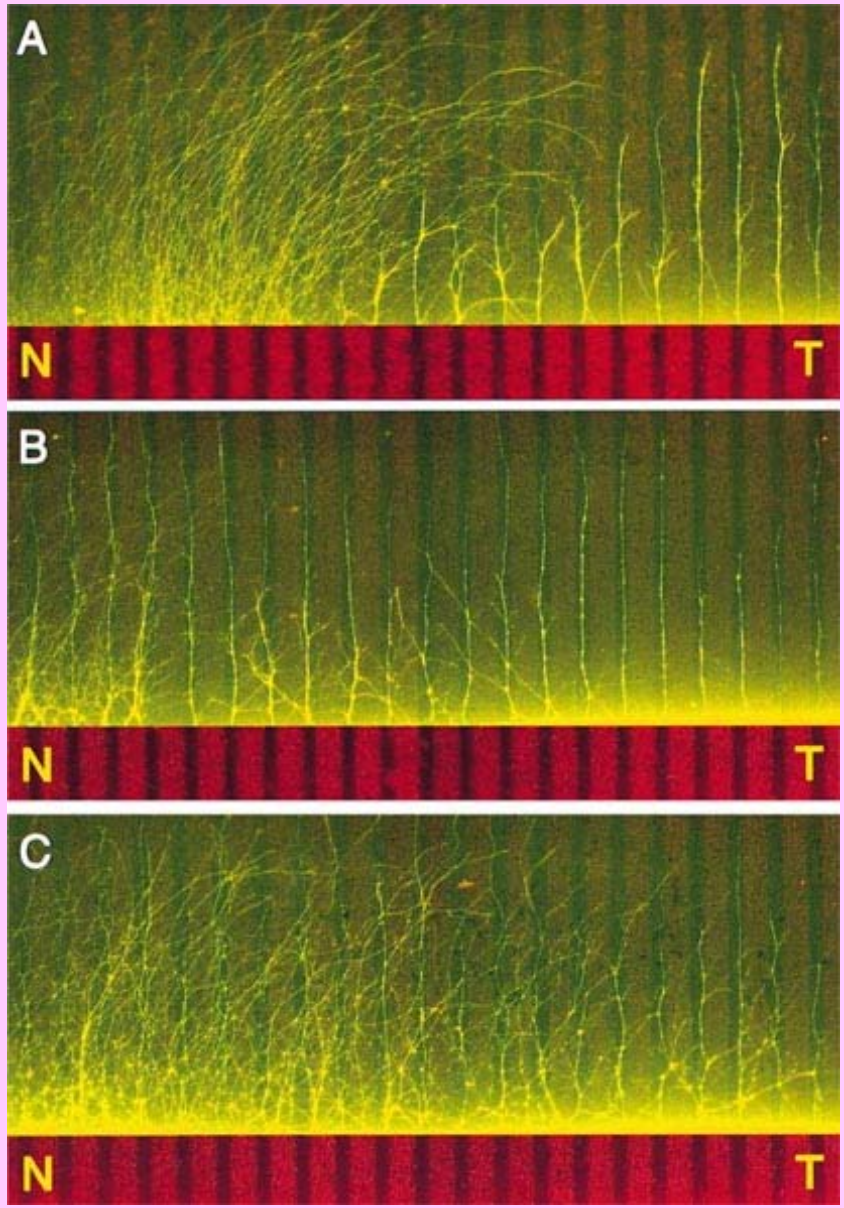

Retina
Tectum
N
T
A
P
EphA3
Ephrin-A2
D
D
V
V
EphB2/B3
Ephrin-B1 +??

Membrane stripe assay of chemorepulsion

Nasal (left) and temporal (right) RGC axons grown in culture and allowed to project over stripes (red) of cell membranes of COS cells transfected with ephrin-A2 (A) or ephrin A5 (B,C).
The red stripes in B have a higher concentration of ephrin-A5 that those in C.

A. Assume temporal RGCs are expressing Eph receptors that are repulsed by binding ephrin-A2, so their axons cannot grow over transfected cells (so respect stripe boundaries). Nasal axons not expressing these Ephs, so grow over stripes.

B.C, note graded response to levels of ephrin-A5.



How are dorsoventral and nasotemporal patterns of transcription factor set up in the developing retina?

Extrinsic factors: retinoic acid has been shown to ventralise the optic cup (Ross et al. (2000) Physiol. Rev. 80, 1021-1054).

Intrinsic factors: the prior expression of other genes

e.g Bmp4 dorsally /Ventropin ventrally (antagonists)

\+ Pax6 (*Pax6*$^{-/-}$ mice - the optic vesicle does not express any dorsal or nasotemporal markers).

Nicole Baumer et al. (2002) Pax6 is required for establishing the naso-temporal and dorsal characteristics of the optic vesicle. *Development* 129, 4535-4545.

Dorso-ventral and naso-temporal specification of the retina

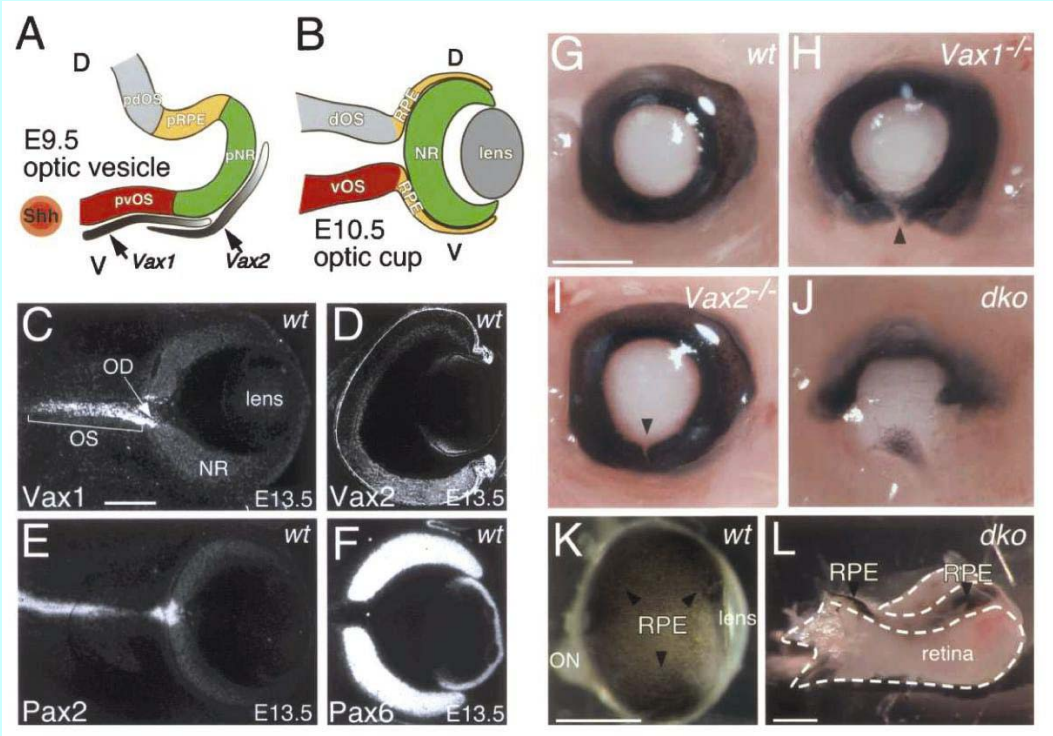
Regionally restricted patterns of expression of **transcription factors** imposes dorso-ventral and naso-temporal specificity in cells within the developing optic cup.

These transcription factors, directly or indirectly, control the expression of different cell surface molecules in developing retinal ganglion cells from different parts of the retina. (Ephrin receptors)

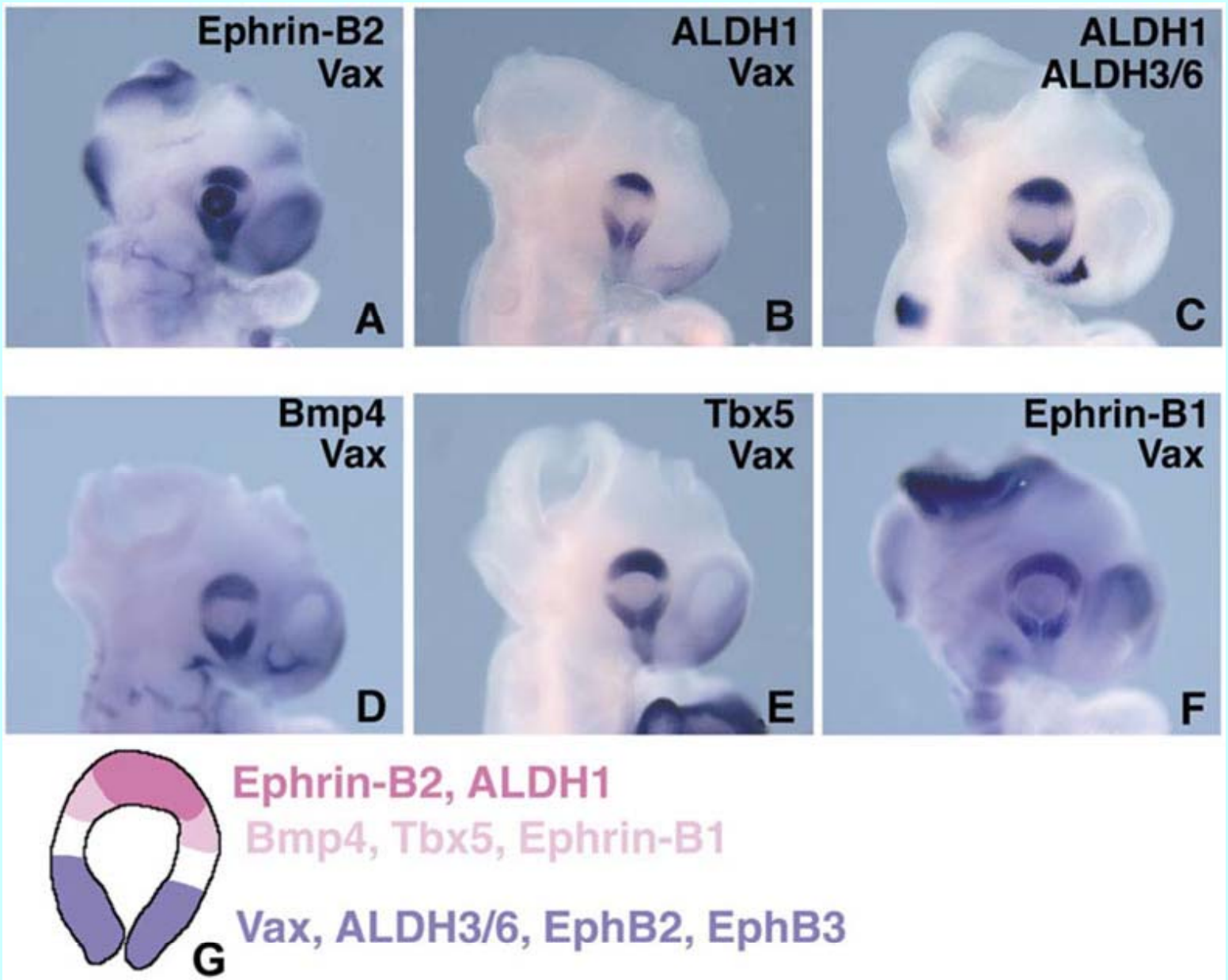
This causes the axons from retinal ganglion cells localised in different parts of the retina to project to different, specific points in the lateral geniculate nucleus and superior colliculus.

Dorso-ventral and naso-temporal specification of the retina

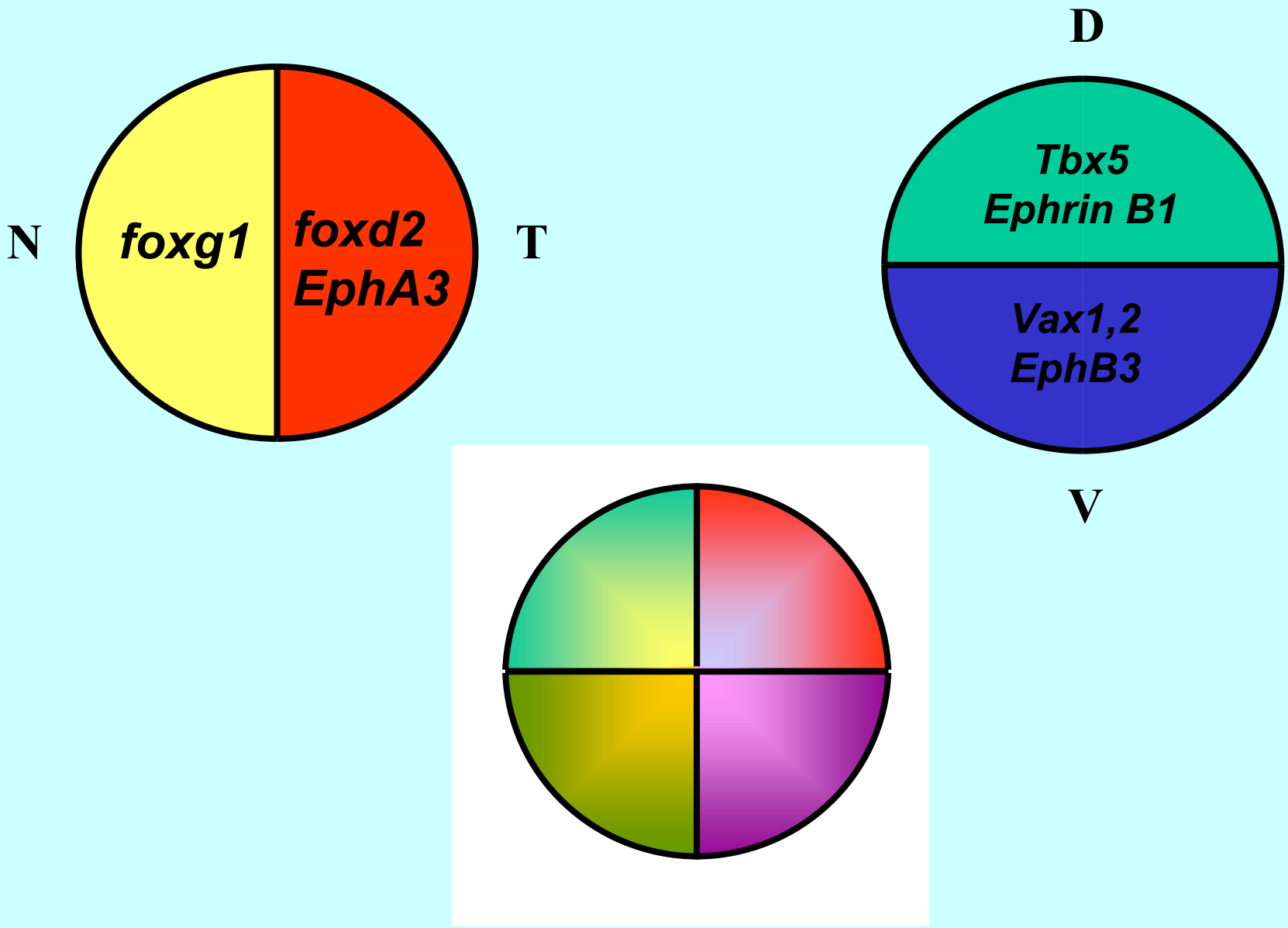
Vax genes ventralise the embryonic eye. (Homeodomain transcription factors)
Mui et al., 2005. Genes Dev. 19, 1249-1259.



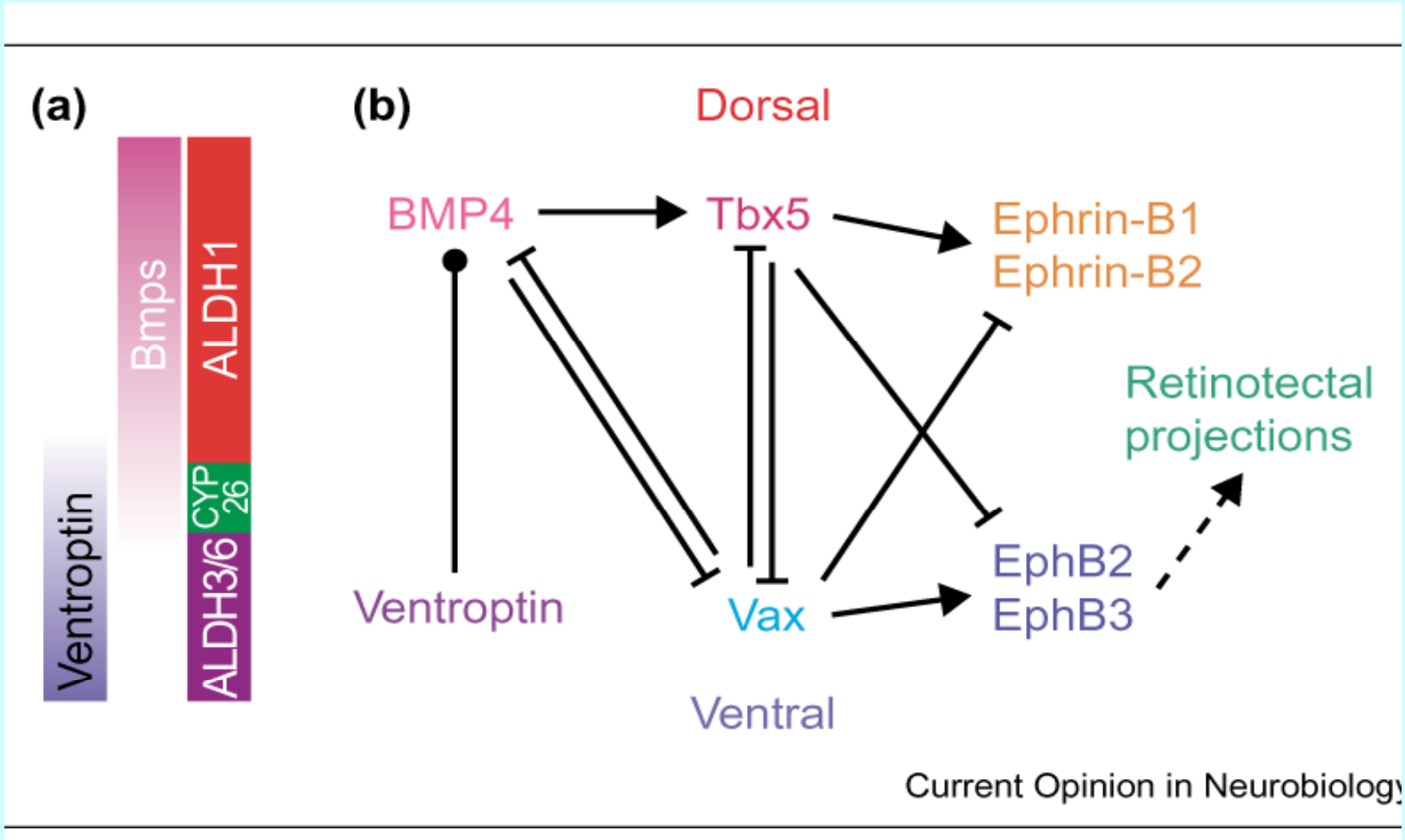
Expression of Vax genes in optic stalk represses Pax6.
Loss of Vax genes – optic stalk retains retinal-like, ventral retina not develop.



Dev. Biol. 251, 59-73. The retina is divided into multiple D/V domains.



Viral misexpression



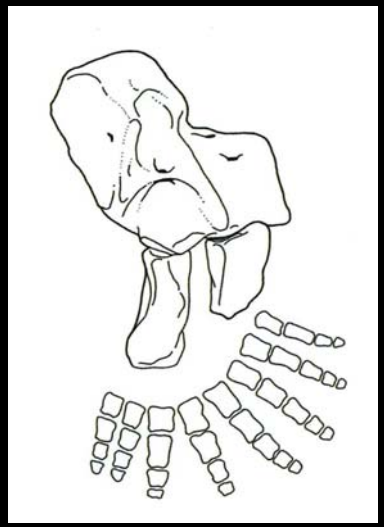
Maureen Peters. 2002. Patterning of the neural retina. *Curr. Opin. Neurobiol*. 12, 43-48.

Conservation of genetic control of eye development

Concept of homology….

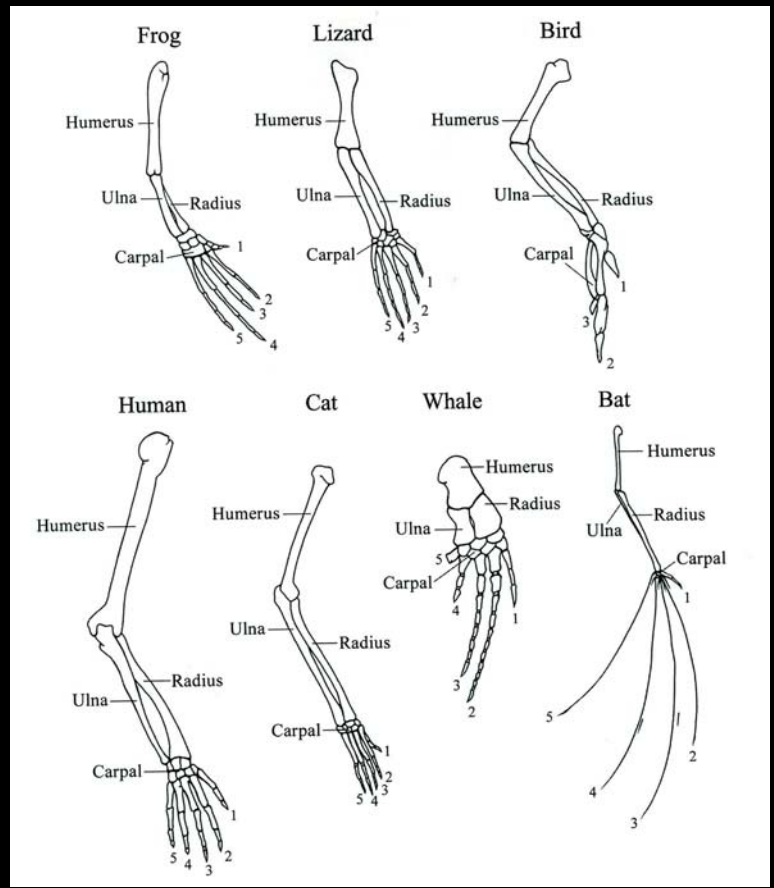
…things 'being the same' because they are evolutionarily related

Homologous structures e.g. vertebrate forelimbs – look different but are all derived from the front legs of a common ancestor.



Acanthostega

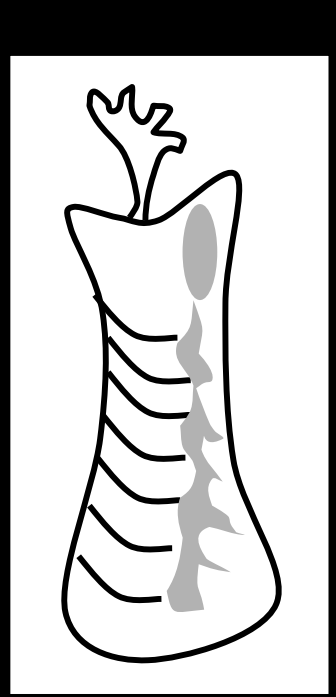
time



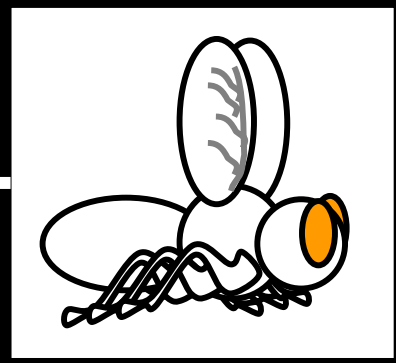
Concept of homology….

…things 'being the same' because they are evolutionarily related

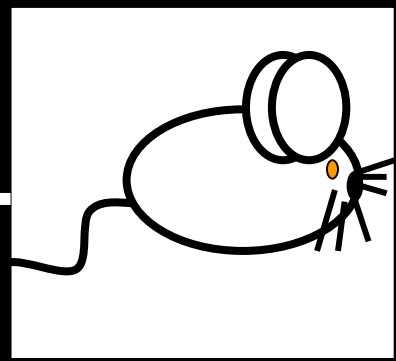
Same principle applies for homologous genes….



Gene X
Gene Y



Gene X*
Gene Y*



Gene X**
Gene Y**

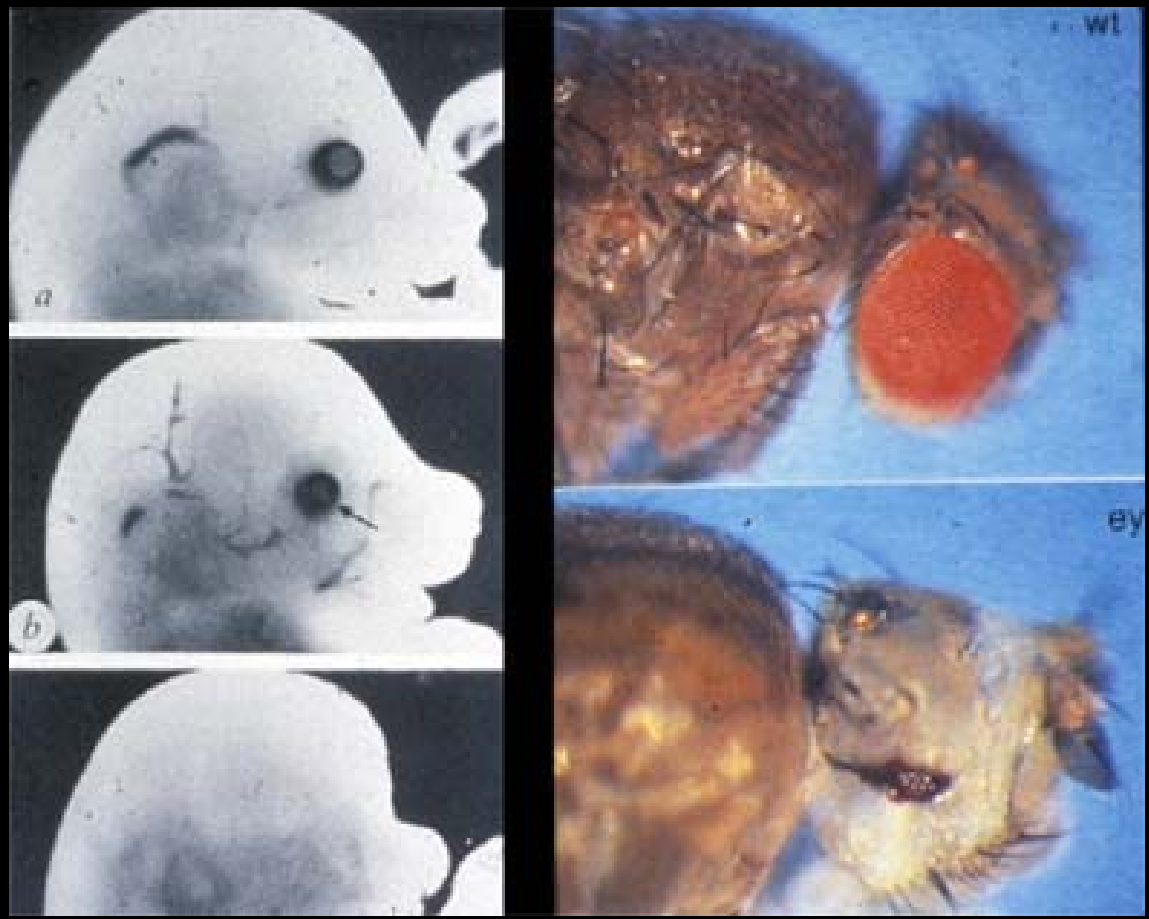
time

Pax6 is required for eye development in mice and flies

$Pax6^{+/+}$

$Pax6^{+/-}$

$Pax6^{-/-}$

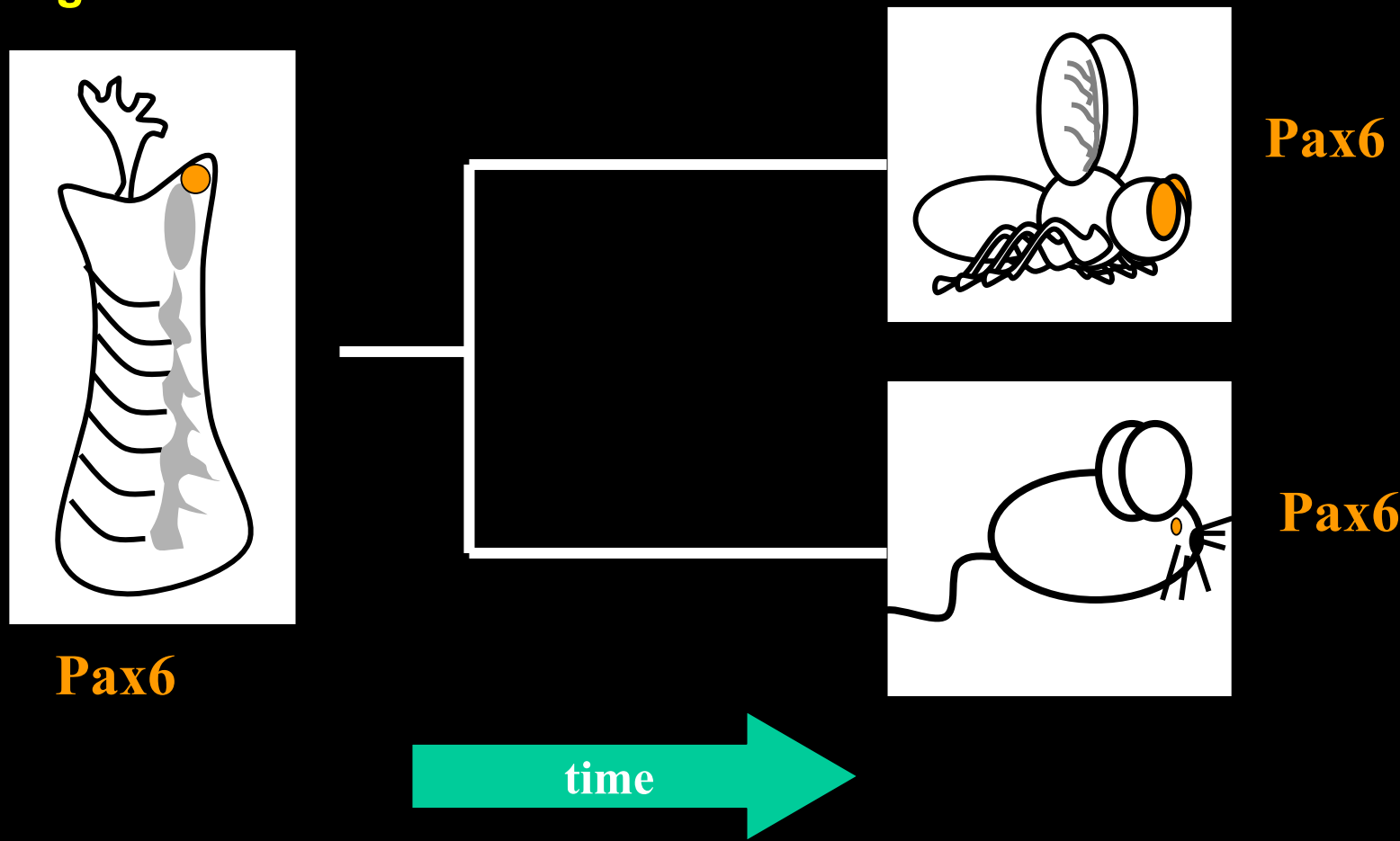


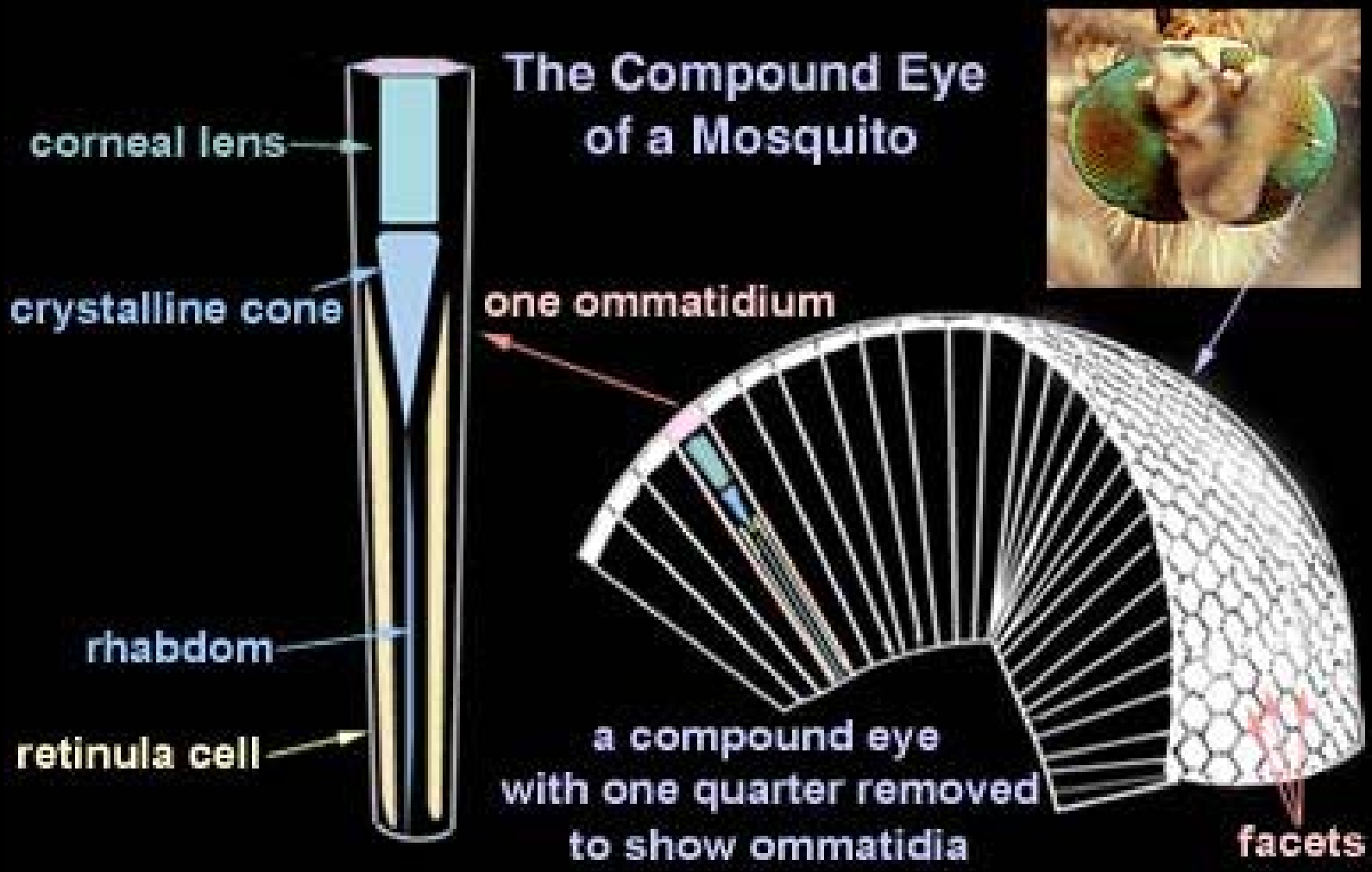
$Pax6^{+/+}$

$Pax6^{-/-}$

Concept of homology….

… so is the development of homologous structures controlled by homologous genes?
… can the eyes of invertebrates and vertebrates be homologous?



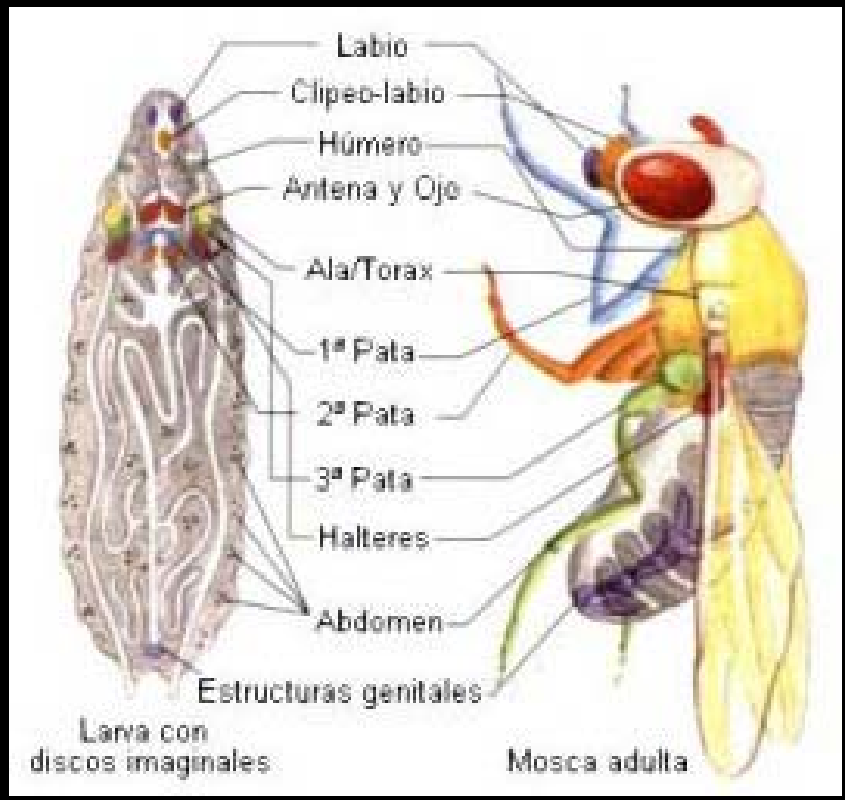


'It requires little persuasion to be convinced that the lens eye of a vertebrate and the compound eye of an insect are independent evolutionary events.' Ernst Mayr, 1961. (but…. ?rhodopsin?).

Fly eye development is very different from vertebrate.

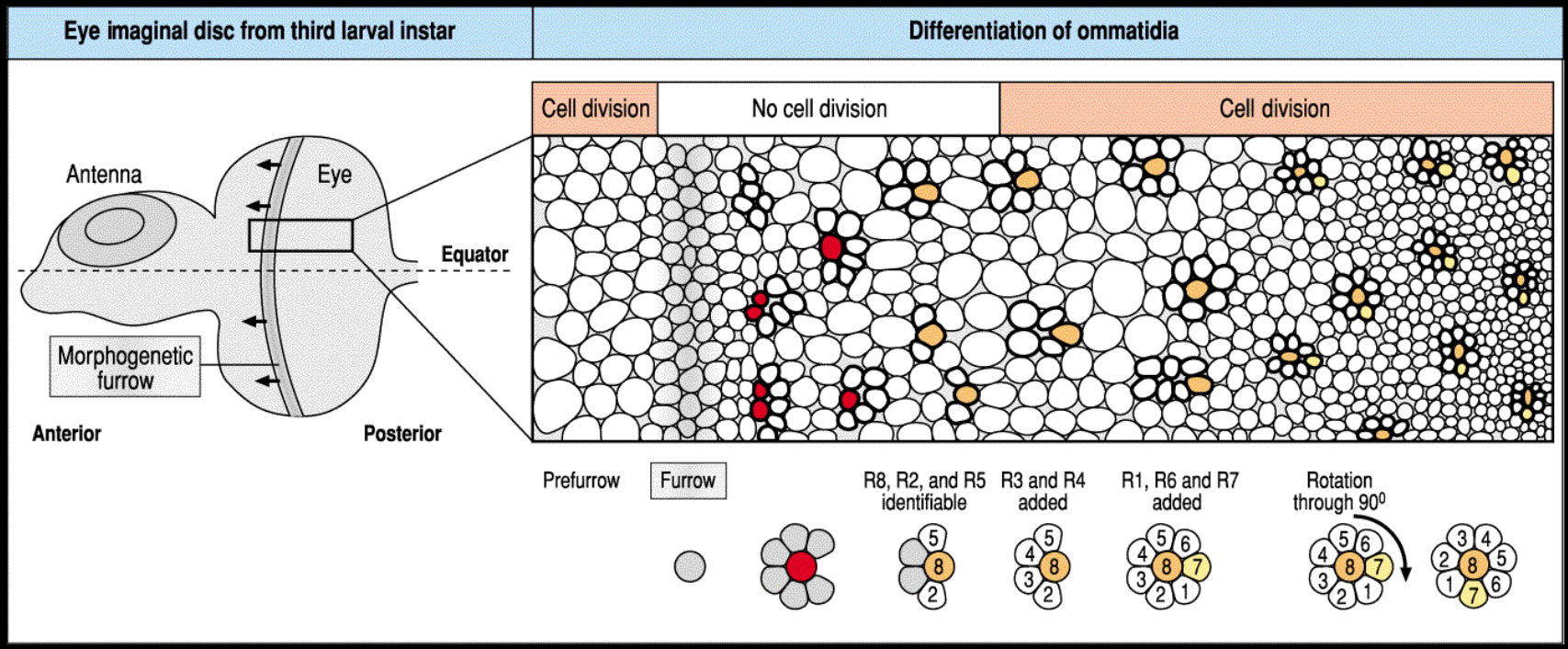
'Imaginal discs' – small epithelial sheets tucked away towards the front of the larva.

When the larva metamorphoses, imaginal discs proliferate and grow and develop into the adult structures.
Separate imaginal discs for labia (jaws), eye/antennae, legs, wings, halteres, genitals.



Fly eye development is very different from vertebrate.

Eye imaginal disc – fused with antennal imaginal disc – expresses fly PAX6



A photoreceptor cell (rhabdomere 8) is specified in sheet of pluripotent cells - recruits all the other photoreceptor and support cells. This happens independently for all ommatidia.

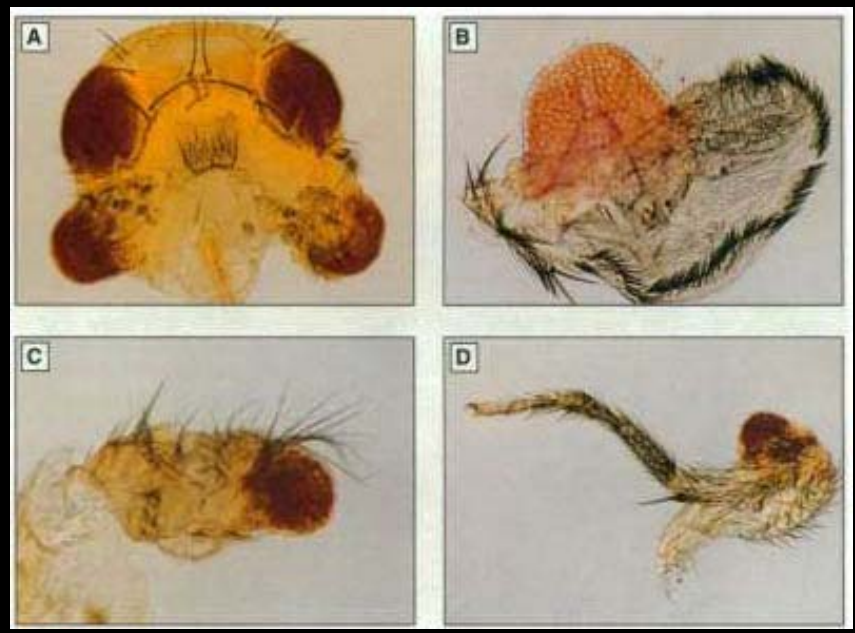
Pax6 has been called the 'master regulatory gene' for eye development.

Required in many tissues throughout eye development

Loss of function leads to loss of eyes in mice and flies.

Expression is conserved in eyes in many different phyla with many different designs of eye, incl. octopus, clams, photosensitive ocellus of Ascidians, flatworms.

Ectopic expression in leg/wing/antennae imaginal discs of *Drosophila* leads to formation of ectopic eyes (Pax6 sufficient to override the genetic programming of imaginal discs and make them form **functional** eyes).



Halder et al., *Science* 1995

But, lots of genes can create ectopic eyes in Drosophila.

Organised into 4 families:

PAX6 family
Members are *eyeless* (= Pax6), *twin of eyeless*, *eyegone*.
DNA-binding transcription factors.

EYA (*eyes absent*)
Has protein-binding domains that interact with members of the following two families.

SIX family
sine oculis, *Optix*
Have DNA binding and protein-binding domains. Binds *eya* to form functional transcription factor.

DACH (*dachshund*)
Shown to interact with *eya*. May be transcriptional cofactor.

Cross-regulation and autoregulatory loops involving the members of the 'master genes' might explain why fly eye development fails if any one of the families is missing.

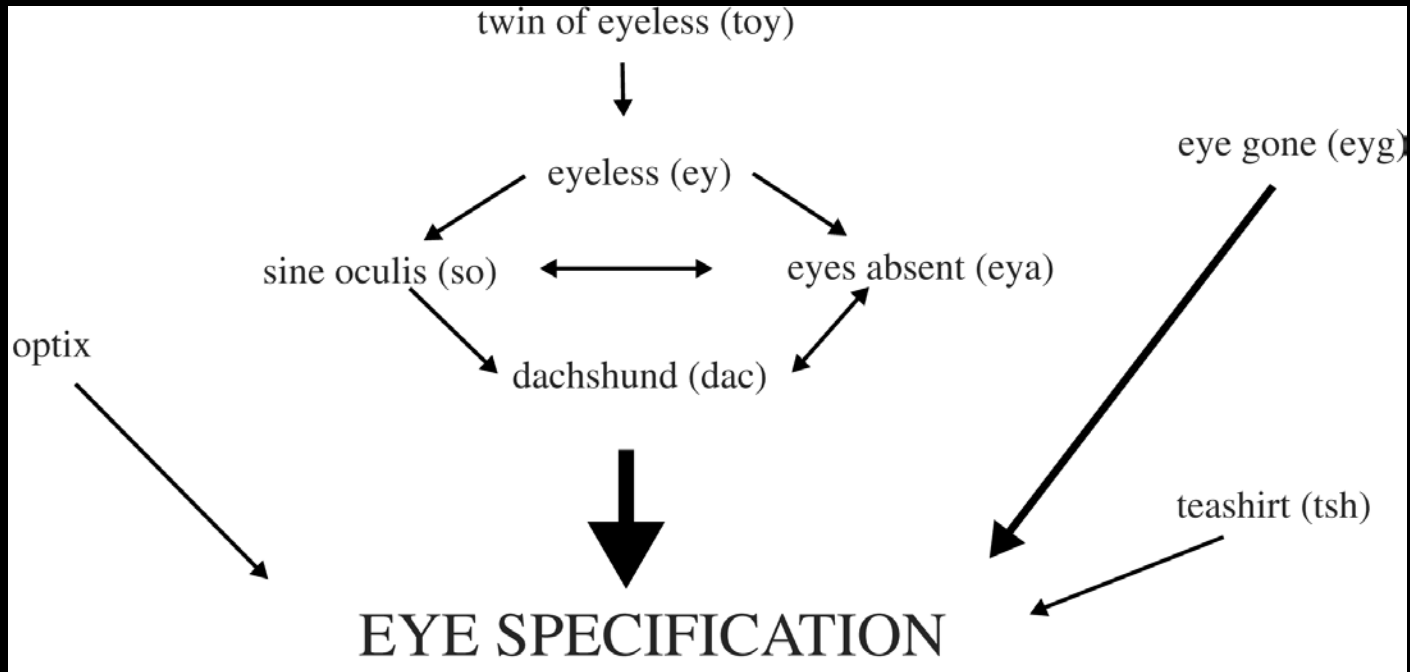


Figure 2. Hierarchy of *Drosophila* eye specification genes. Eight nuclear factors are known to affect the specification of the *Drosophila* compound eye. The arrows indicate either genetic, transcriptional or biochemical interactions (see text for details).

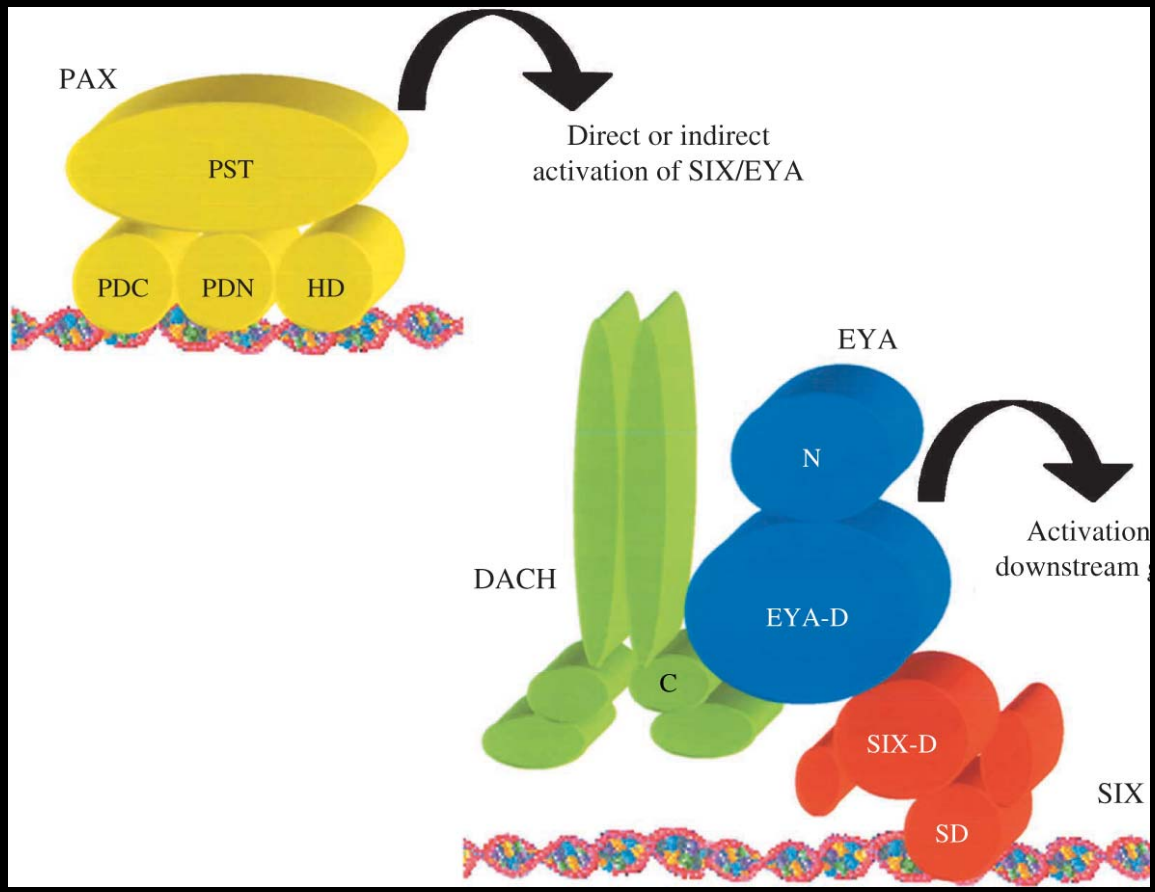
Members of the PAX6, EYA, SIX and DACH families all show some characteristics of 'master regulators' of *Drosophila* eye development.
i.e.

Loss of function of any of the genes leads to loss of eyes.

Ectopic expression in leg or wing primordia leads to formation of ectopic eyes.

Vertebrates have homologues of all these families, many of which are expressed in the eyes. Gene duplications have occurred.
PAX6: *Pax6.*
EYA: *Eya1-4*, *Eya2*, *Eya3*, *Eya4*.
SIX: *Six1-6*
DACH: *Dach1*, *Dach2*.

A conserved team at the centre of eye development?



Hanson, I. M. (2001) *sem. in Cell & Dev. Biol*. 12, 475-484.

PAX6, SIX, EYA and DACH genes in vertebrate eye development

PAX6 - Expressed throughout eye development.
Loss of function leads to failure of eye development.
Ectopic expression leads to ectopic eye structures.

EYA - *Eya1*, *Eya2*, *Eya3* expressed in eye development.
Human *EYA1*$^{-/-}$ leads to ocular abnormalities.

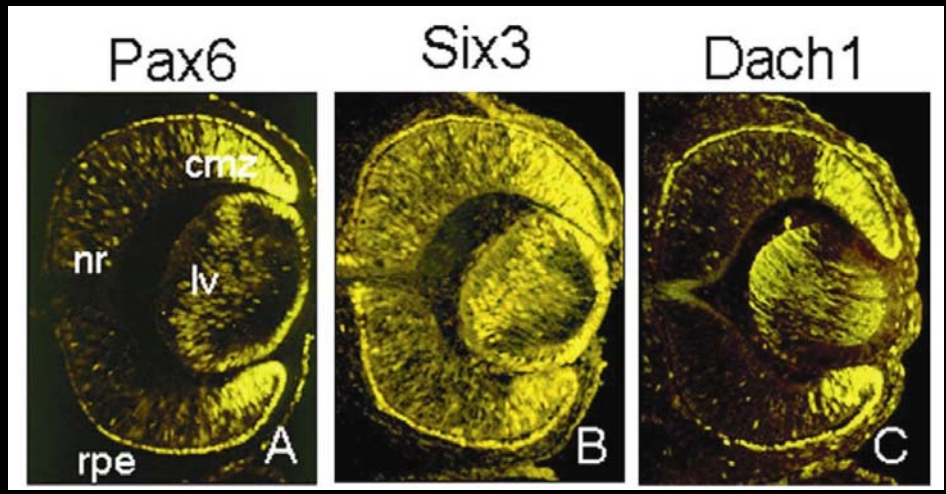
SIX - *Six3, Six4, Six5, Six6* expressed in eyes
Six3 expression is dependent on Pax6 in lens, but not retina.
Six3$^{-/-}$ leads to eye abnormalities
Ectopic expression of *Six3* and/or *Six6* in brain leads to ectopic expression of retinal markers/structures.
Six3 and *Six6* both important for determination of retinal cell fates.

DACH- *Dach1*, *Dach2* expressed in eyes. Function unknown.

DETAILS NOT IMPORTANT. JUST REMEMBER THAT THE GENES THATARE IMPORTANT FOR EYE DEVELOPMENT IN DROSOPHILA ARE OFTEN IMPORTANT FOR EYE DEVELOPMENT IN VERTEBRATES TOO!

PAX6, SIX, EYA and DACH genes in vertebrate eye development

Look carefully at gene expression and interactions…
…things not THAT simple.
Purcell *et al*., 2005. Gene Expression Patterns



Sequence and general ocular expression of e.g. Six3, Eya1, Dach 1, Pax6 is conserved.
But not all genes expressed together in same cells all the time (note CMZ).
The expression of one gene doesn't always require expression of the others.
Dach1 and Eya1 not absolutely required for eye development.

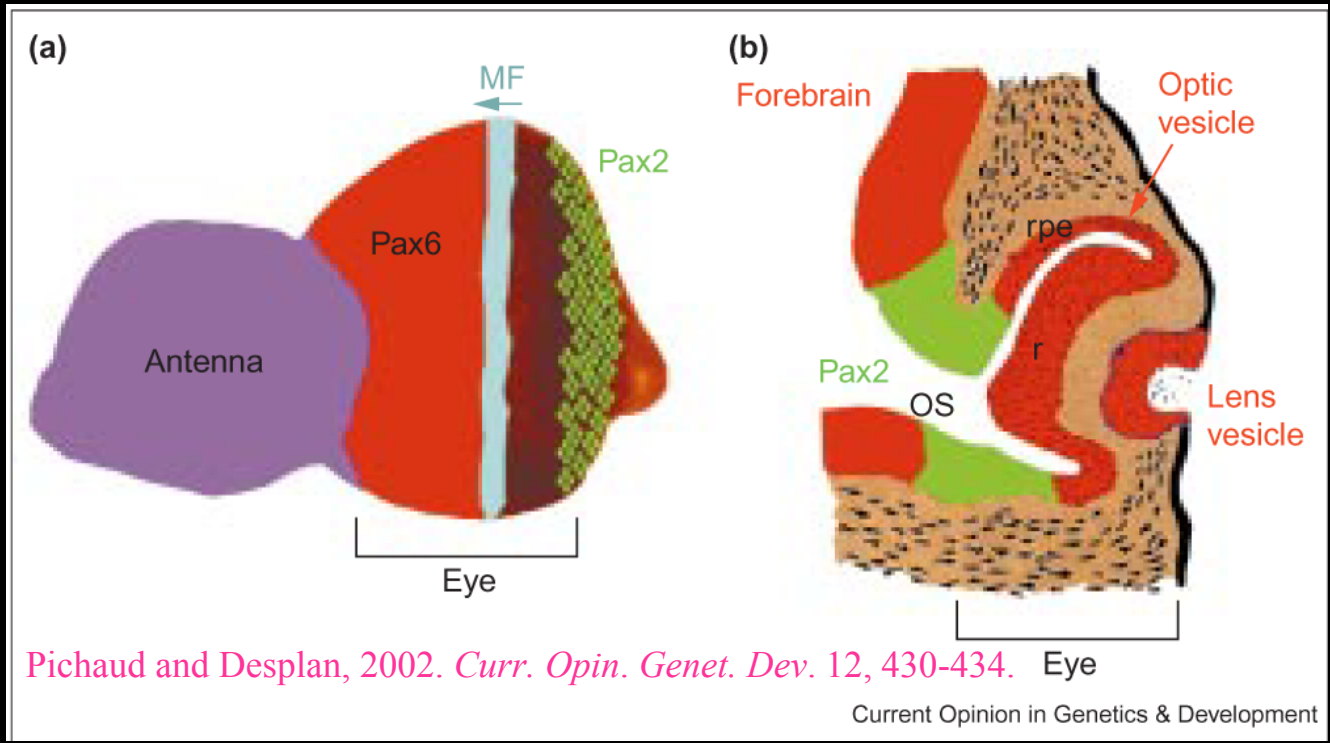
Even in *Drosophila*…
not all tissues that normally express the PAX6, EYA, SIX, DACH genes go on to form eyes

The PAX6, EYA, SIX, DACH interaction might be a conserved regulatory network that can drive differentiation of many tissues, with specificity depending on other extrinsic or intrinsic signals.

E.g. during vertebrate limb development, *Six1*, *Eya2*, *Dach2* and *Pax3* are all co-expressed and together drive myogenesis.

Eya-1 and Pax6 genes interact during development in *C. elegans*, which doesn't have eyes (Furuya, M. et al., 2005)

Expression of Pax2 and Pax6 is non-overlapping in *Drosophila* and vertebrate eyes.



Drosophila: Pax6 in undifferentiated multipotent cells ahead of morphogenetic furrow.
Pax2 in differentiating support cells.

Vertebrate: Pax6 in multipotent retinal progenitor cells.
Pax2 in differentiating optic stalk (support cells).

Prox 1 (remember that?) is the vertebrate homologue of the Drosophila gene ***prospero (Pros)***

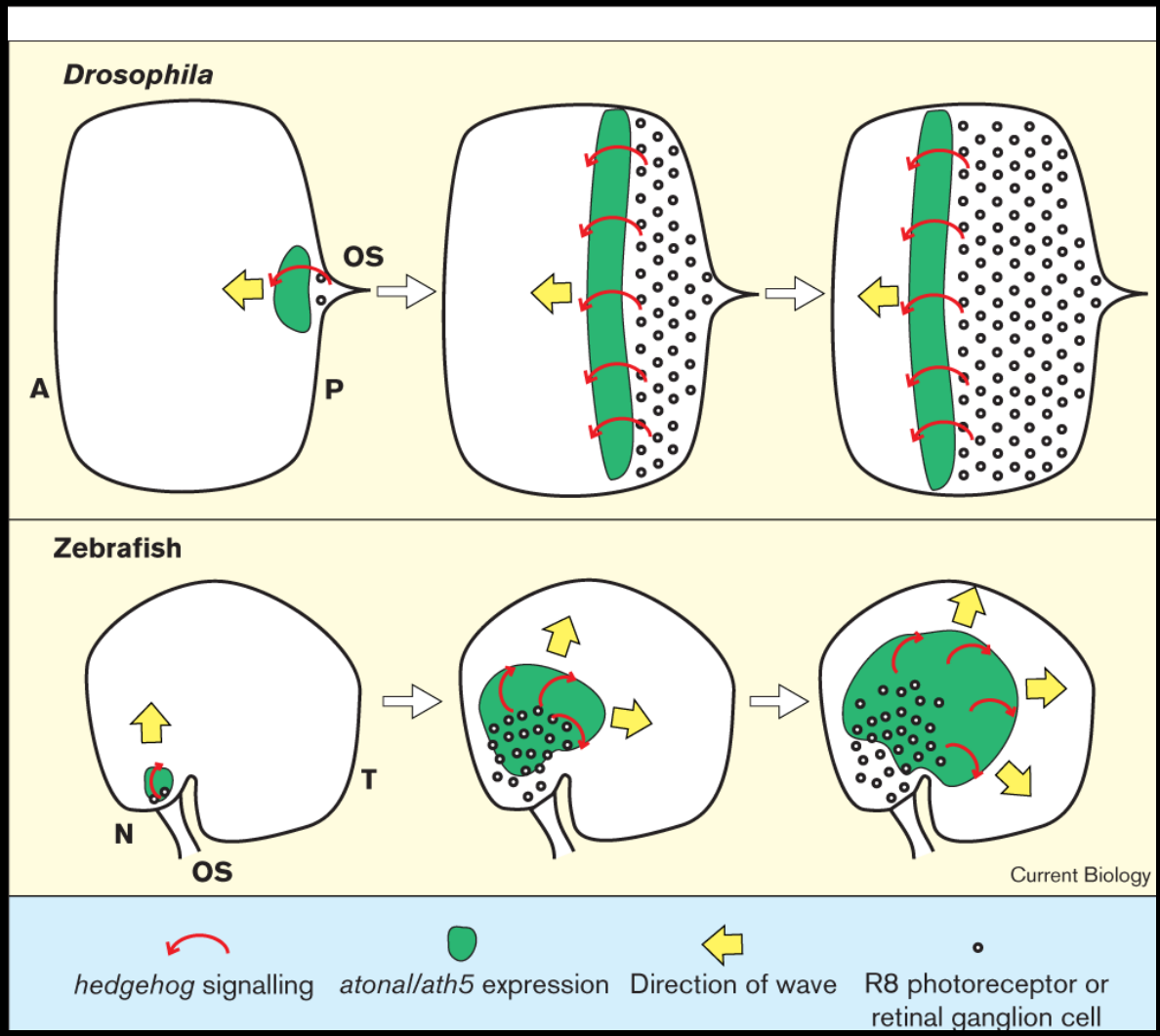
prospero is expressed in the developing photoreceptors (rhabdomeres) of the Drosophila eye.

Loss of *Pros* transforms rhabdomere 7 into rhabdomere 8
I.e. transforms one type of retinal cell into another

just like in vertebrates

See Cook, 2003. *BioEssays* 25, 921-925

atonal (= *ath5*, *Math5*) and *hedgehog* (= *Shh*) in vertebrate and invertebrate eyes



A Mexican wave of atonal / ath5 and hh / Shh expression proceeds from optic stalk outwards through the undifferentiated retina and precedes the differentiation of the first retinal neurons (R8 or RGCs).

hh > atonal > neurogenesis

Jarman, A. P. (2000) *Curr. Biol.* 10, R857-R859.

Summary:

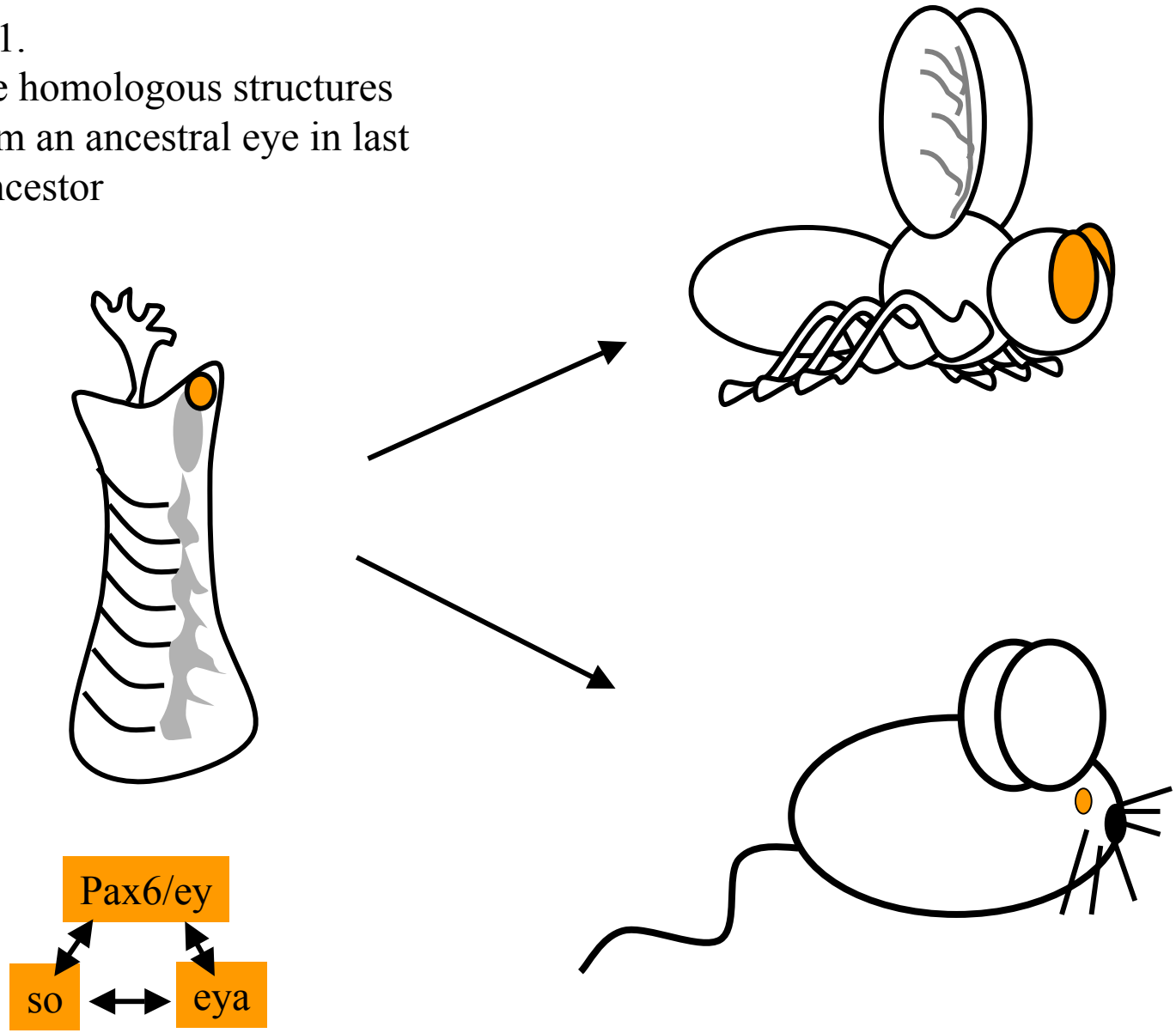
Vertebrate and invertebrate eyes use same or similar genetic pathways during development.
Homologous genes, deployed in similar areas doing similar jobs.

Implies that these genetic pathways were used to build eyes in last common ancestor (a disgusting worm-like thing).

BUT.
There are important differences...
e.g. vertebrate *Rx* gene, critical from earliest stages for formation of optic vesicle and retina - Drosophila homologue *Drx* not expressed in eyes.
When examine ***details*** of action of genes, or tissues where genes expressed, apparent similarities become a lot more complicated/less convincing.

Reading:
Jarman, A. P. (2000). Vertebrates and insects see eye to eye. *Curr. Biol*. 10, R857-859.
Kumar J. P. and Moses, K. (2001) Eye specification in *Drosophila*: perspectives and implications. *Sem. In Cell Dev. Biol*. 12, 469-474.
Peters, M. A. (2002). Patterning the neural retina. *Curr. Opin. Neurobiol*. 12, 43-48.

Possibility 1.
All eyes are homologous structures derived from an ancestral eye in last common ancestor



Possibility 2.
Last common ancestor didn't have eyes, but used Pax6/so/eya genes to pattern anterior CNS. Eyes evolved independently, but hijacked the same developmental 'motor'.