

Teil I (Fischbach):

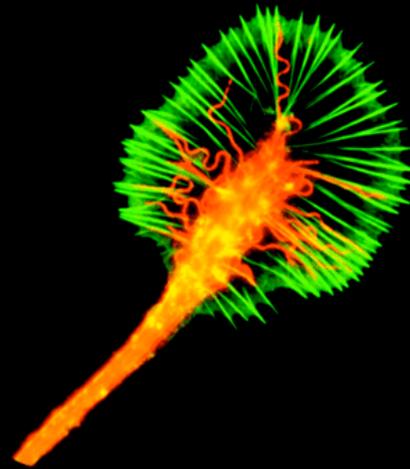
Drosophila als Modellsystem der Entwicklungsgenetik

Termine

20.10. 2010	Reichweite der Entwicklungsgenetik
27.10. 2010	Die Festlegung der Körperachsen
26.01. 2011	Neurogenese
02.02. 2011	Augenentwicklung
09.02. 2011	Verhaltensgenetik

Axonale und dendritische Wegfindung revisited

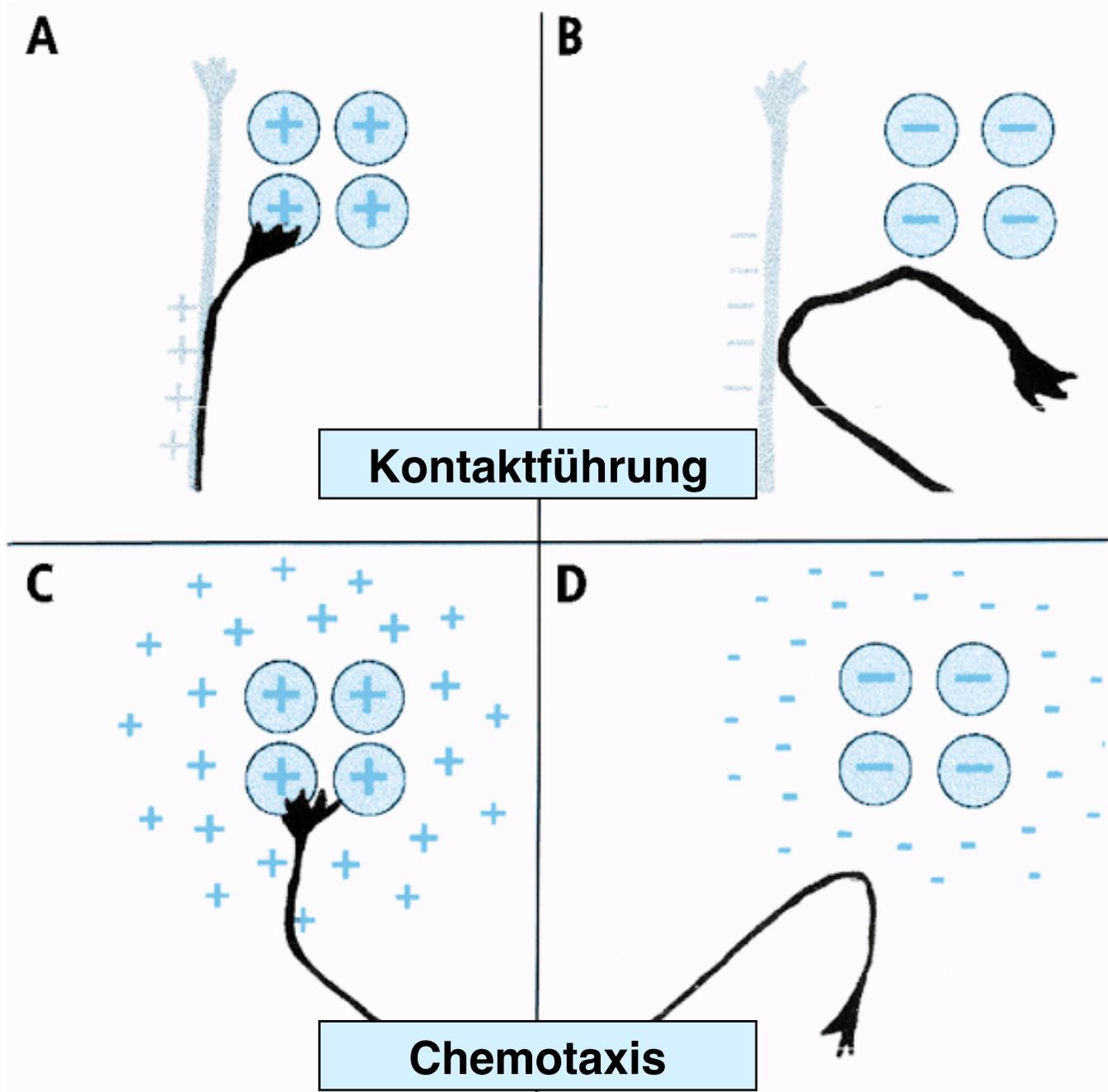
**Wachstumskegel an der
Axonspitze**

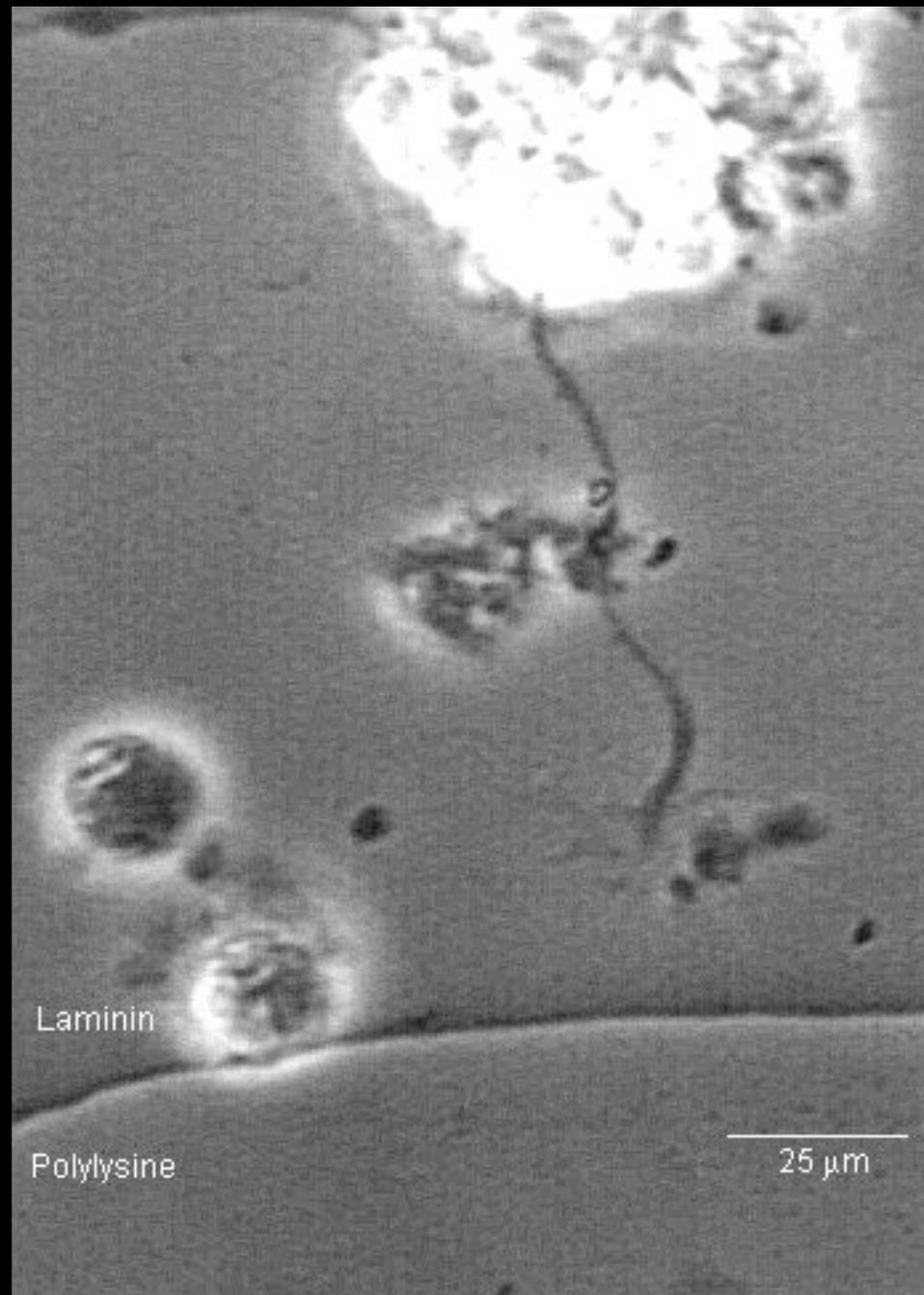


<http://www.fmi.ch/groups/AndrewMatus/video.actin.dynamics.htm>

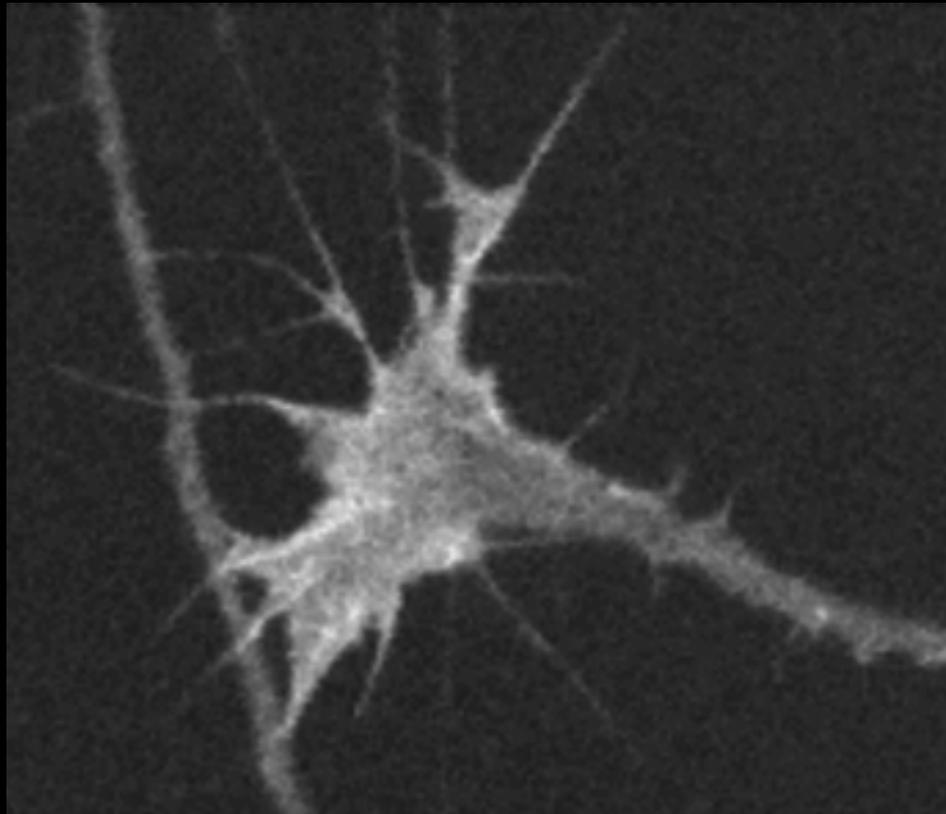


Mechanismen der Wegfindung

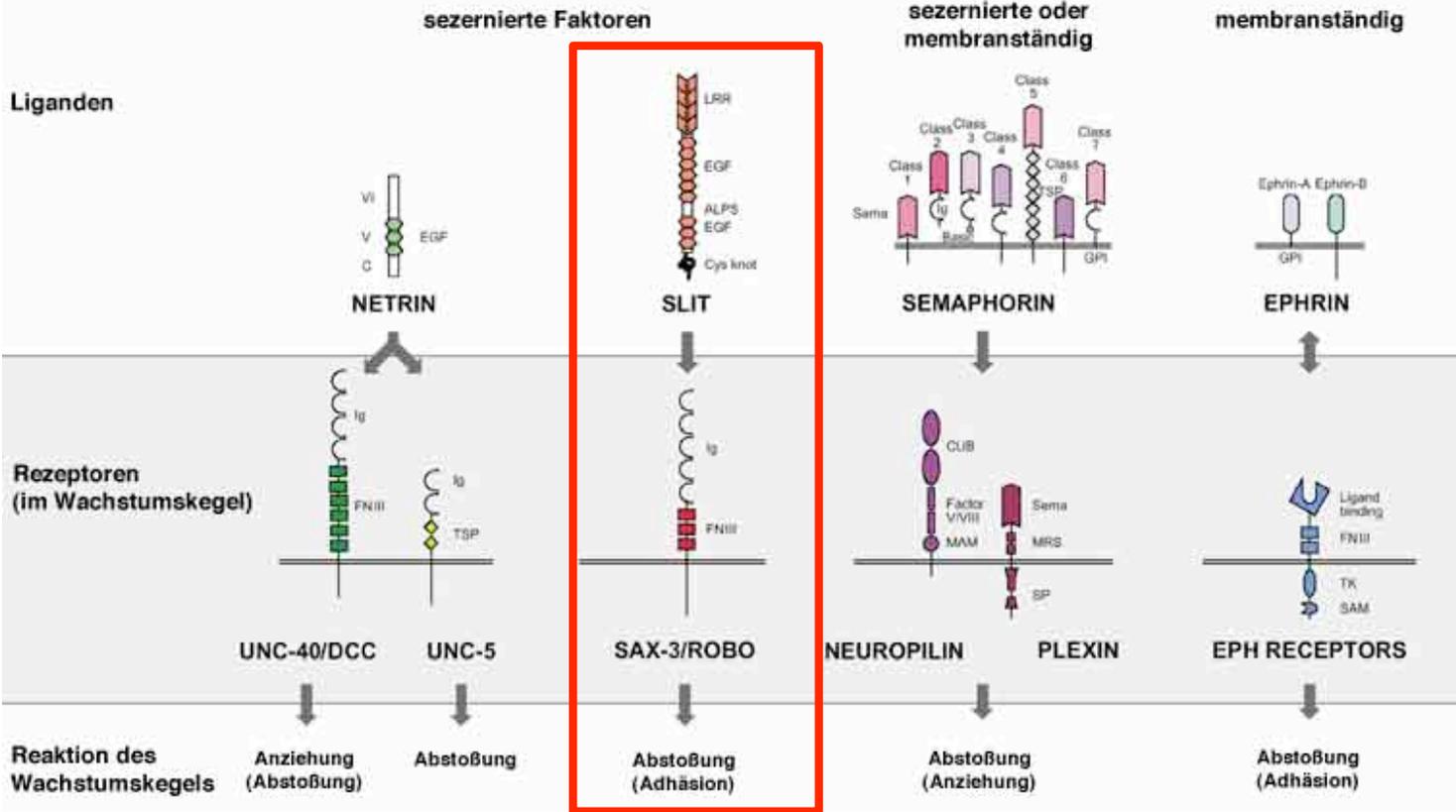




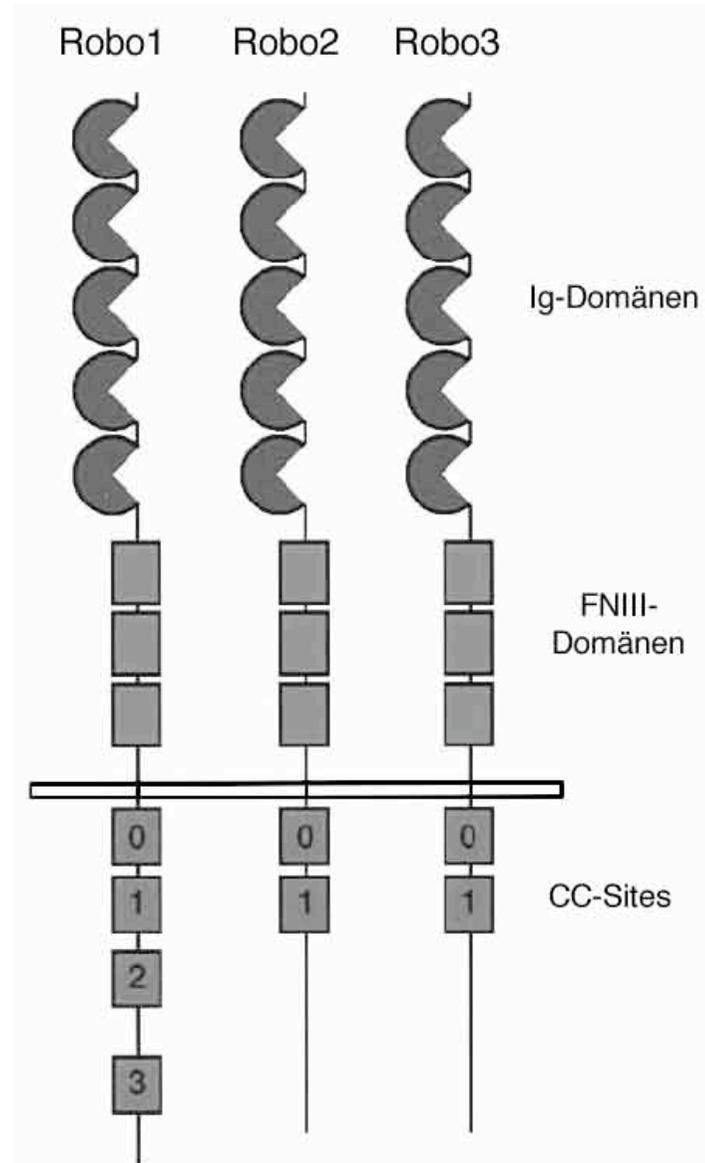
Wachstumskegel bei Kontaktführung



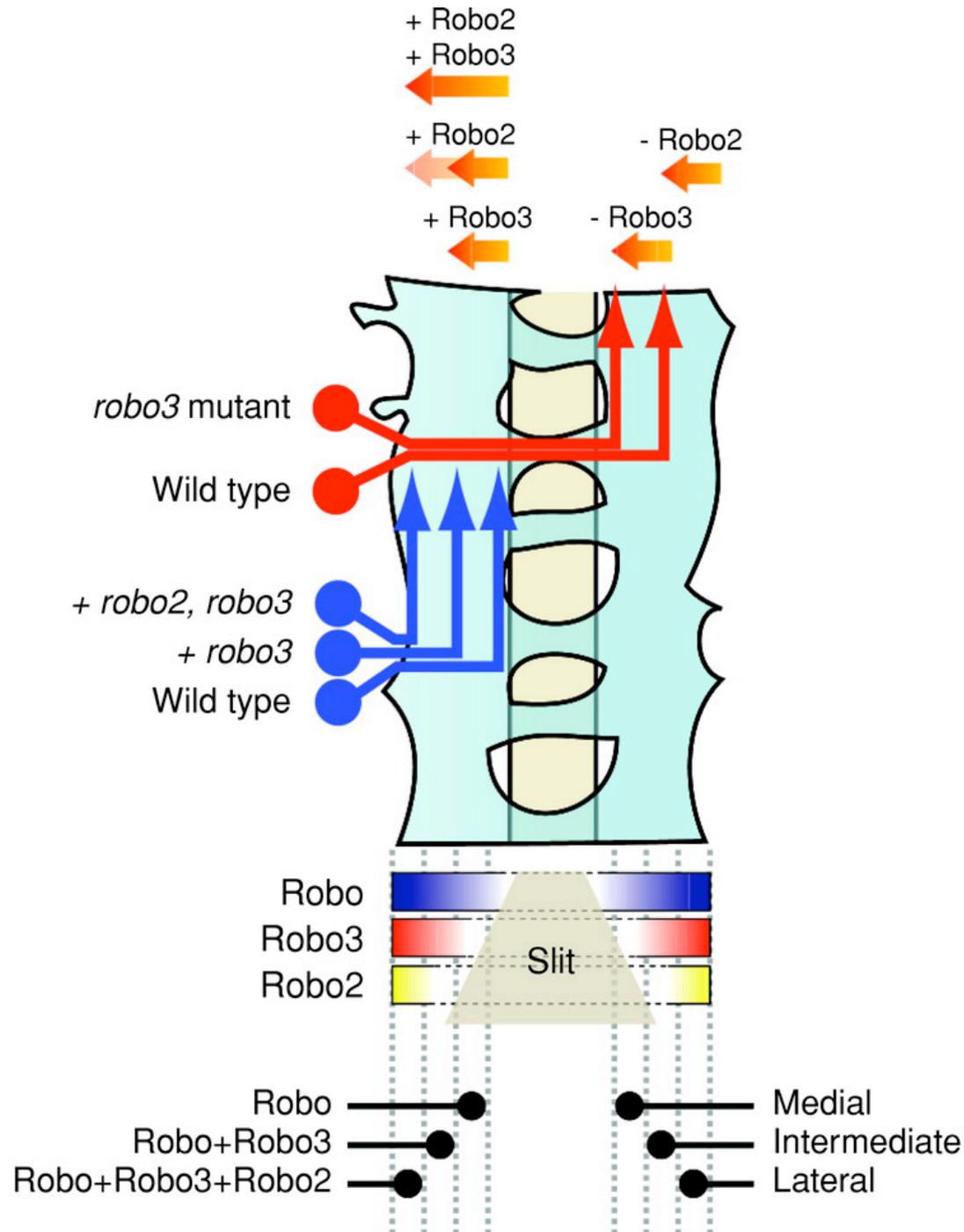
Einige konservierte Navigationssysteme für Wachstumskegel



Struktur der Robo-Rezeptoren



Robo Protein Expression

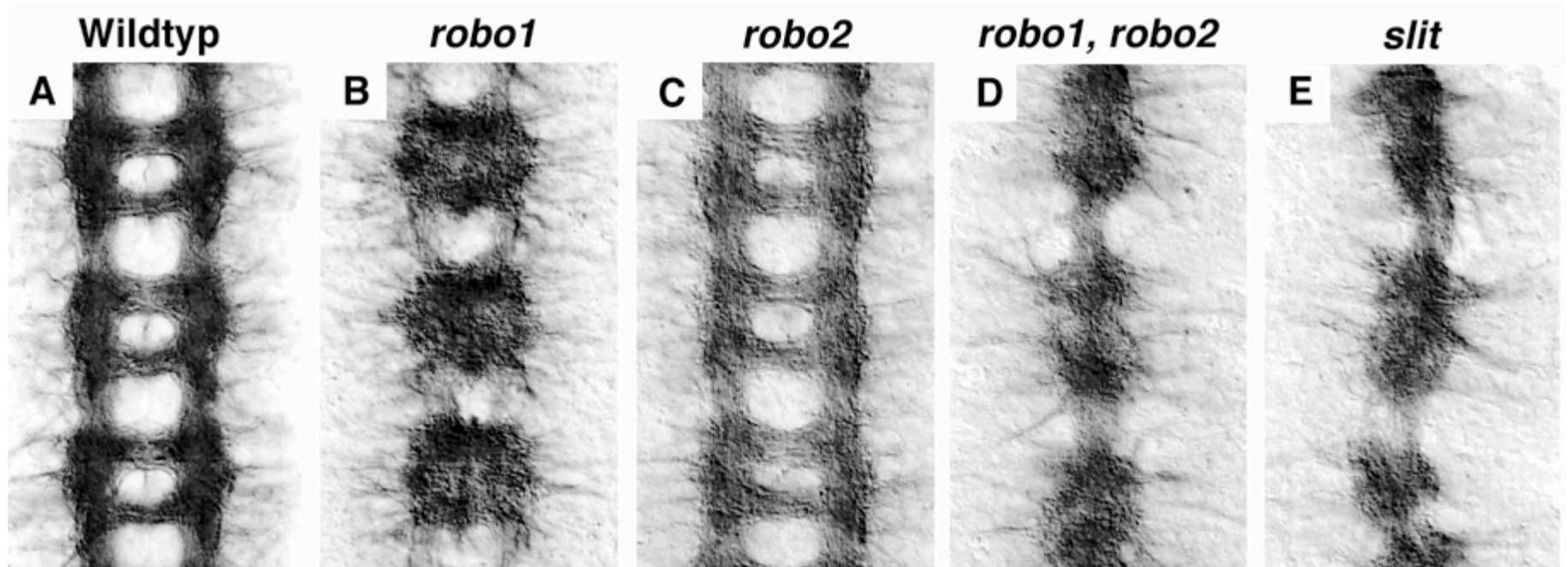


Die drei Roborezeptoren sind in unterschiedlichen, aber überlappenden lateralen Streifen exprimiert.

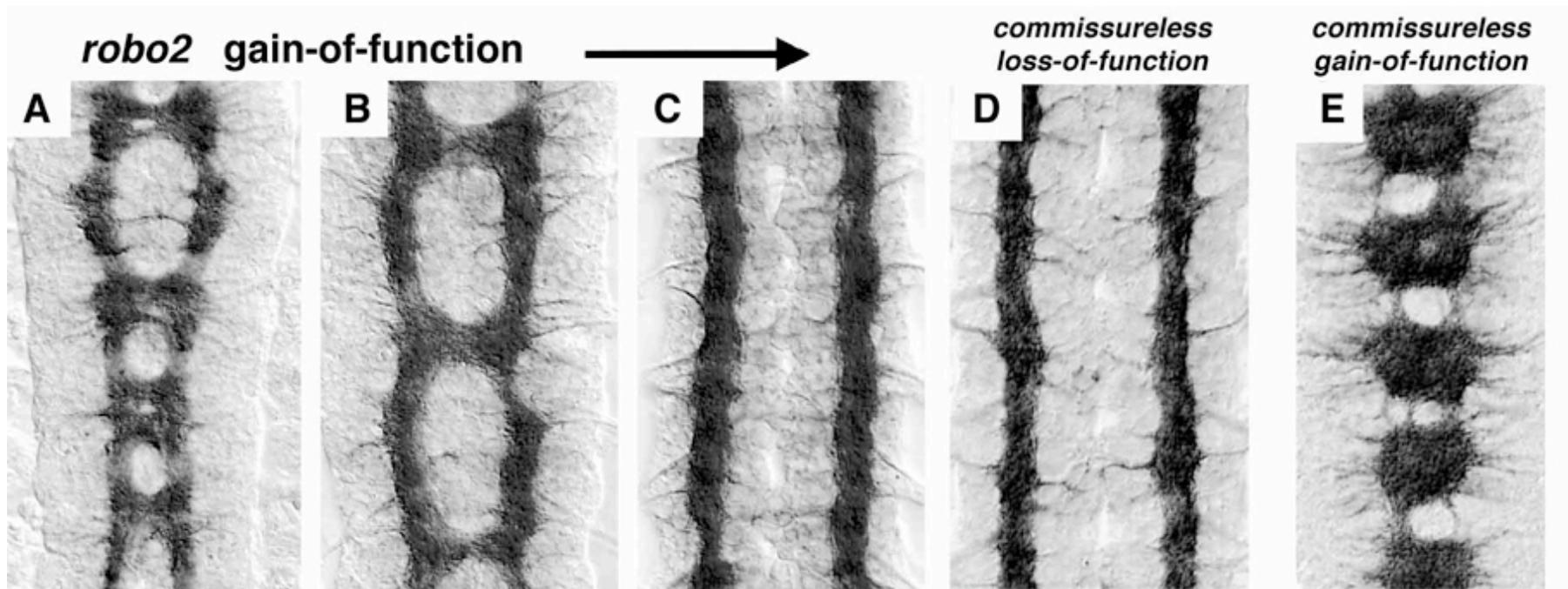
1. In einer medialen Zone wird nur Robo exprimiert.
2. In einer intermediären Zone exprimieren Axone Robo and Robo3
5. In einer lateralen Zone exprimieren Axone Robo, Robo2, Robo3

→ Je mehr Robos, umso weiter weg von der Mittellinie

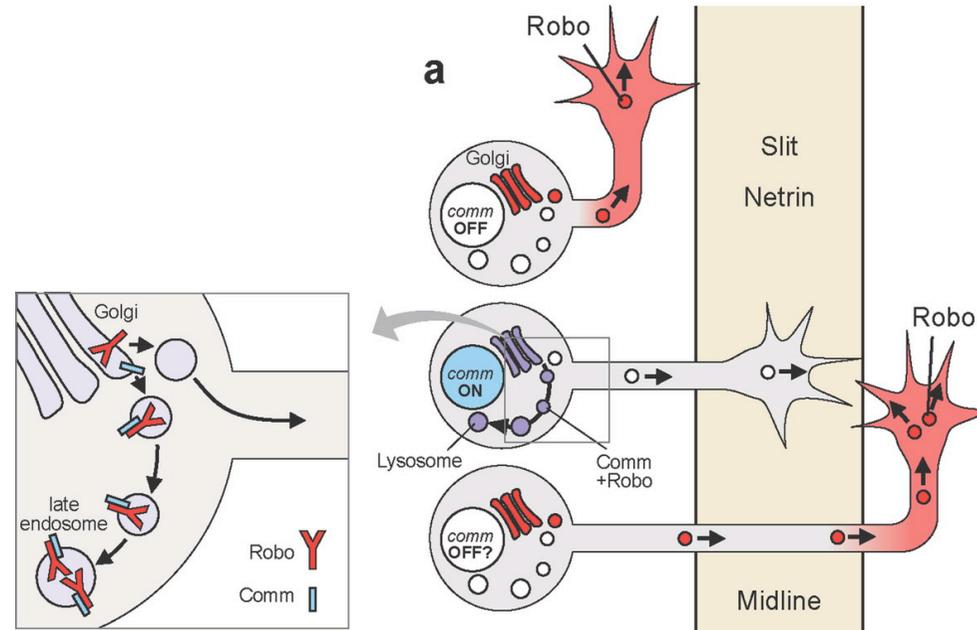
genetische Analyse der Kommissurausbildung I



genetische Analyse der Kommissurausbildung II



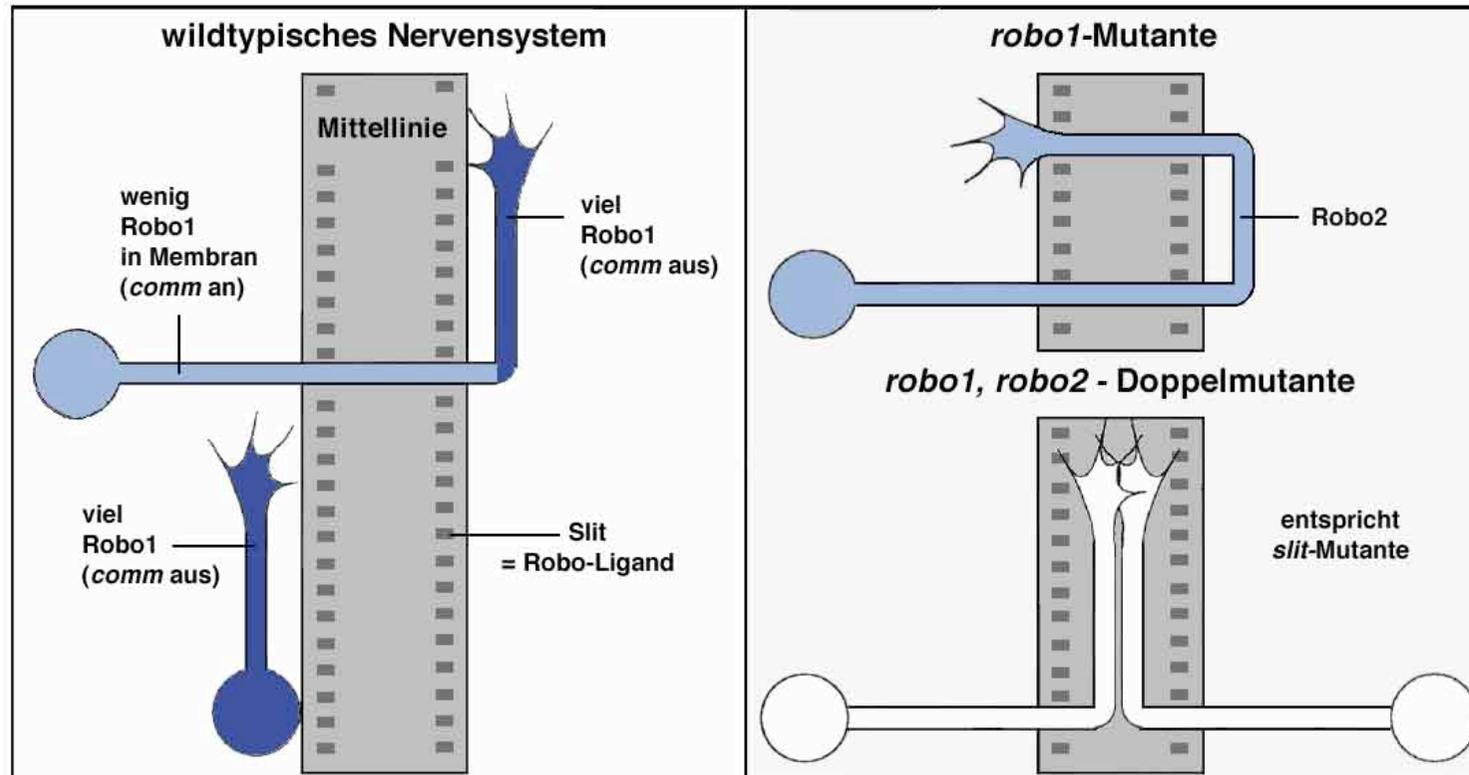
Comm hält Robo Konzentration in Wachstumskegeln niedrig



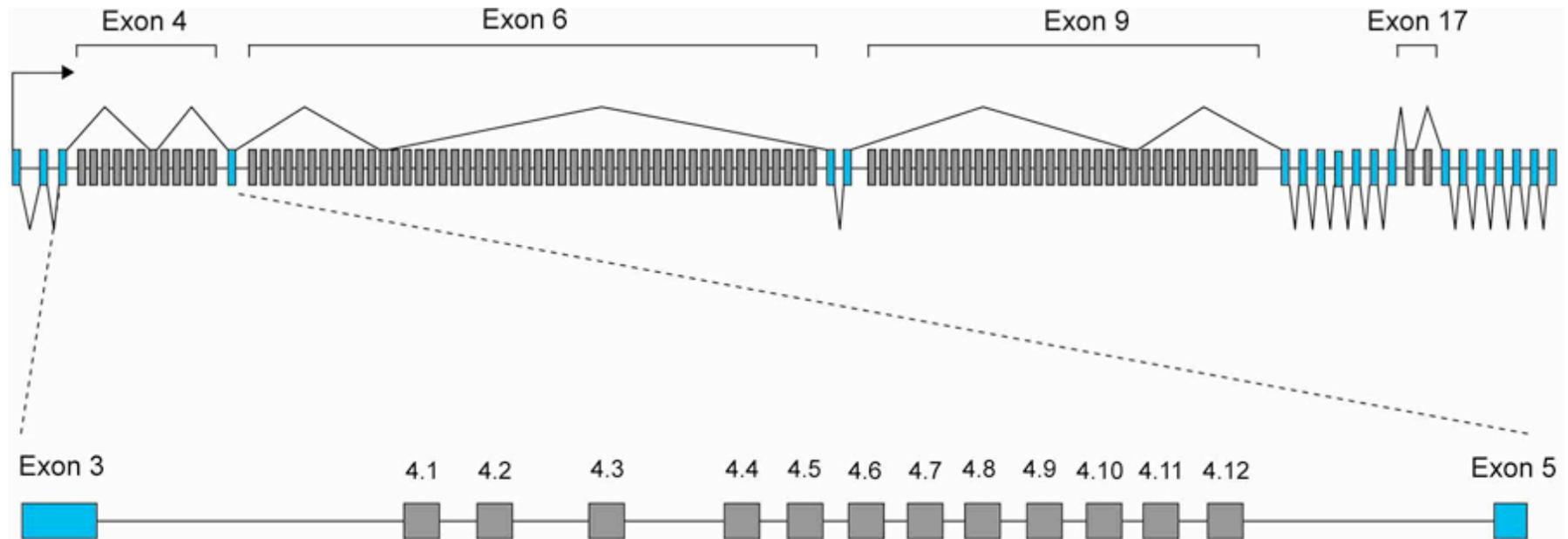
A Dickson BJ, Gilestro GF. 2006.
R Annu. Rev. Cell Dev. Biol. 22:651–75

- Comm lokalisiert im Golgi und in späten Endosomen
- Es bindet Robo und rekrutiert es in die Endosomen
- Robo ohne Comm wird in die Axone transportiert. Das findet in Gegenwart von Comm nicht statt.

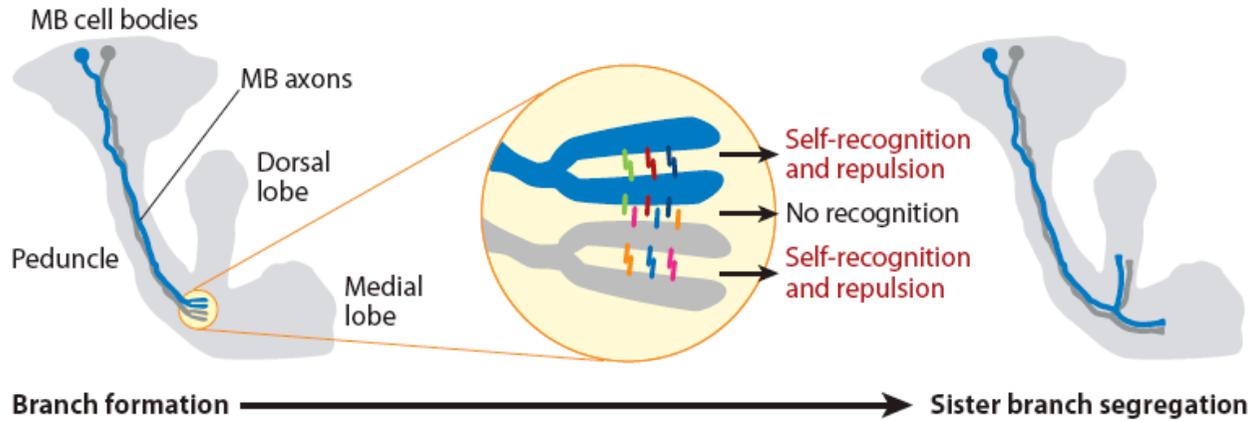
genetische Analyse der Kommissurausbildung III



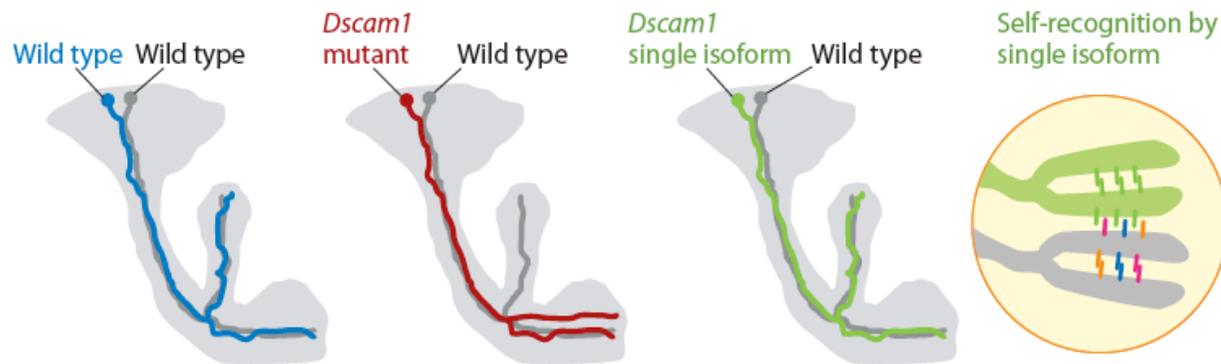
genomische Organisation des Down-Syndrom-Proteins von *Drosophila* (Dscam)



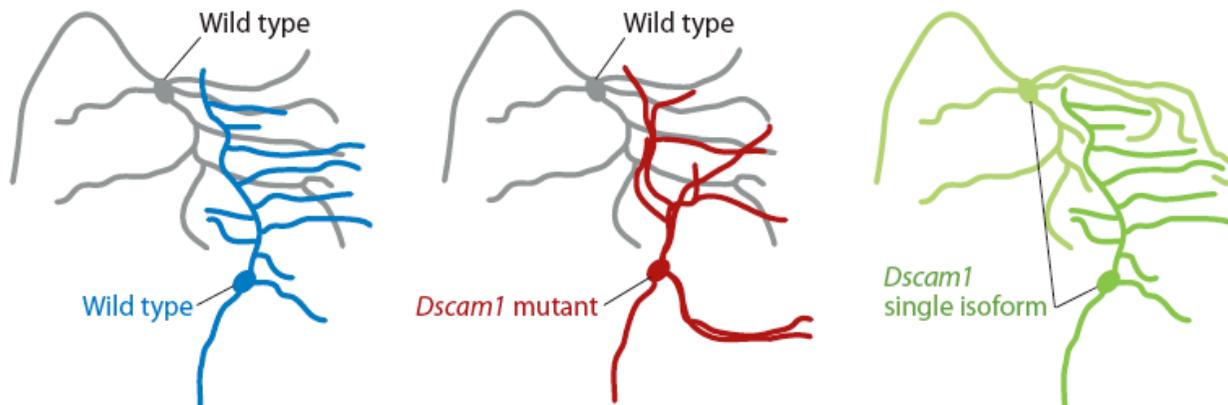
a Dscam1 mediates self-avoidance in MB axons



b The morphology of MB neurons of different genotypes



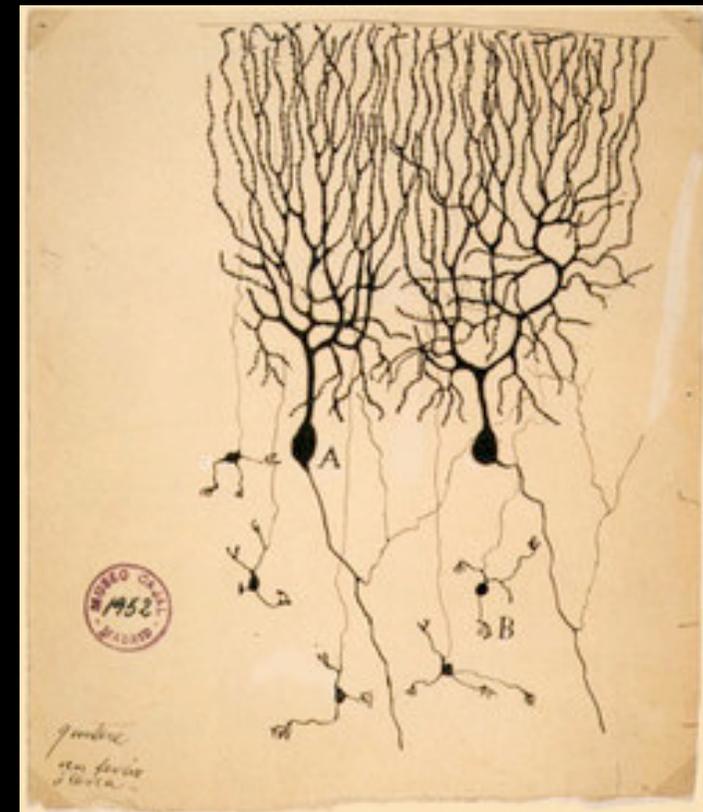
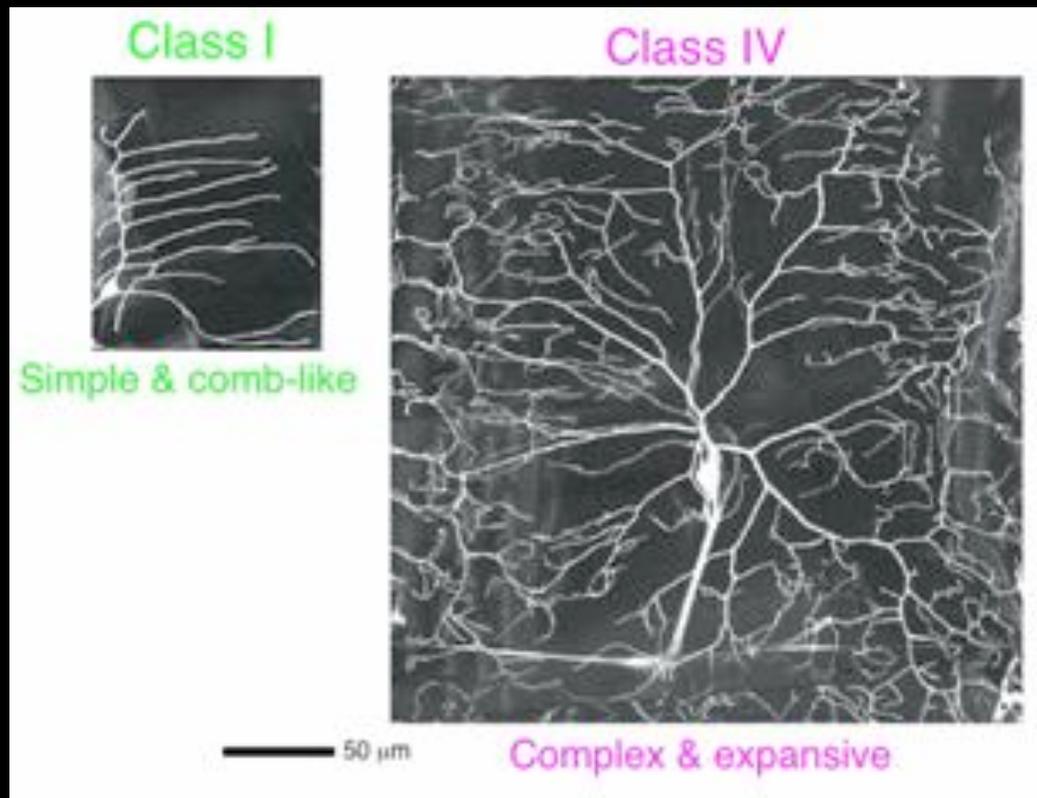
c Dscam1 mediates self-avoidance in da neurons



„self-avoidance“ von
Kenyonzellaxonen in den
Pilzkörpern von
Drosophila

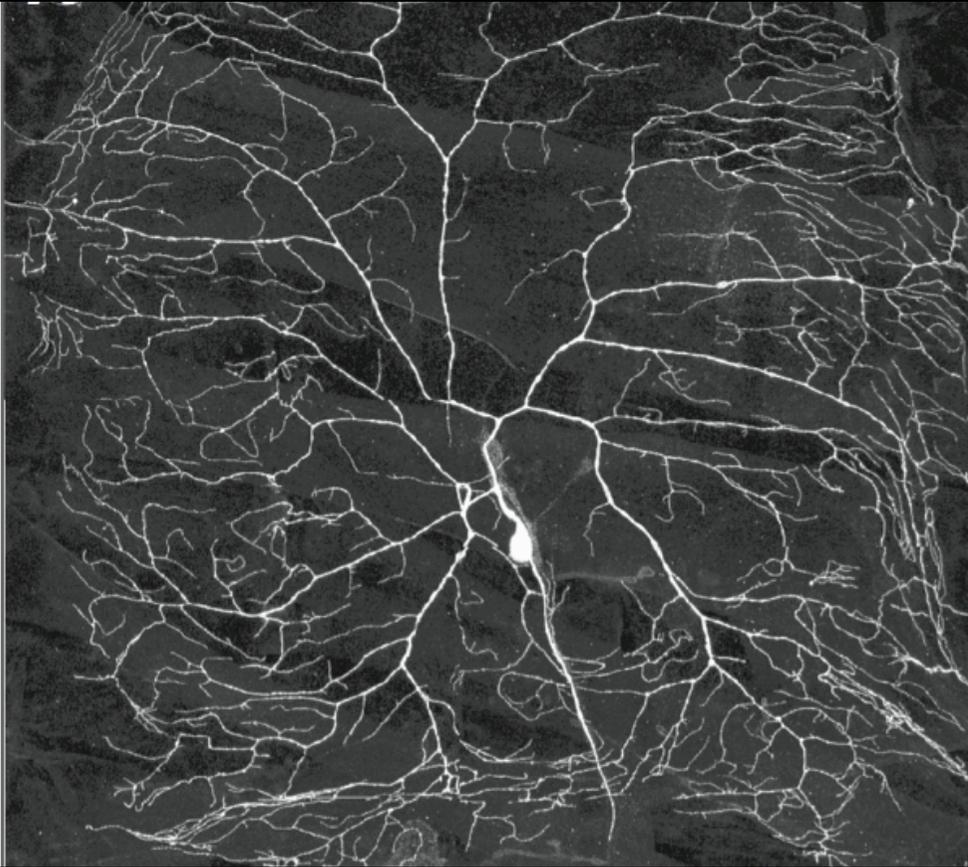
„self-avoidance“ von
Dendriten in da Neuronen

Genetische Programmierung dendritischer Baumstrukturen

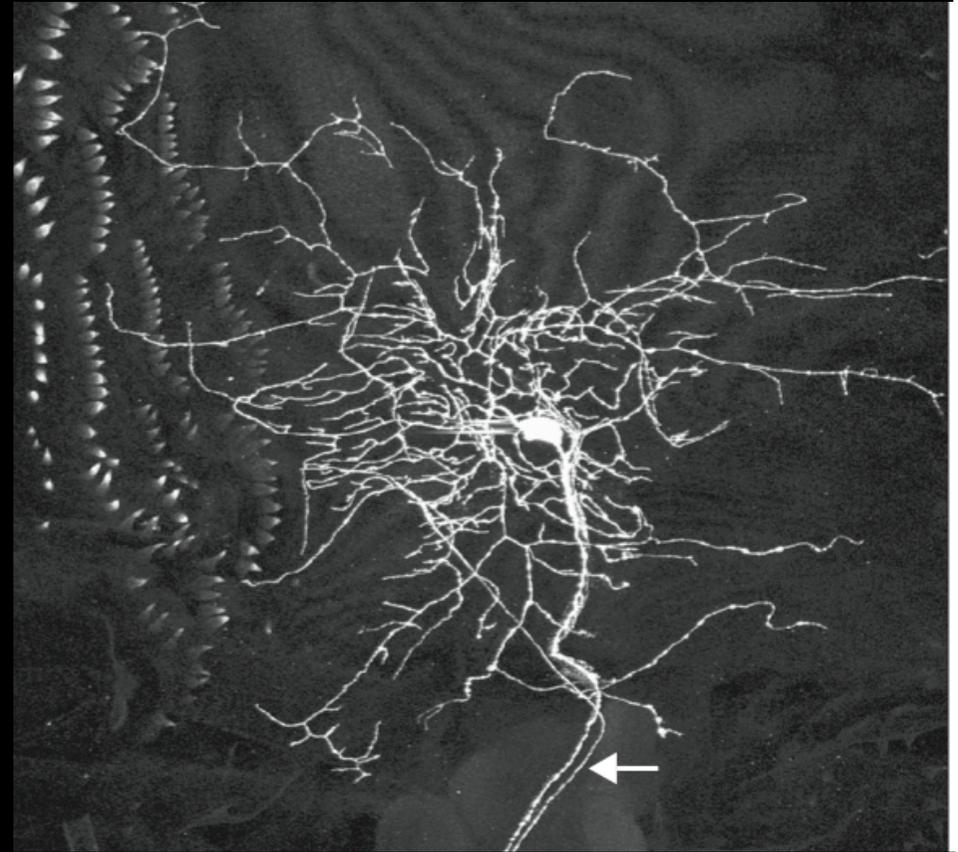


Genetische Programmierung dendritischer Baumstrukturen

Kontrolle

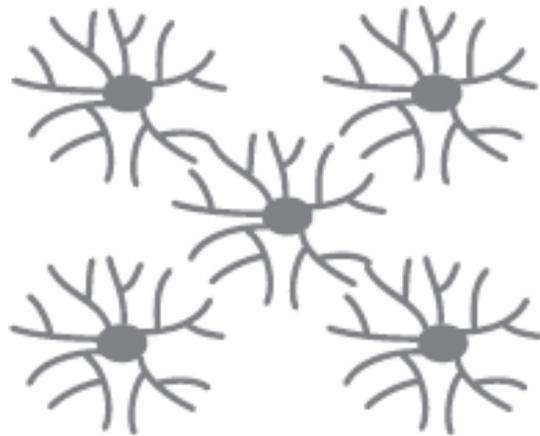


mutant



b DSCAM regulates tiling and self-avoidance in the mouse visual system

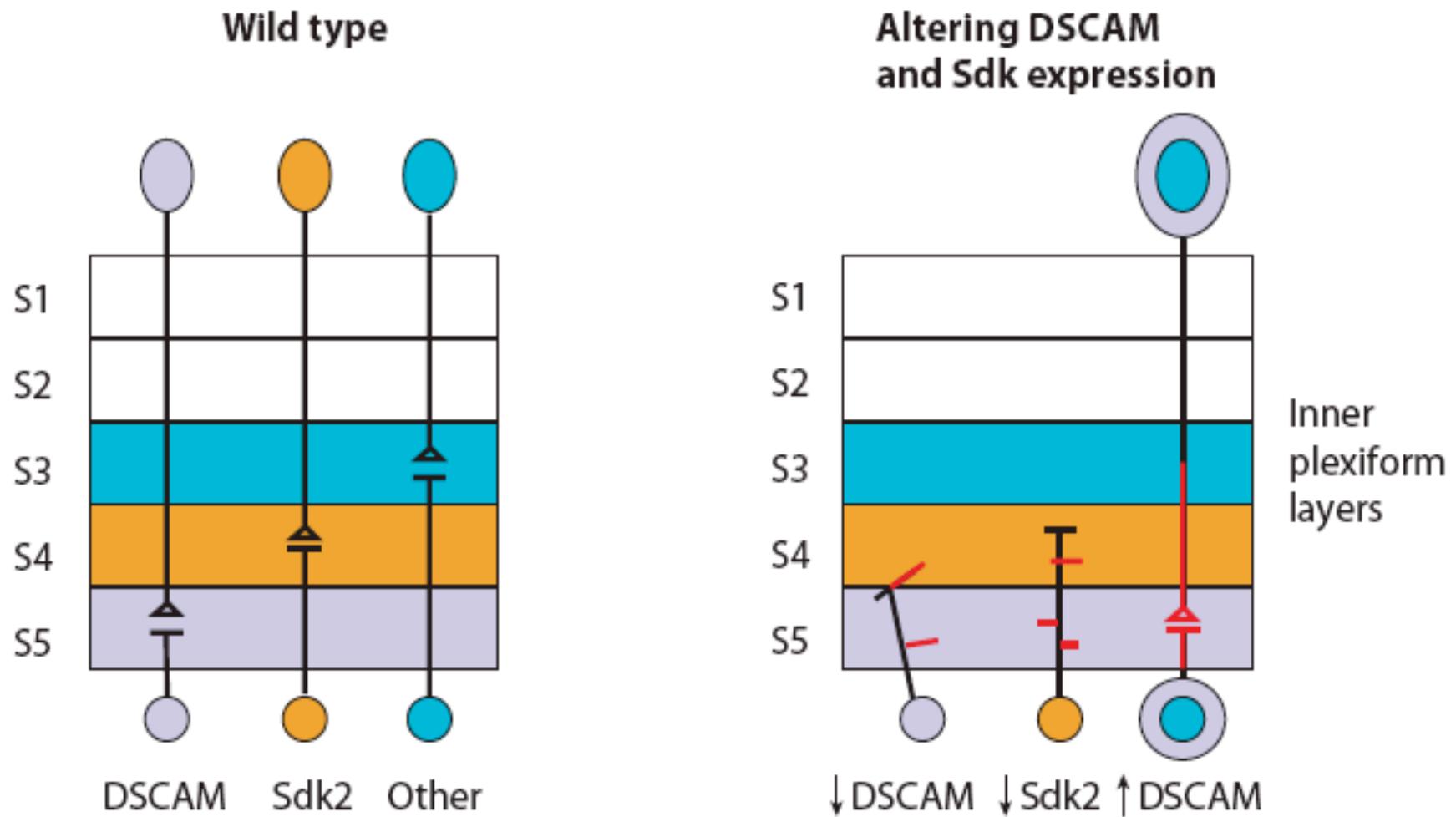
Wild type



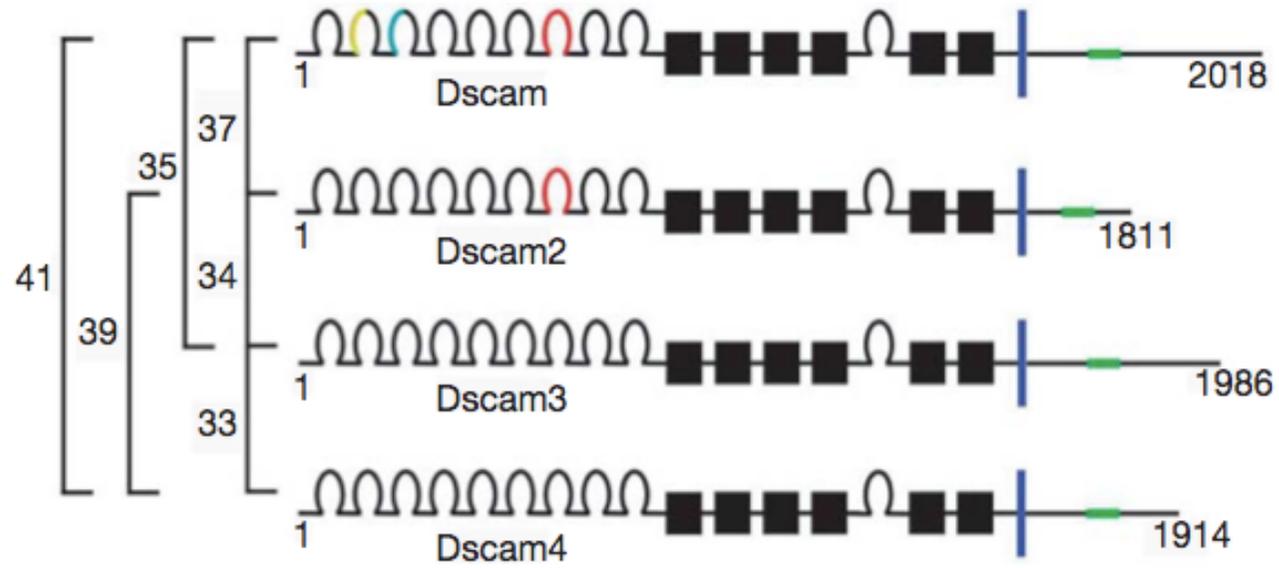
***DSCAM* mutant**



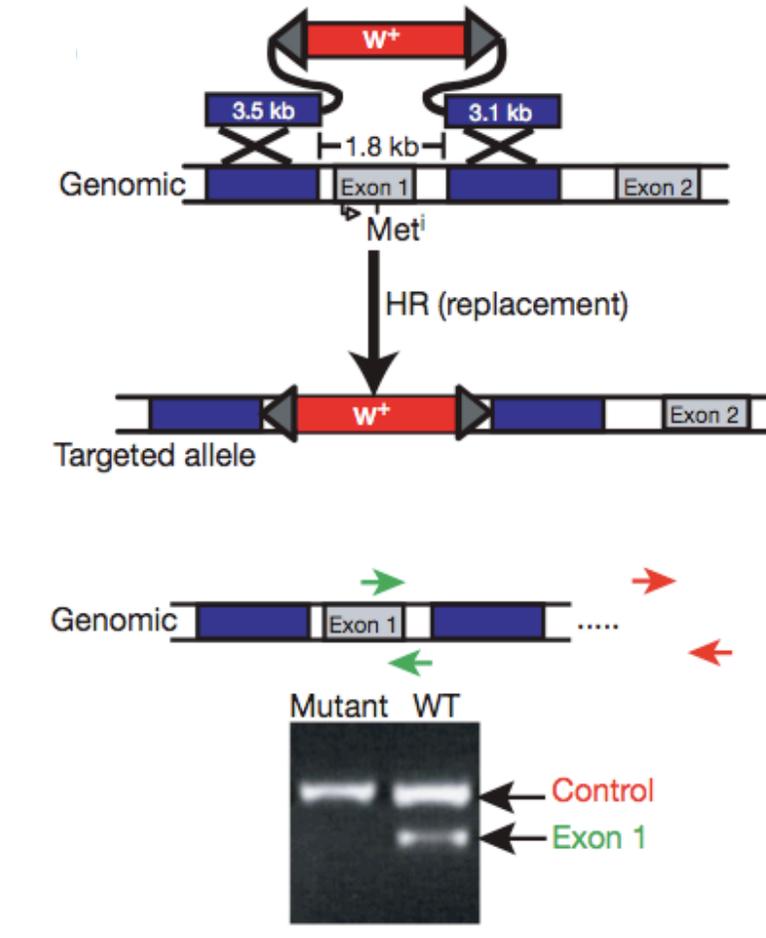
C DSCAM regulates layer specificity in the chick visual system

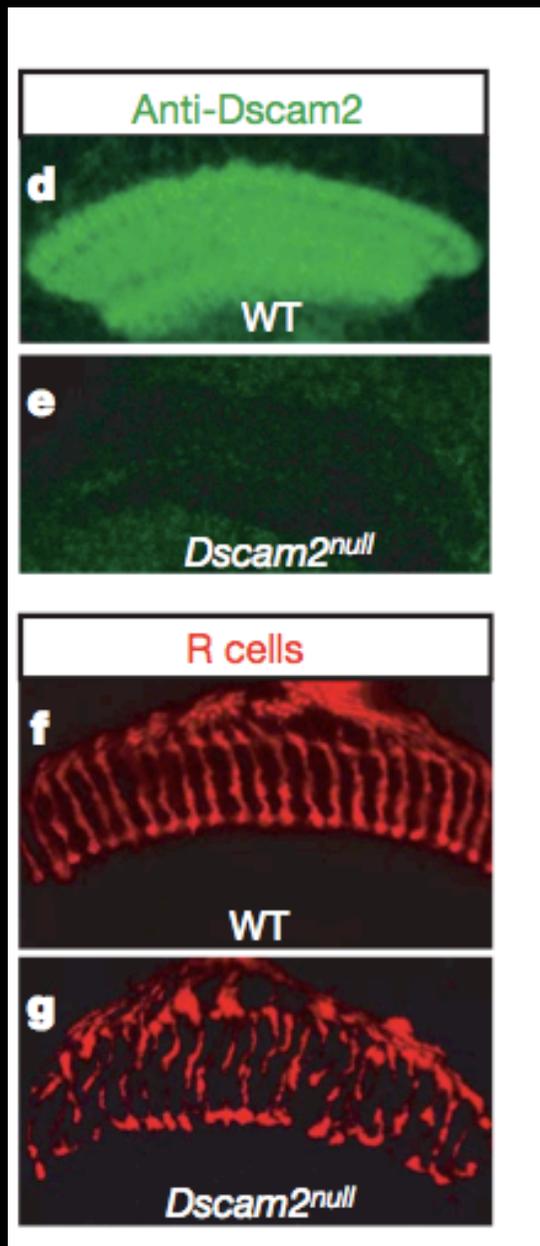


Es gibt vier DSCAM-Gene in Drosophila



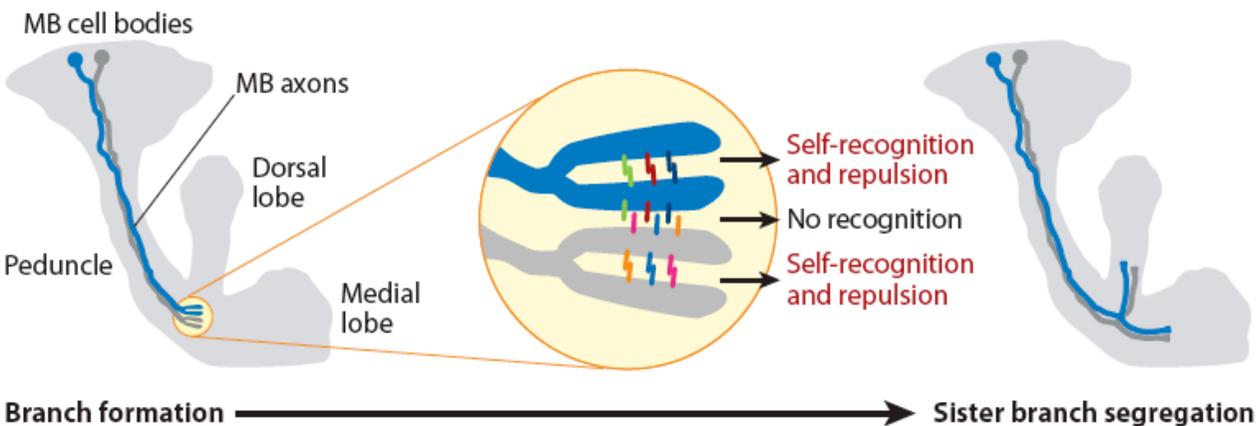
Experimentelle Inaktivierung von DSCAM2 durch homologe Rekombination



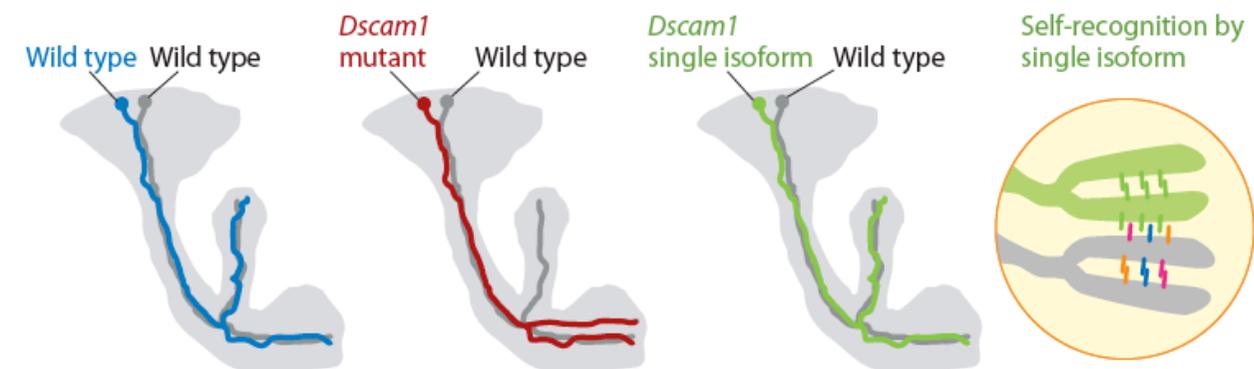


Effekt von
Dscam2^{null} auf die
Anordnung der
axonalen
Terminalien von
Retinulazellen

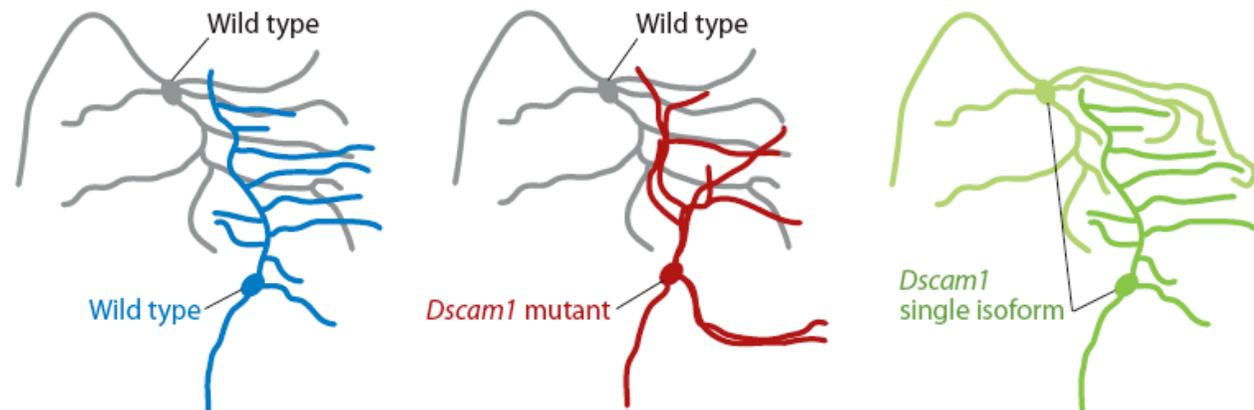
a Dscam1 mediates self-avoidance in MB axons



b The morphology of MB neurons of different genotypes



c Dscam1 mediates self-avoidance in da neurons

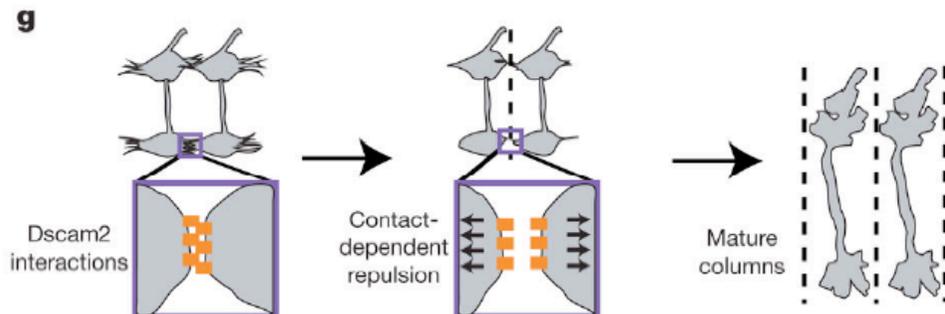
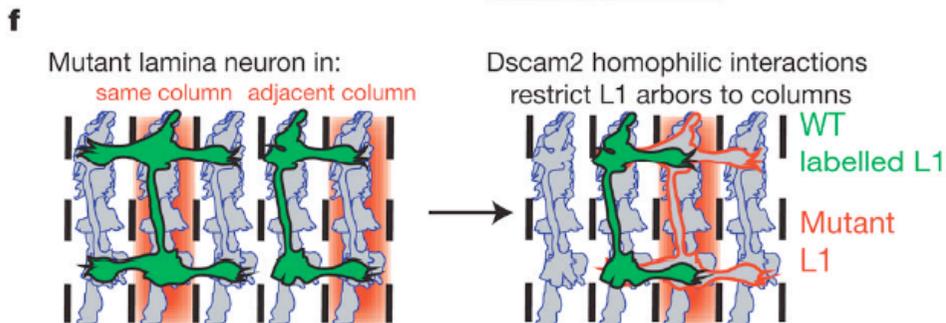
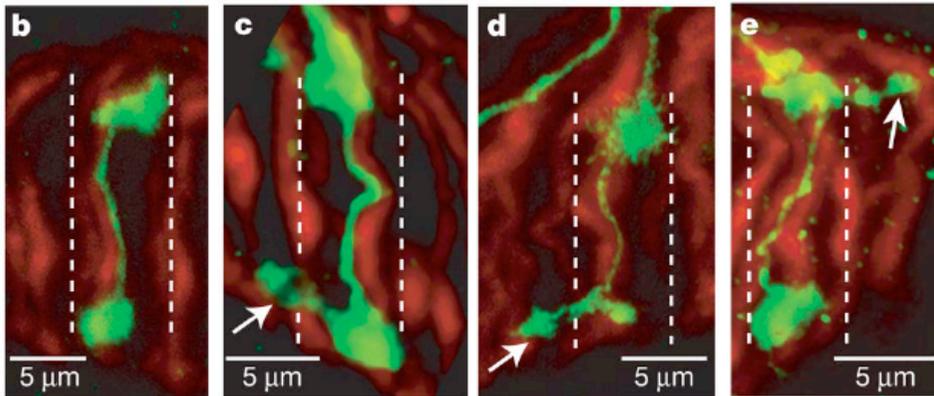
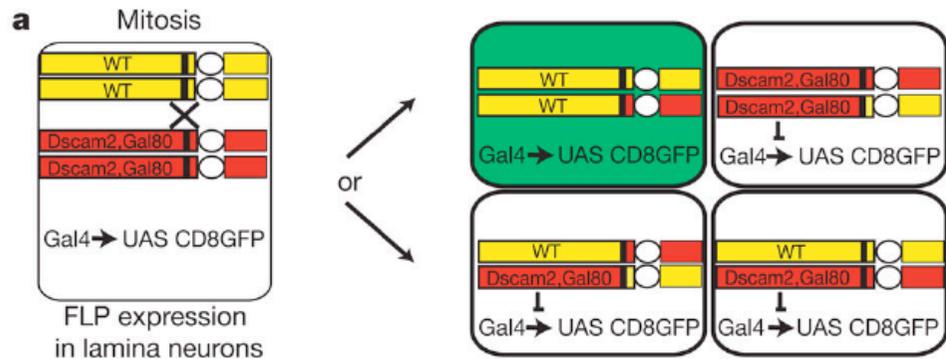


„self-avoidance“ von
Kenyonzellaxonen in
den Pilzkörpern von
Drosophila

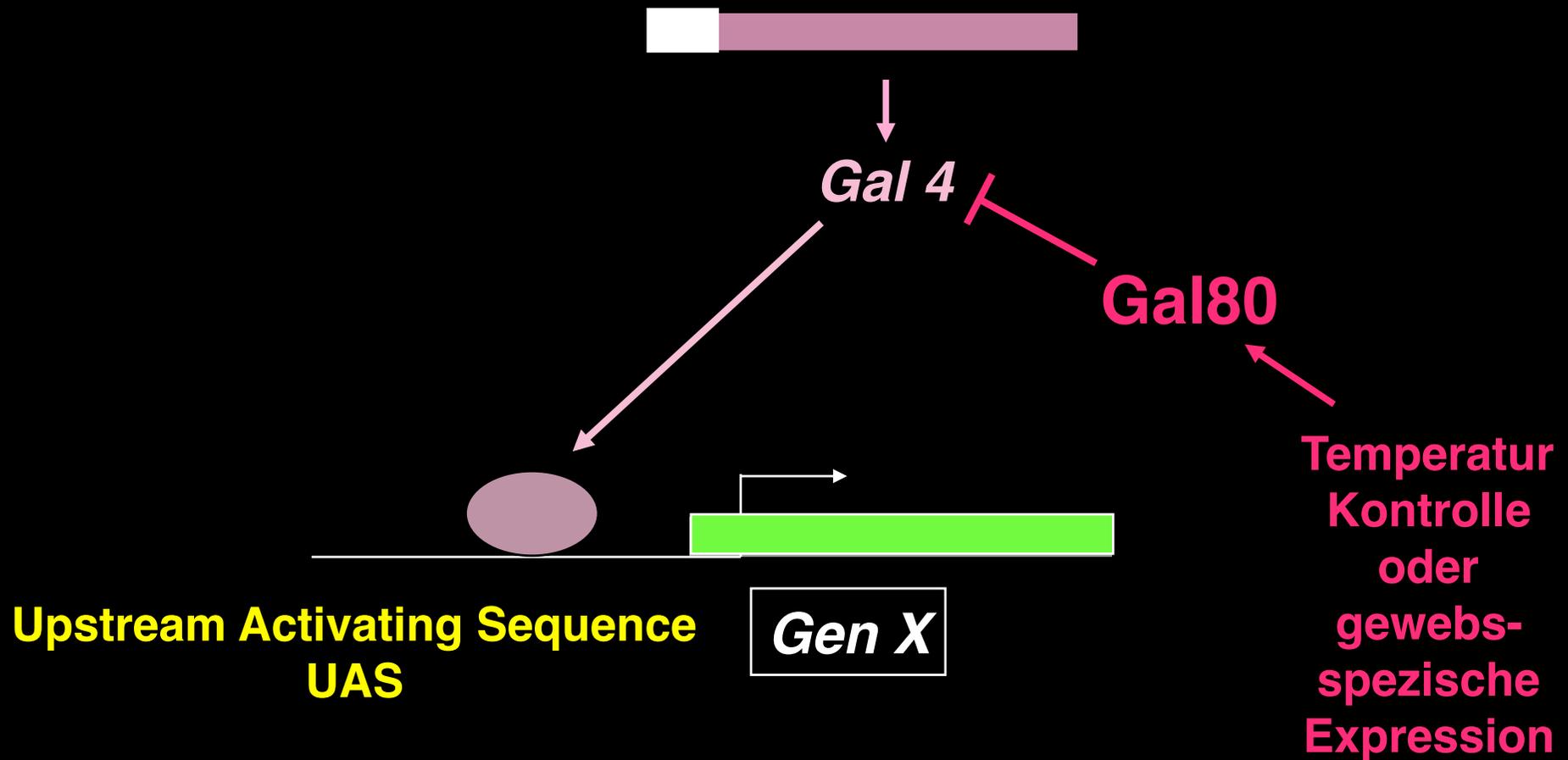
„self-avoidance“ von
Dendriten in da
Neuronen

„reverse MARCM“

wildtypische Zelle in
mutanter Umgebung

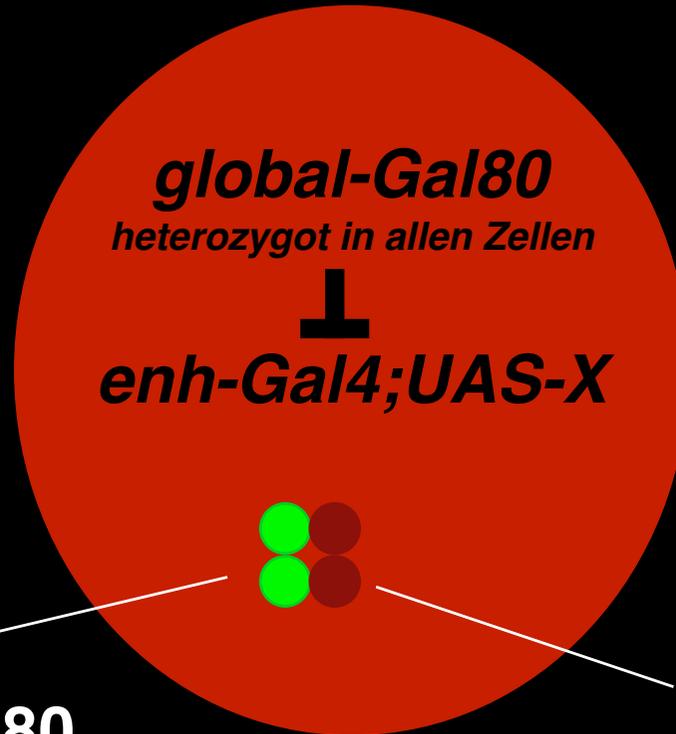


Erhöhung der Spezifität des Gal4/UAS-Systems



MARCM-Methode

Mosaic Analyse with a repressible cell marker



Verlust von Gal80
durch mitotische
Rekombination

homozygoter Gal80
Zwillingsklon

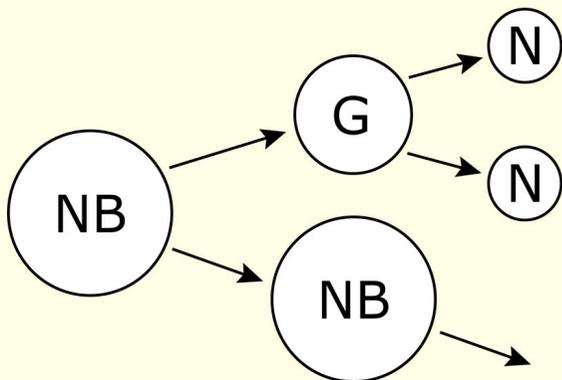
Mosaic Analyse mit einem reprimierbaren Zellmarker (MARCM-Methode)

Mutterzelle

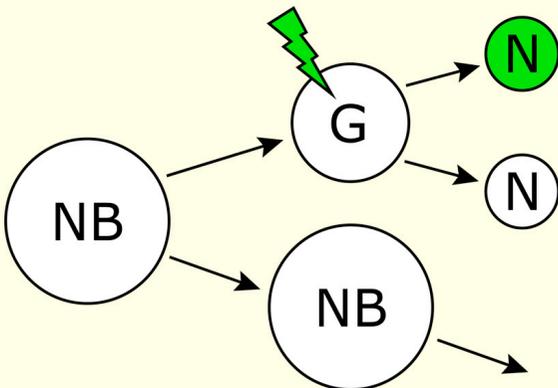


Wichtig ist, in welchen Zellen die mitotische Rekombination stattfindet

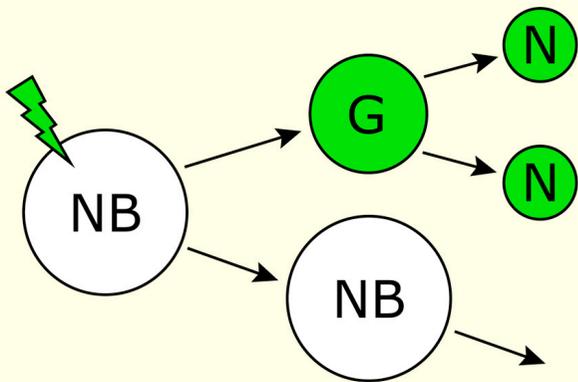
No recombination



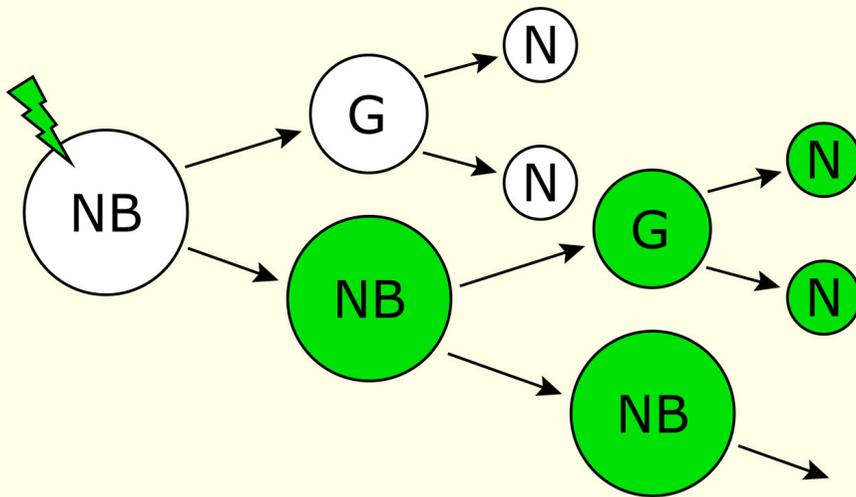
Recombination in Ganglion mother cell



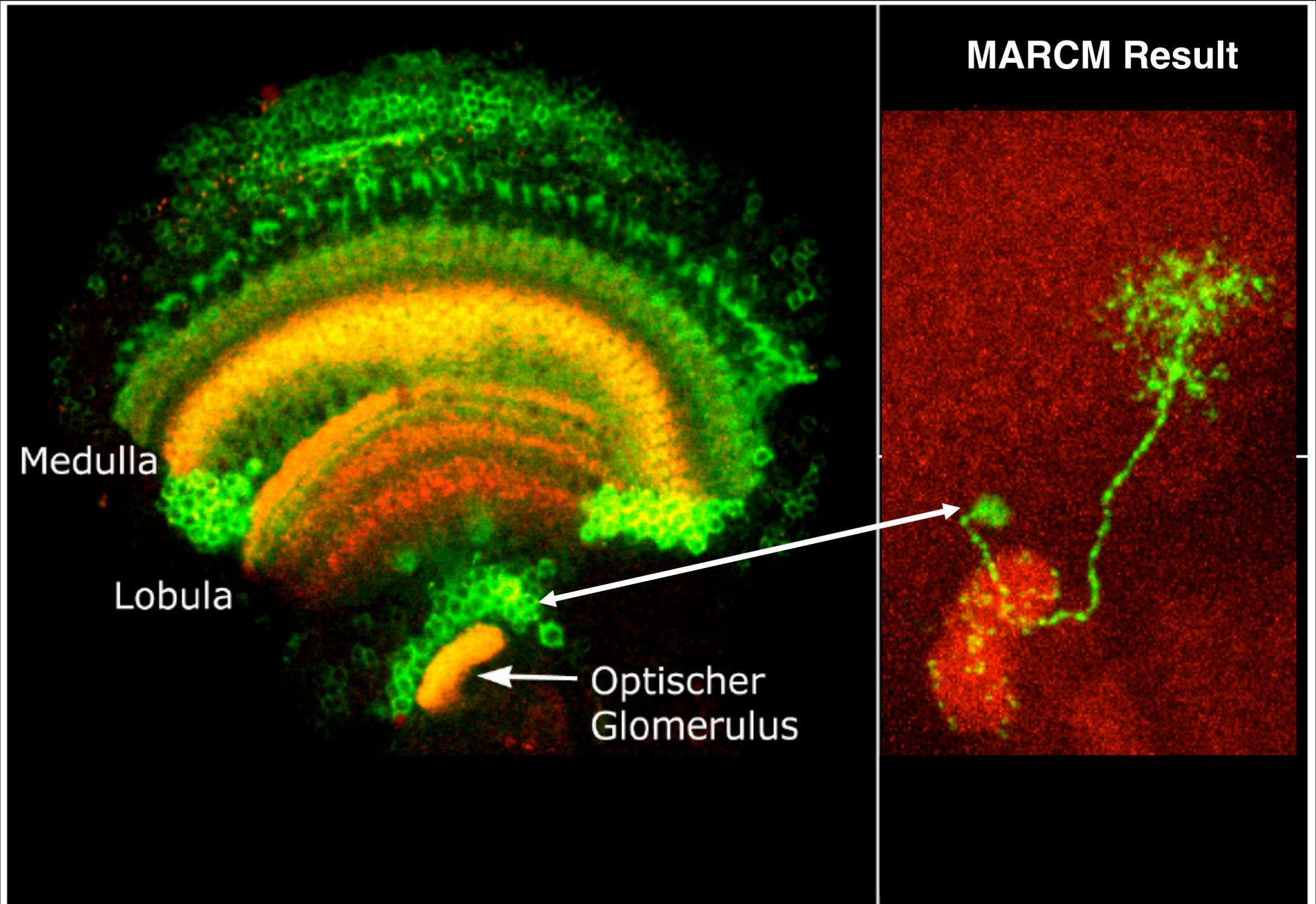
Recombination in Neuroblast

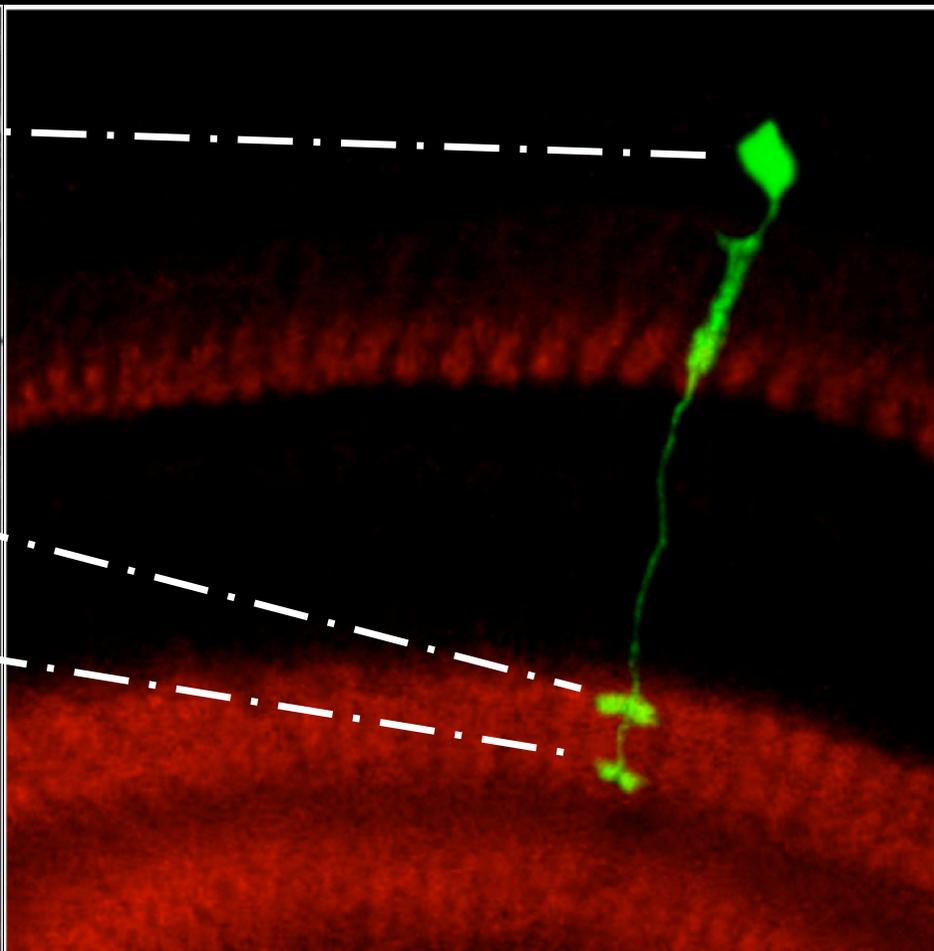
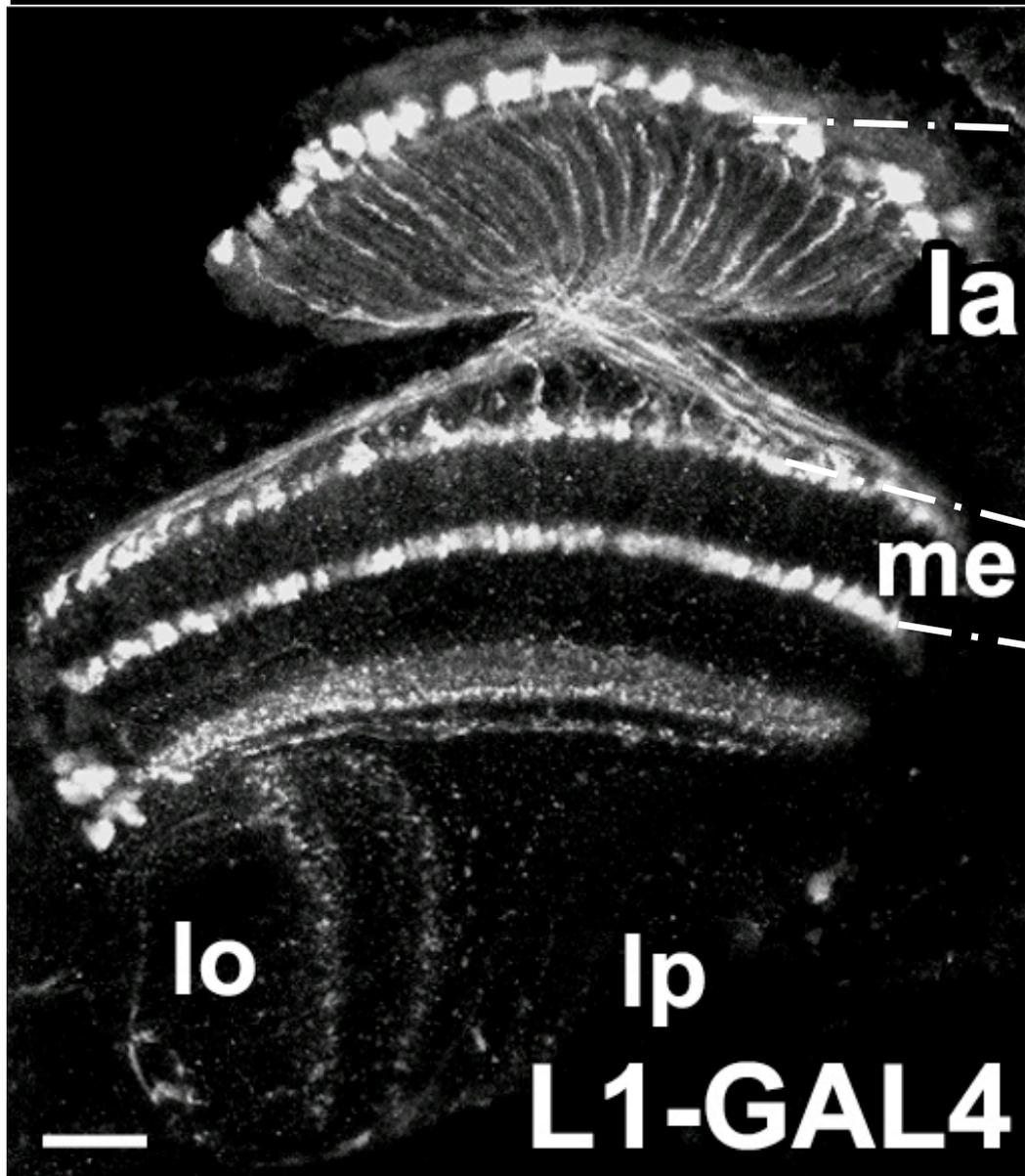


Recombination in Neuroblast



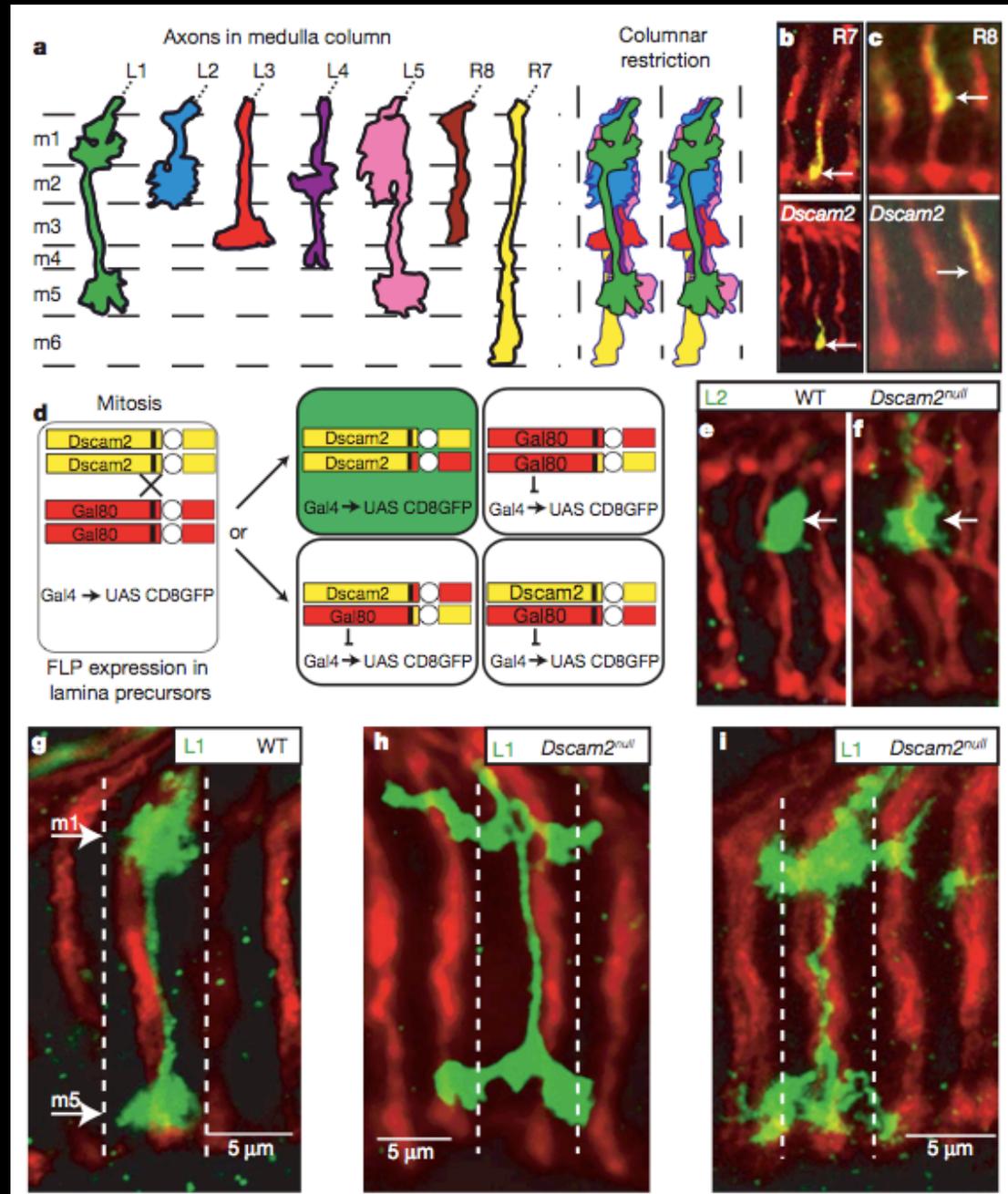
MARCM isoliert einzelne Neurone aus einem gegebenen Gal4-Stamm



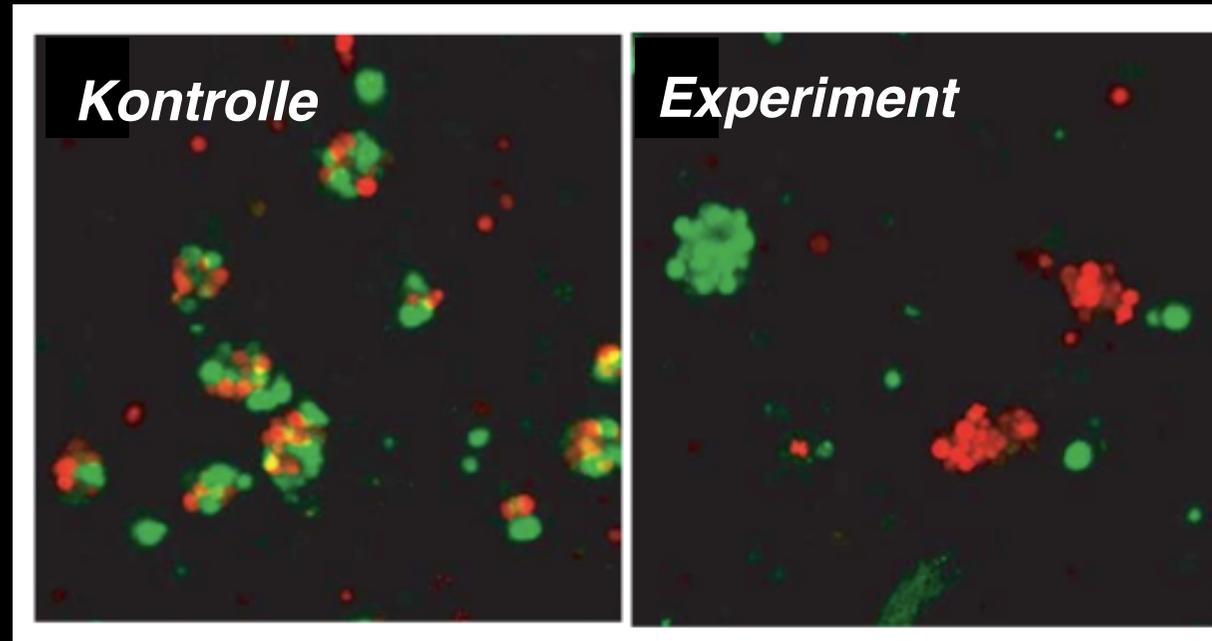


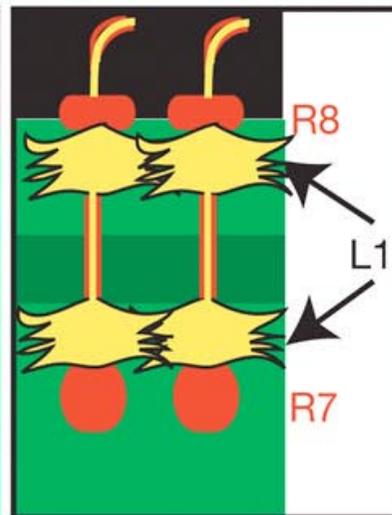
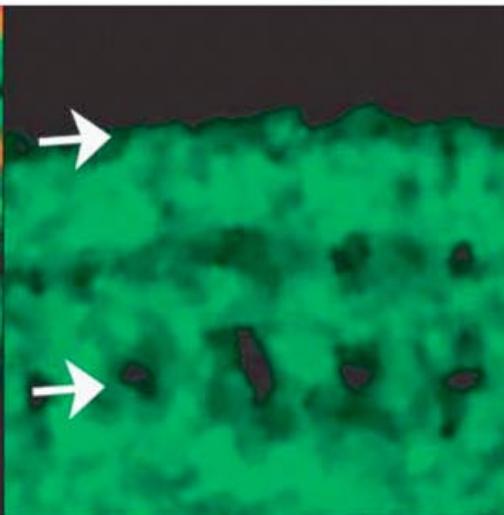
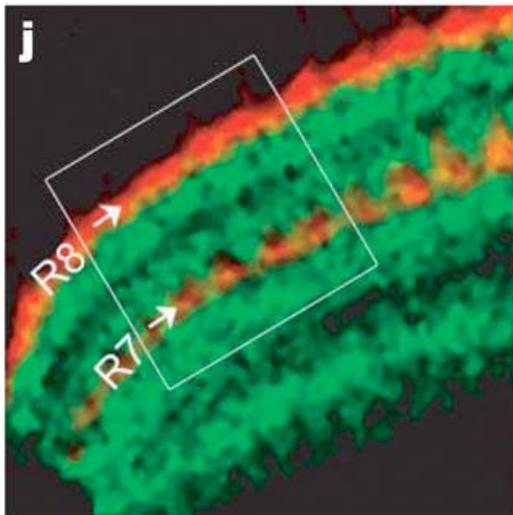
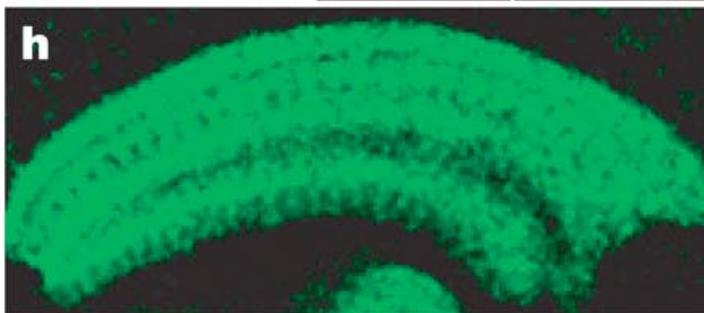
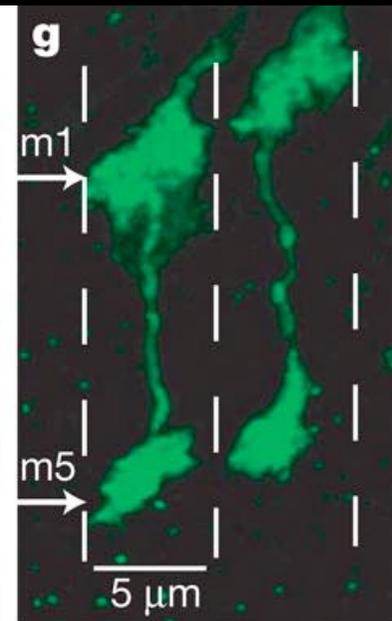
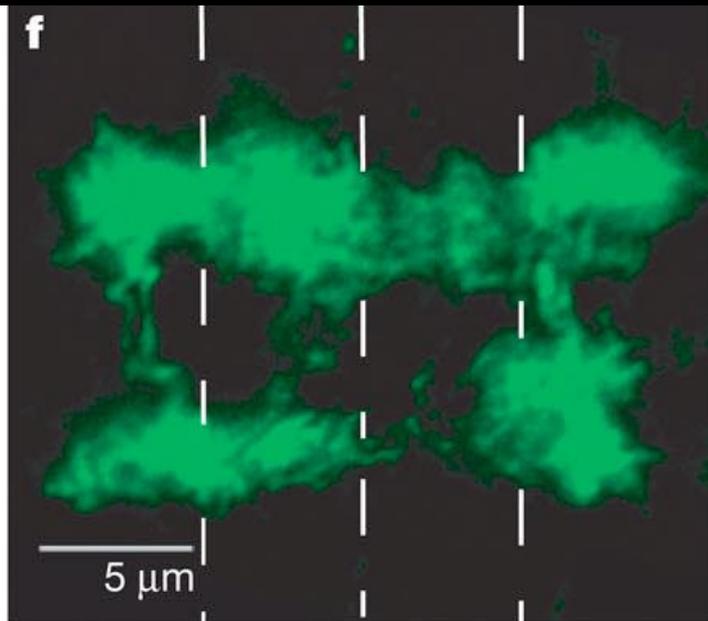
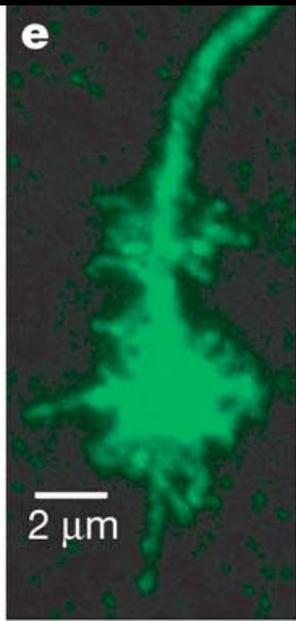
MARCM result
(in the pupa P50%)

Dscam2 ist für das „Pflaster“ der Medulla mit L1-Terminalien notwendig



Dscam2A und Dscam2B Spleißvarianten segregieren homophil in Zellkultur

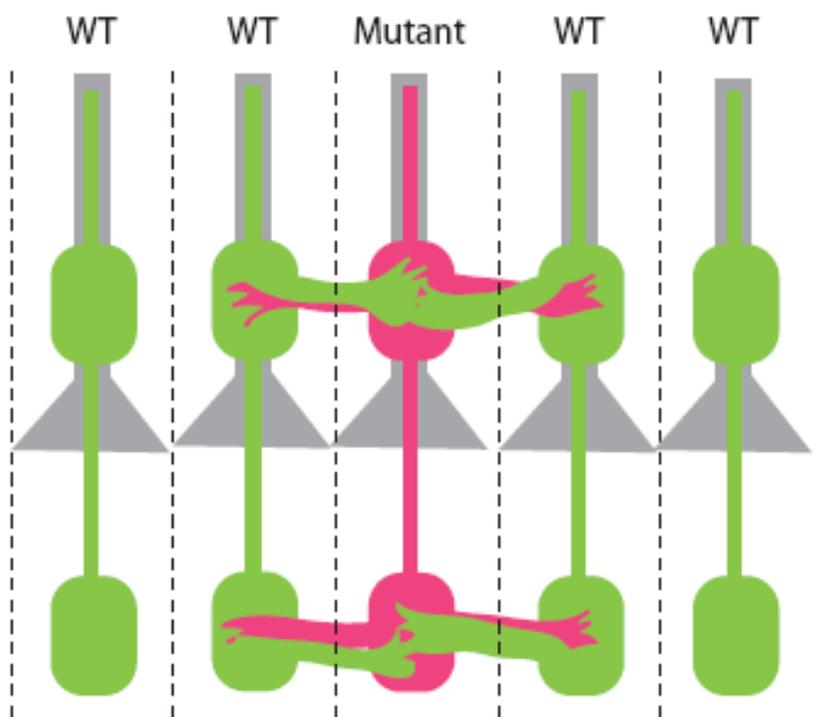




„Tiling“ ist ein sekundärer Effekt in der Entwicklung.

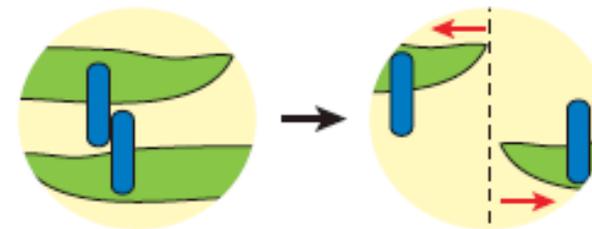
a Dscam2 regulates tiling in the fly visual system

Mature medulla columns



Tiling of L1 axons during development

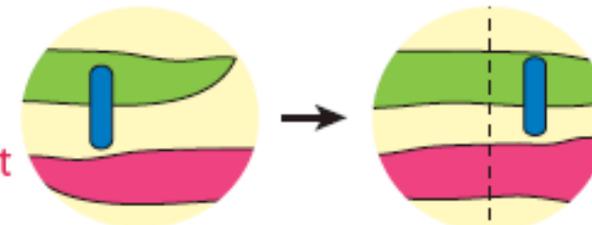
WT development



Dscam2 recognition

Repulsion

WT development adjacent to *Dscam2* mutant column



No recognition

No repulsion

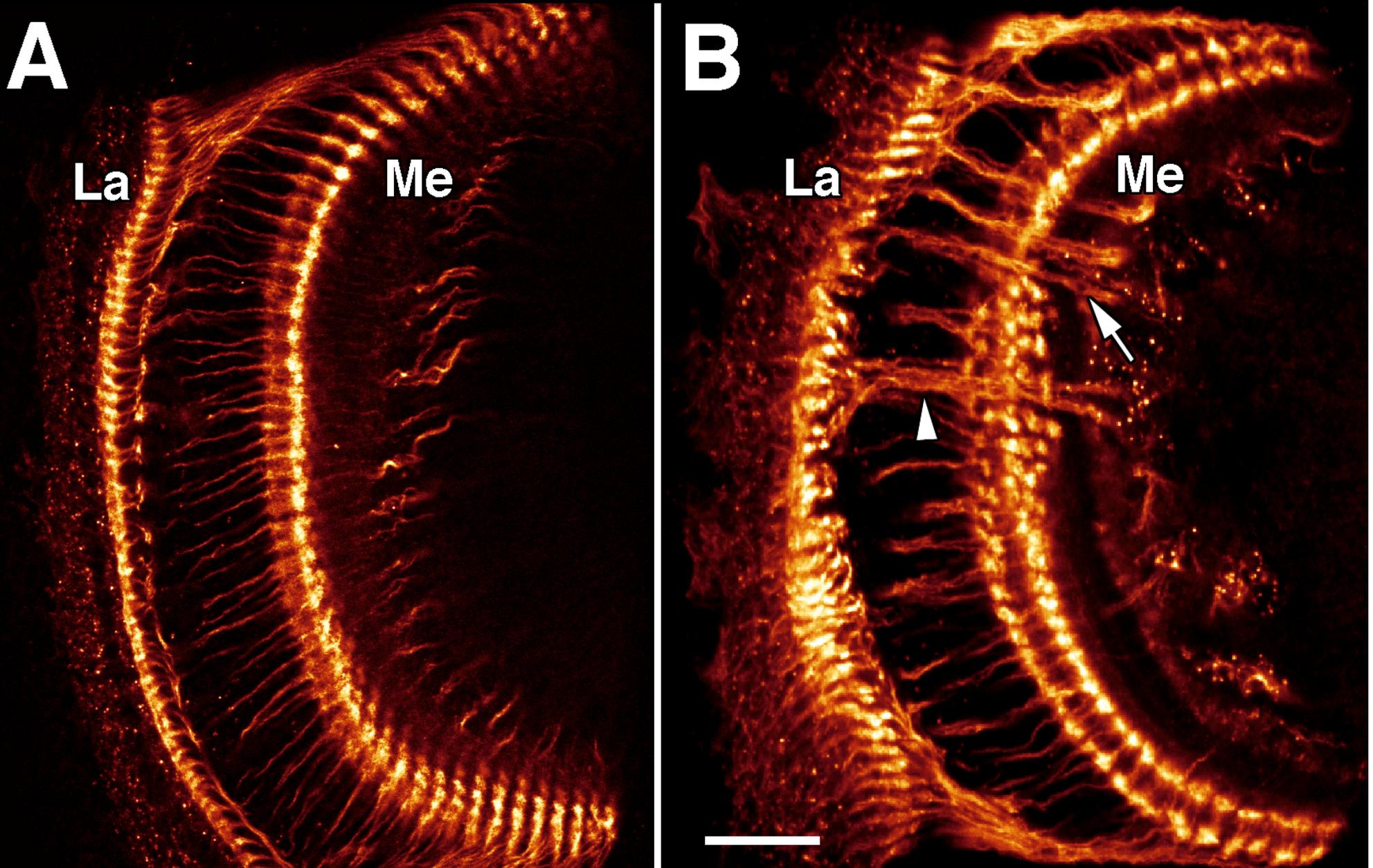
**Irreguläre optische Chiasmen,
ein häufiger mutanter Phänotyp bei Drosophila**



Phenotype of UB883 mutant (P-element mutagenesis)



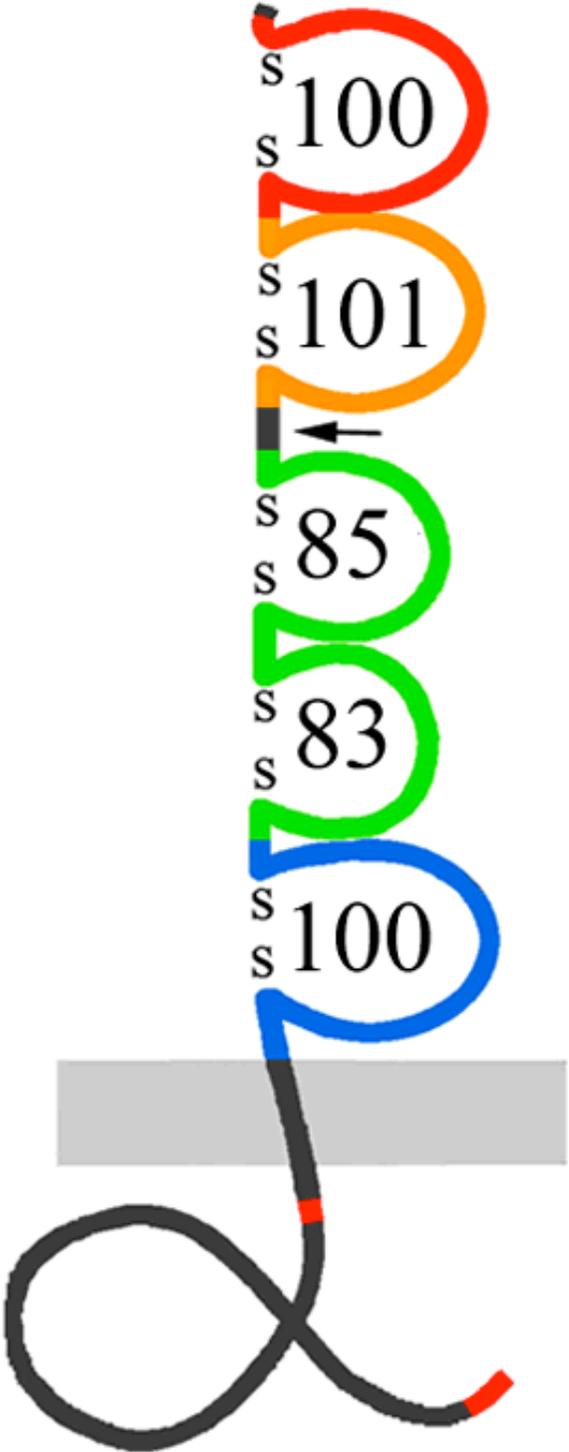
Wild type (A) and *UB883* mutant phenotype (B) visualized using fascicline II immunoreactivity in the midpupal brain



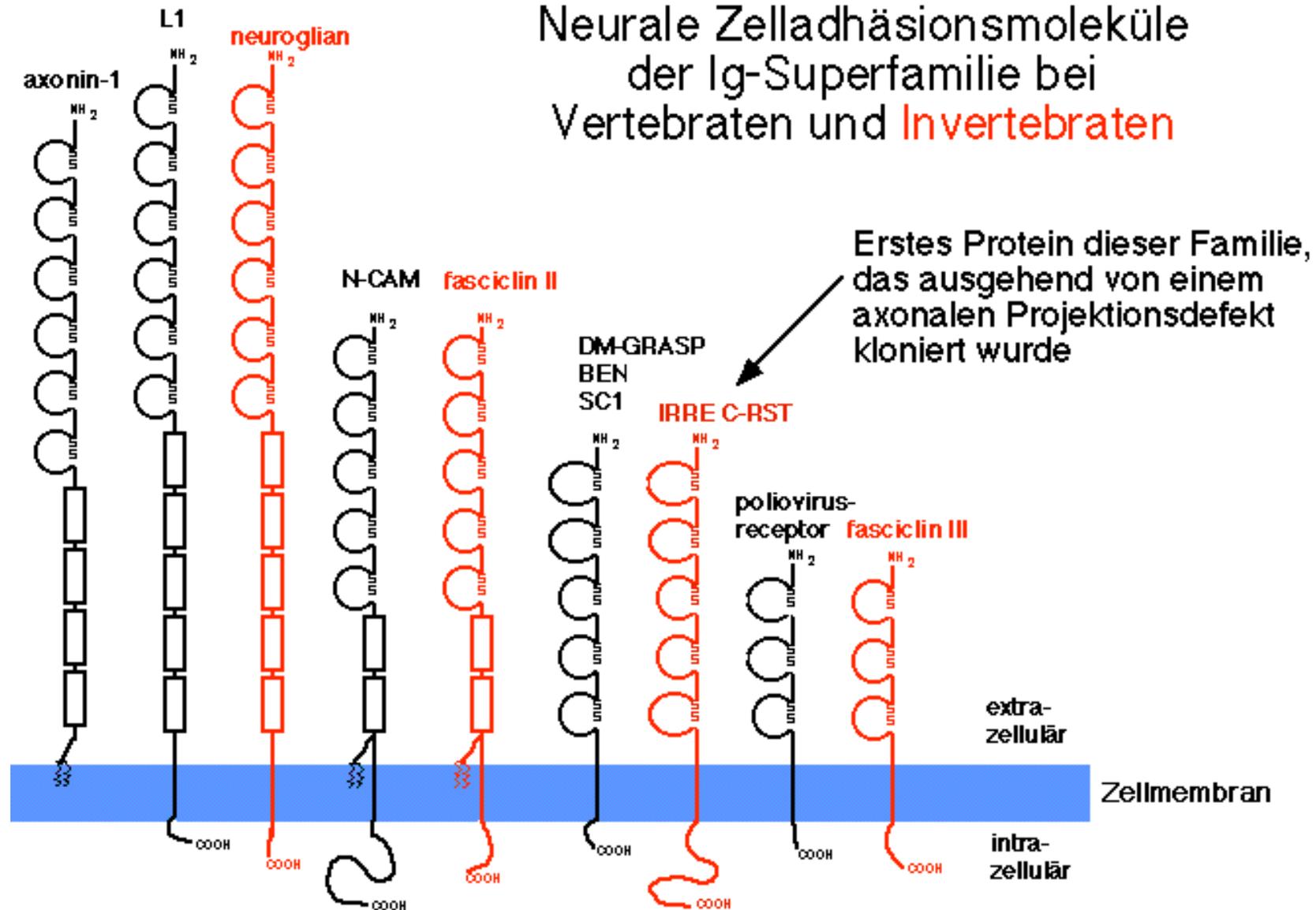
Struktur von IrreC-rst

5 extrazelluläre
Ig-Domänen

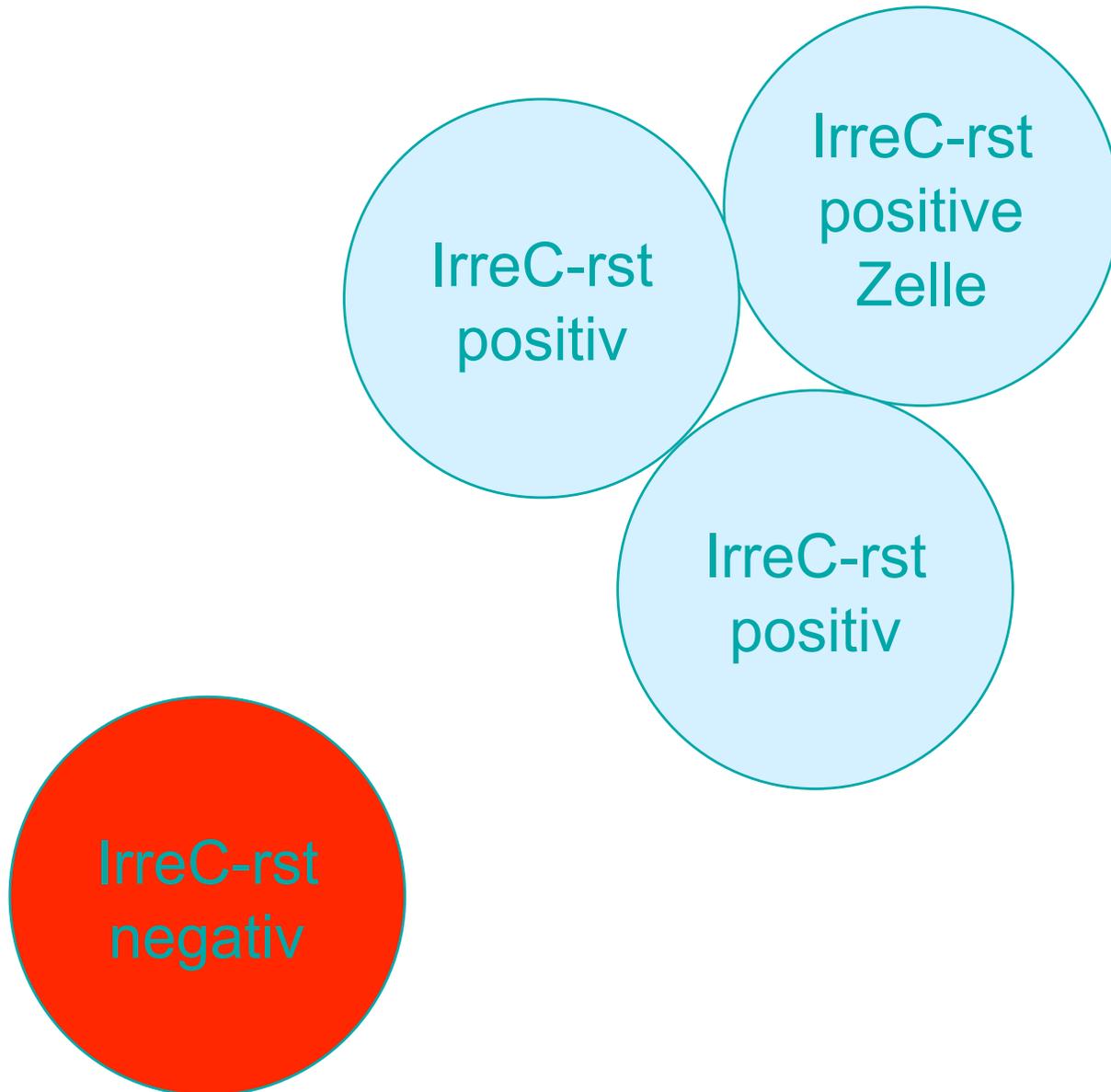
intrazelluläre Domäne



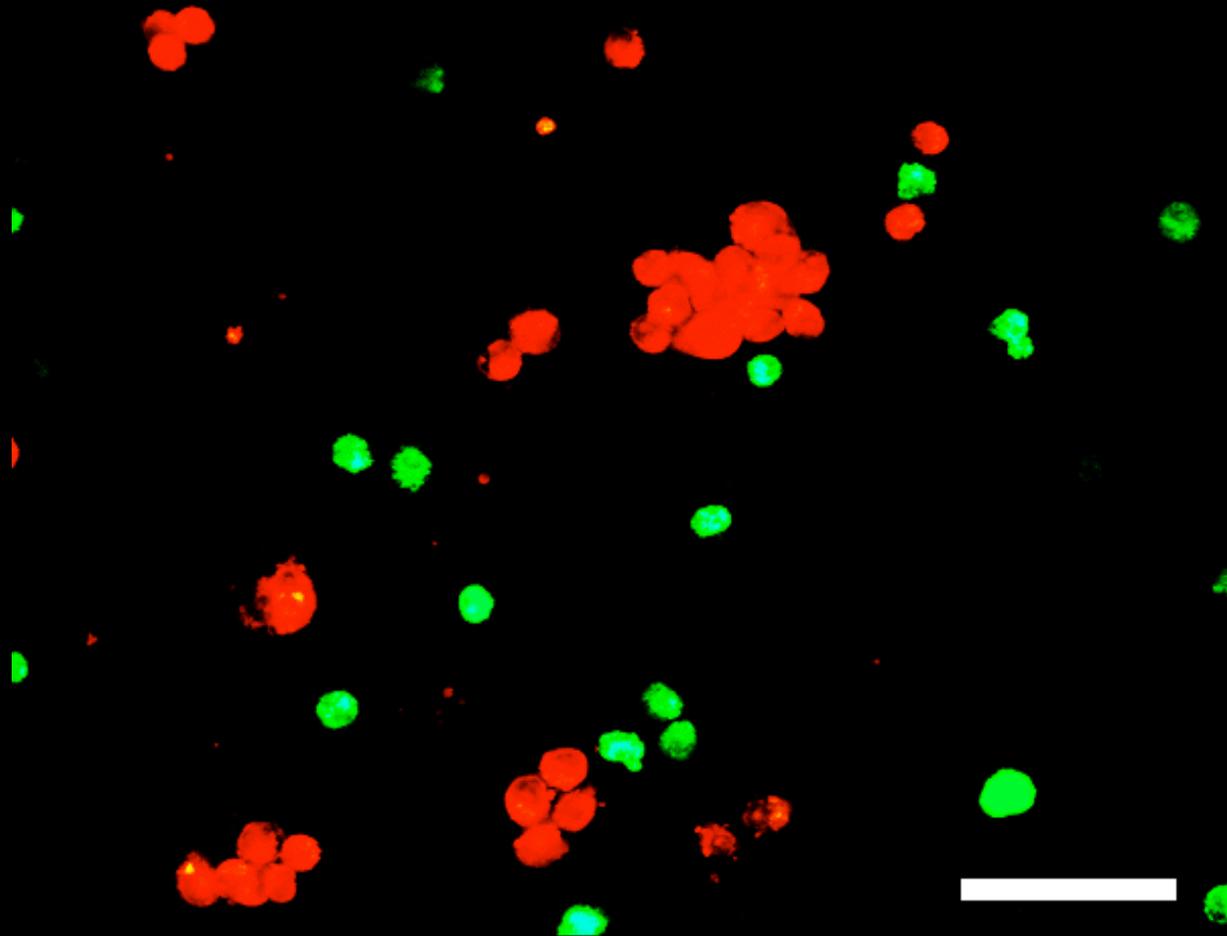
Neurale Zelladhäsionsmoleküle der Ig-Superfamilie bei Vertebraten und **Invertebraten**



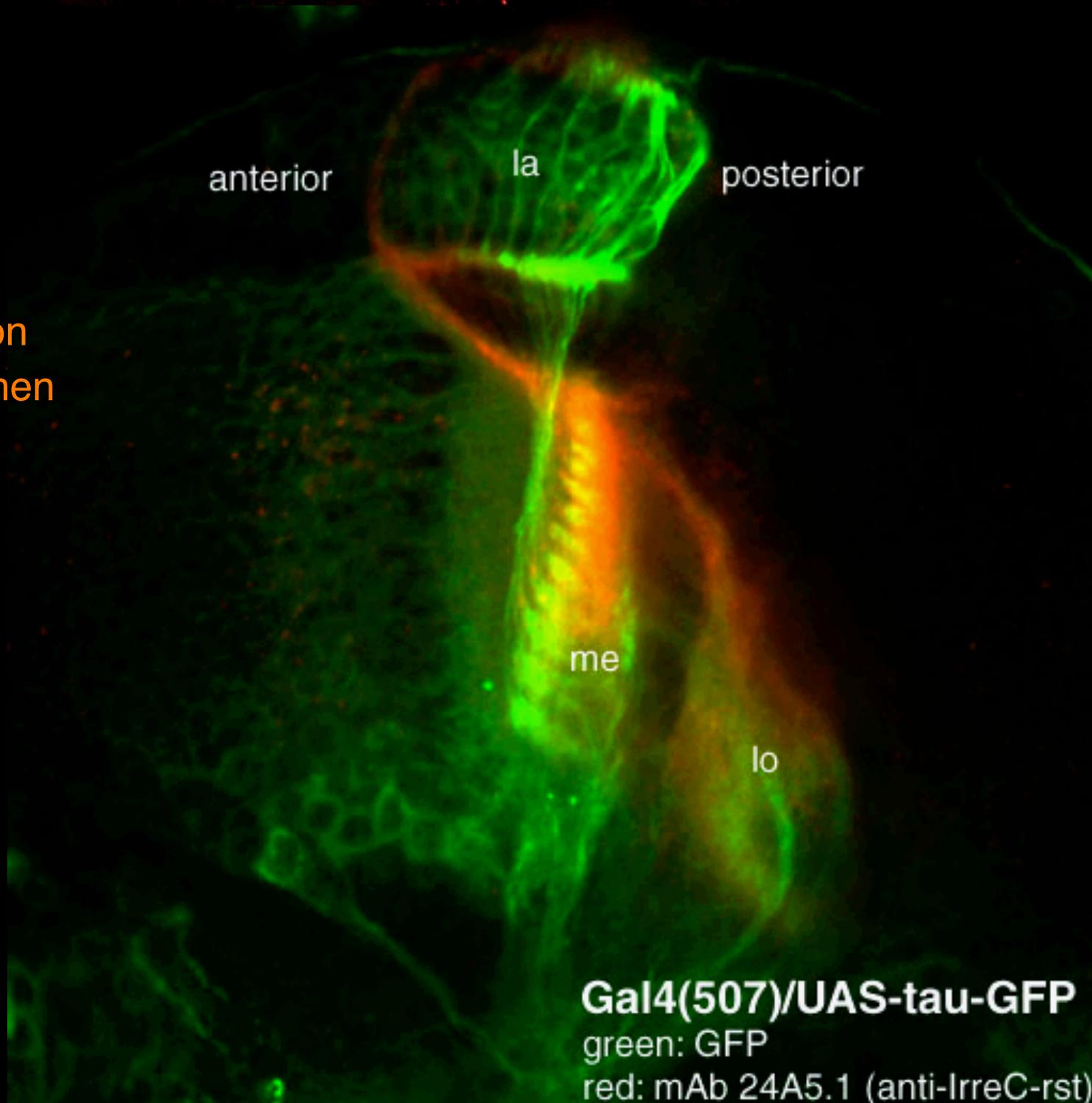
Zelladhäsionsmoleküle machen Zellen klebrig



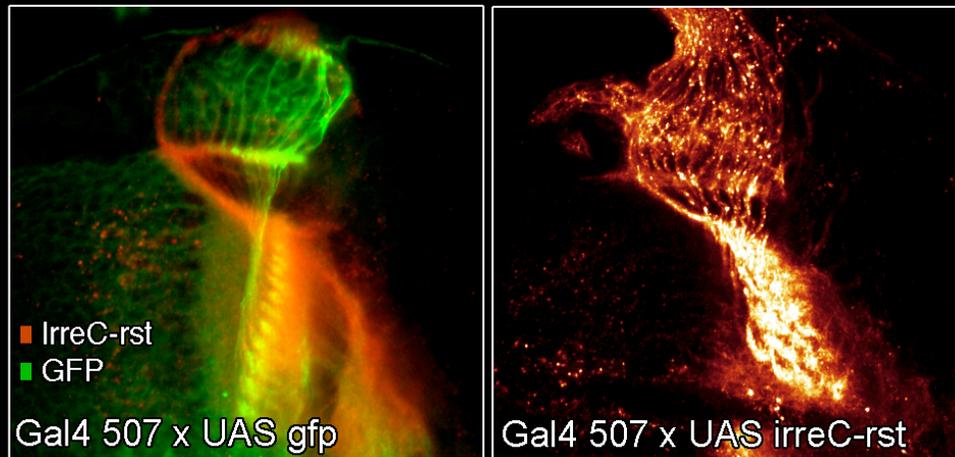
IrreC-rst vermittelt in S2-Zellen eine schwache homophile Interaktion



Larvale
Expression
im optischen
Lobus



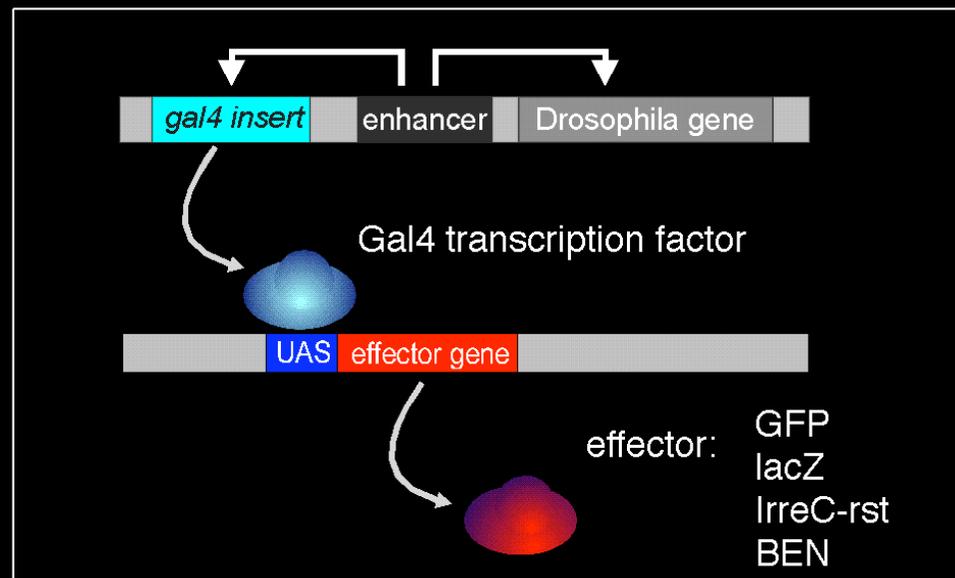
Specificity of IrreC-rst expression can be modified by use of the Gal4-UAS expression system



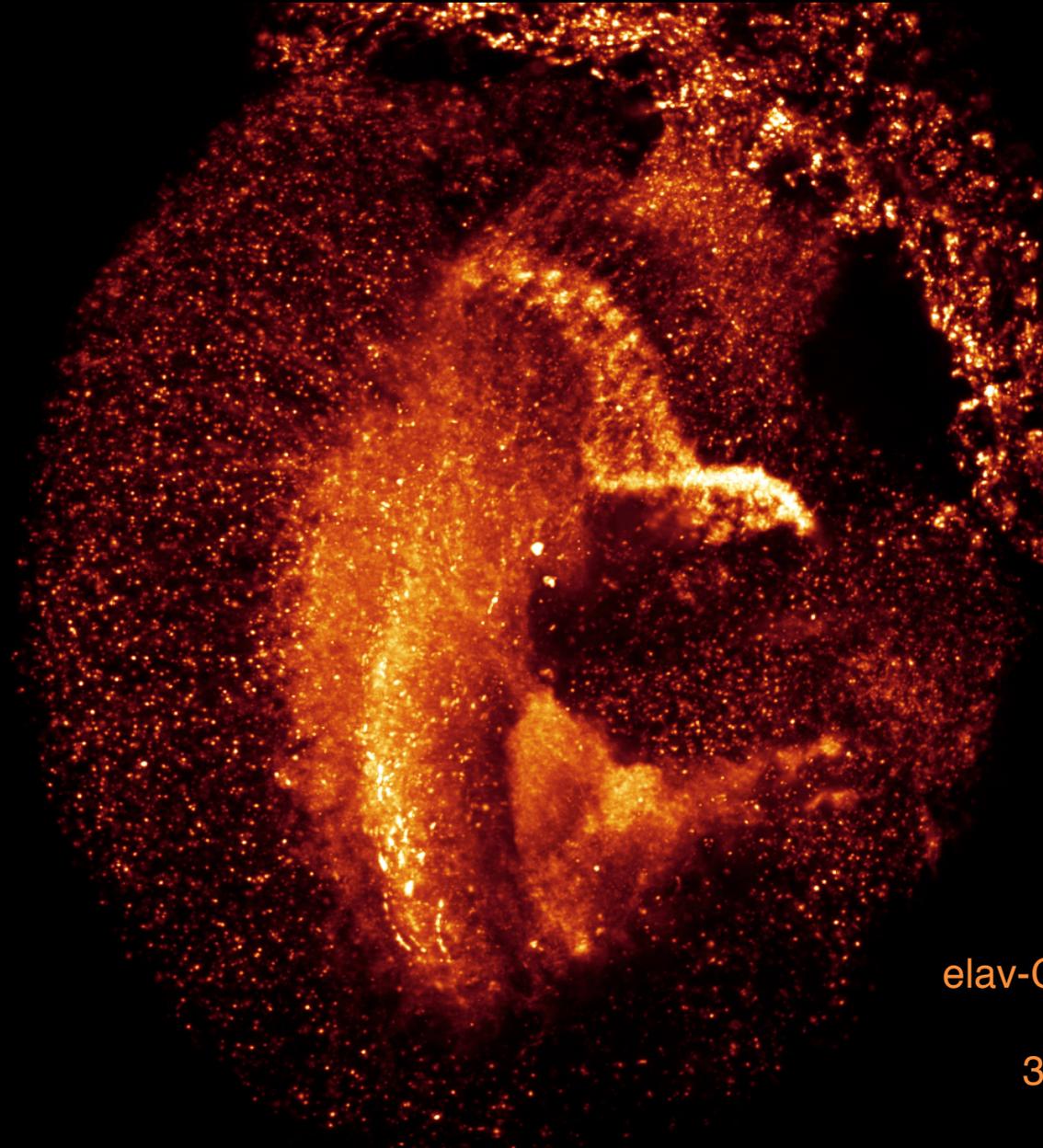
abolishment of temporal specificity by a Gal4 enhancer trap with complementary regulation



Use of different Gal4 expression patterns to establish a map of sensitivity for IrreC-rst expression in the nervous system



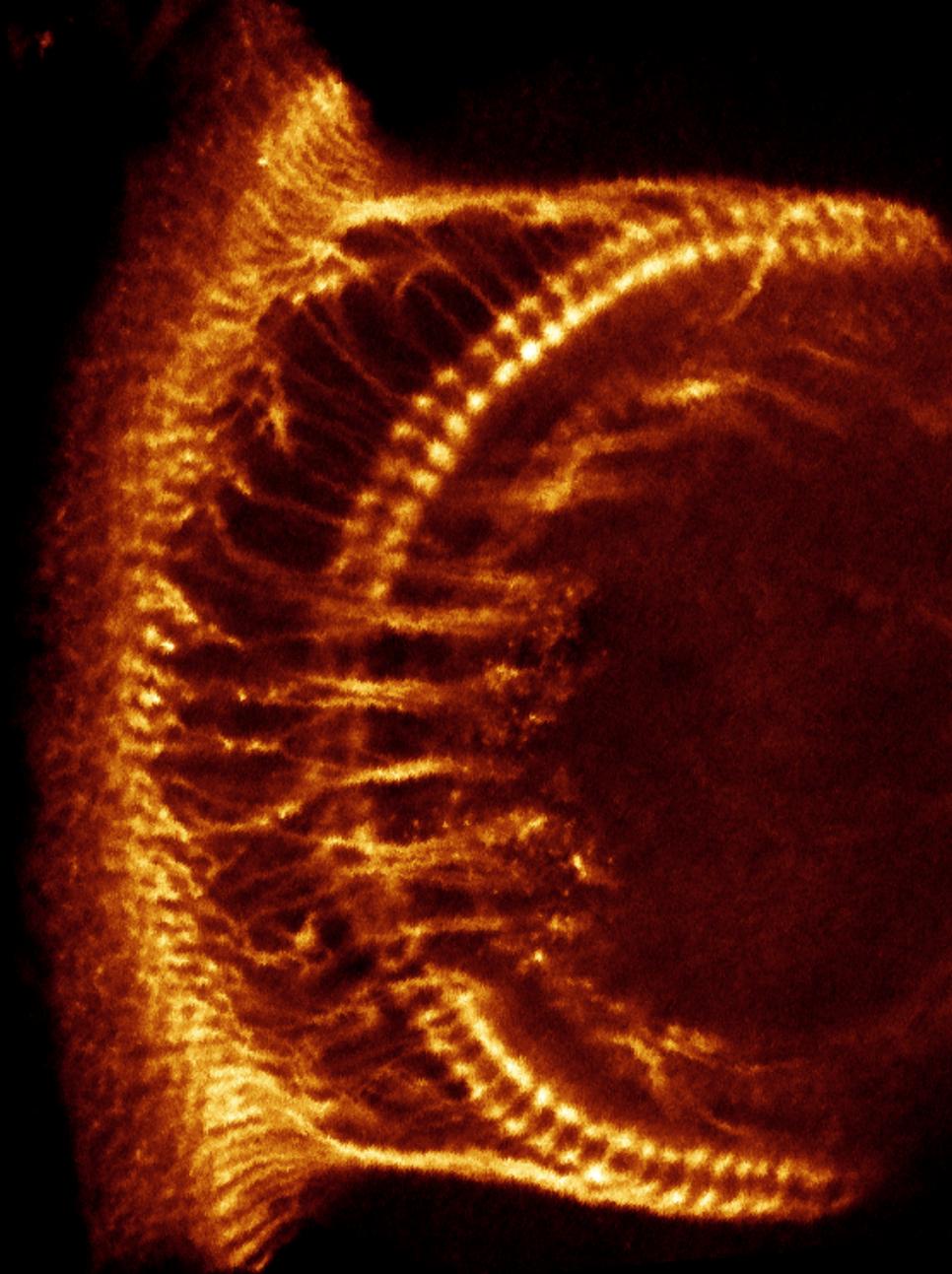
Eliminierung der IrreC-rst vermittelten Positionsinformation im optischen Lobus durch globale Expression



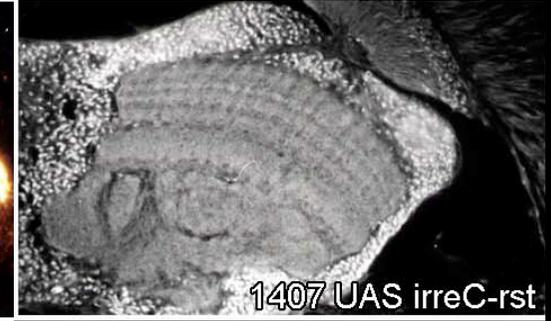
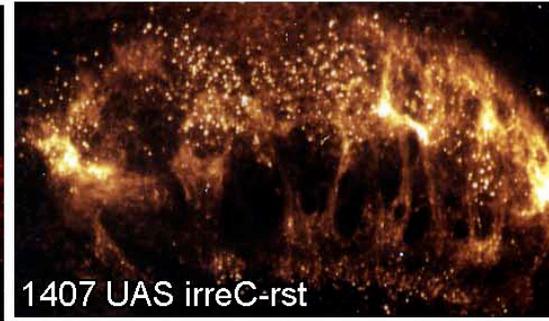
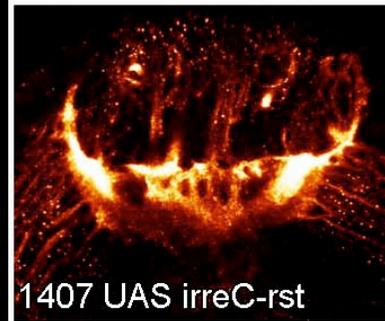
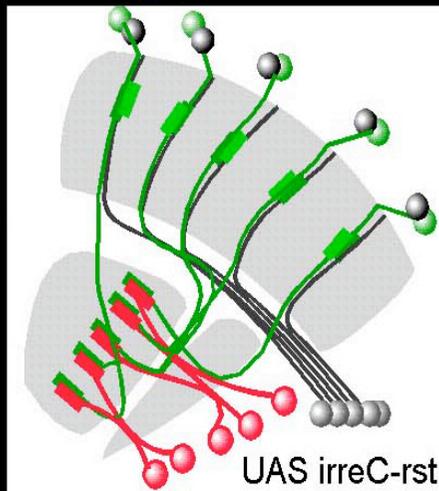
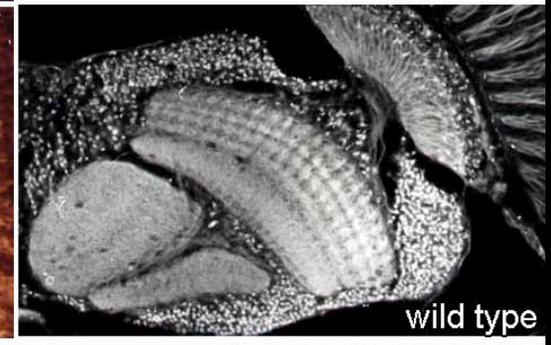
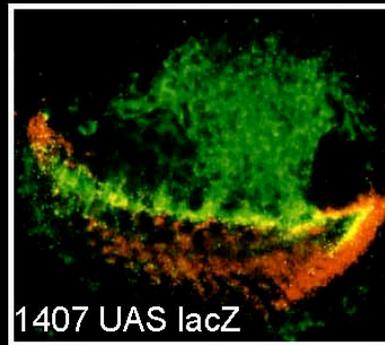
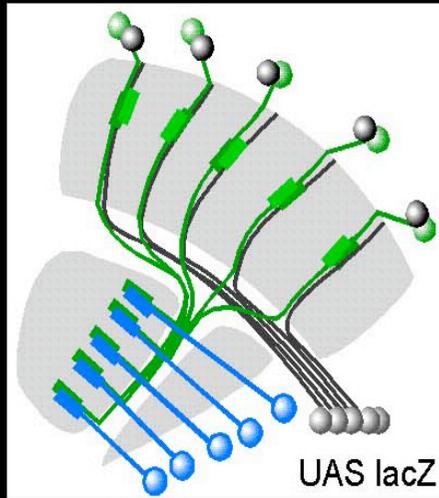
elav-Gal4/UAS-irreC-rst
anti-IrreC-rst
3rd instar larva

Eliminierung der IrreC-rst vermittelten Positionsinformation im optischen Lobus durch globale Expression führt zu einer Phänokopie des loss-of-function Phänotyps

elav-Gal4/UAS-irreC-rst
anti-fascicline II
Puppe 50%



Expansion of IrreC-rst expression to a new cell population causes massive axonal misprojections



larva

pupa

adult

expression of

■ reporter gene (lacZ)

■ effector gene (irreC-rst)

Targeting of IrreC-rst misexpression to the forming inner chiasm causes irregular fasciculation patterns and selectively disturbs neuropil organization of the lobula complex. The outer chiasm is left unaffected.

Augenentwicklung Teil II

Ommatidienentwicklung und Ausbildung des biologischen Kristalls „Komplexauge“

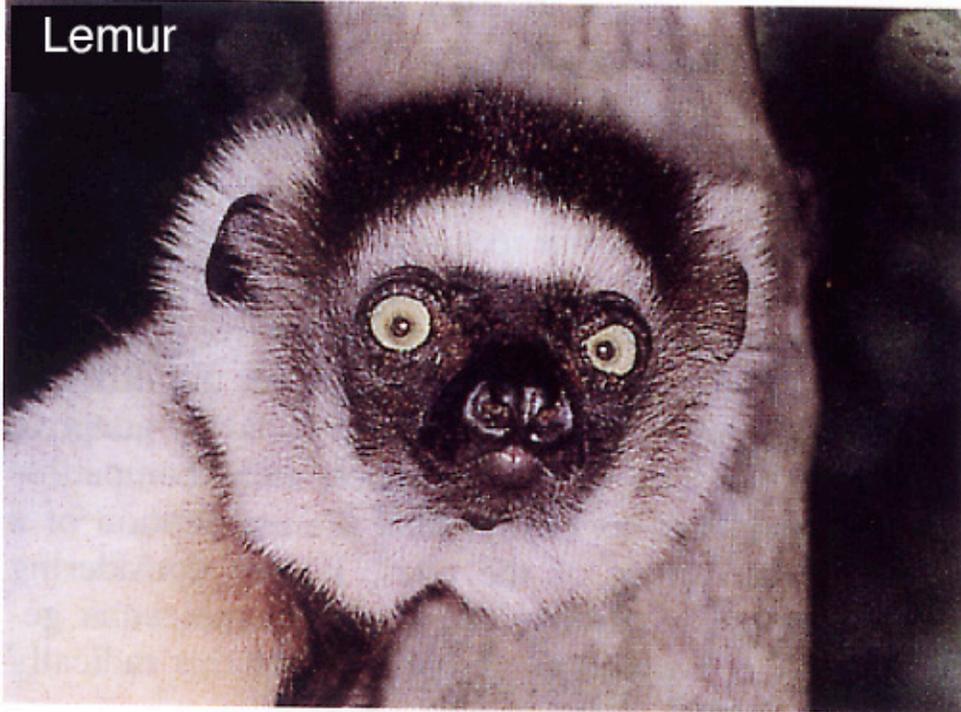
Augenentwicklung

bei Drosophila

Wo nachzulesen?

- www.zum.de/neurogenetik
- Kapitel 34 im Seyffert 1. Auflage
- Kapitel 29 im Seyffert 2. Auflage

Lemur



Gottesanbeterin



Cephalopode

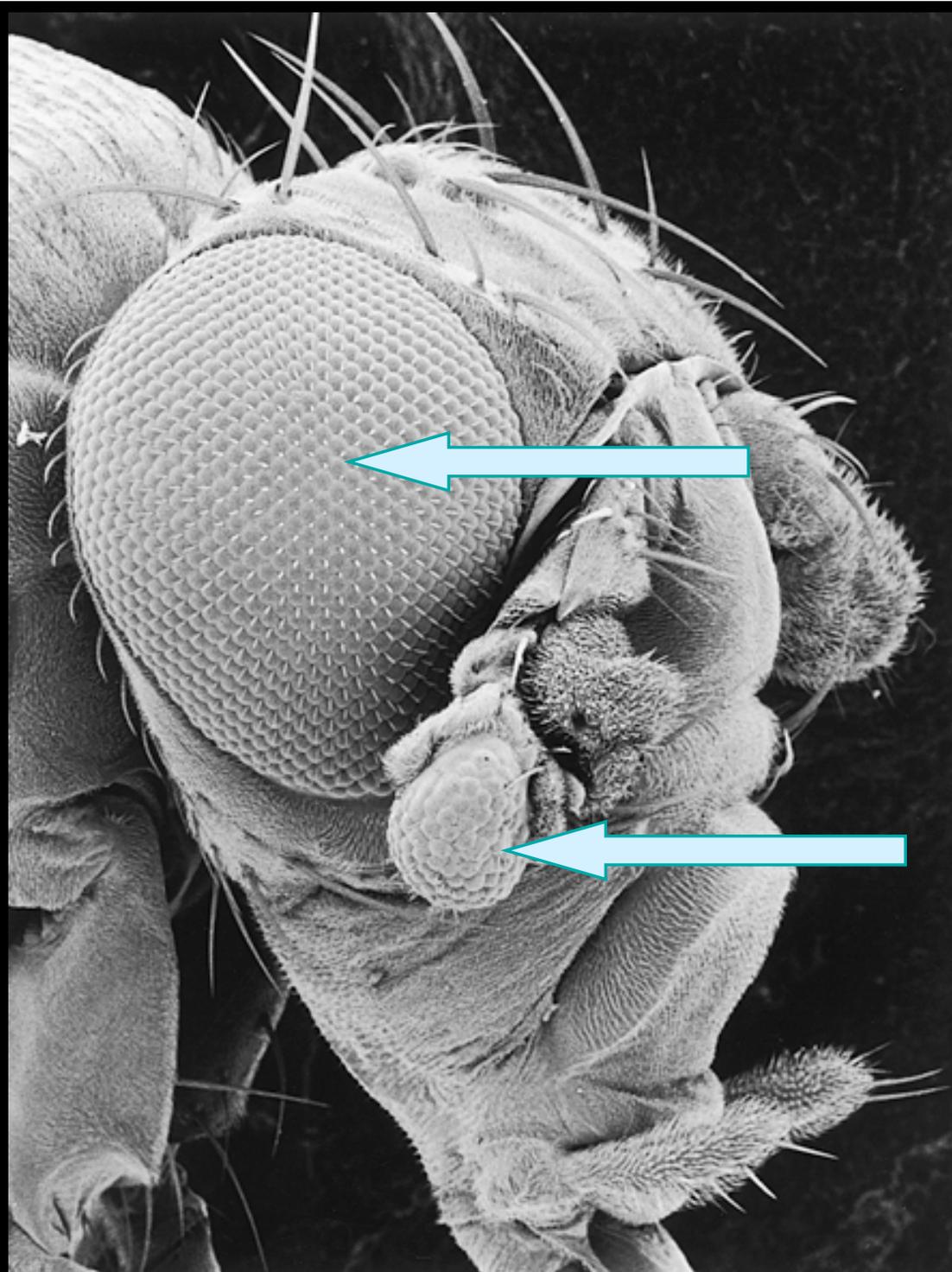


Muschel



Ohne *eyeless* keine Komplexaugen

Das *eyeless*-Gen wurde im Labor von W. Gehring in Basel isoliert und gehört zu einer Familie von Transkriptionsfaktoren, die sowohl eine **Homöobox** als auch eine **Paired Box** aufweisen

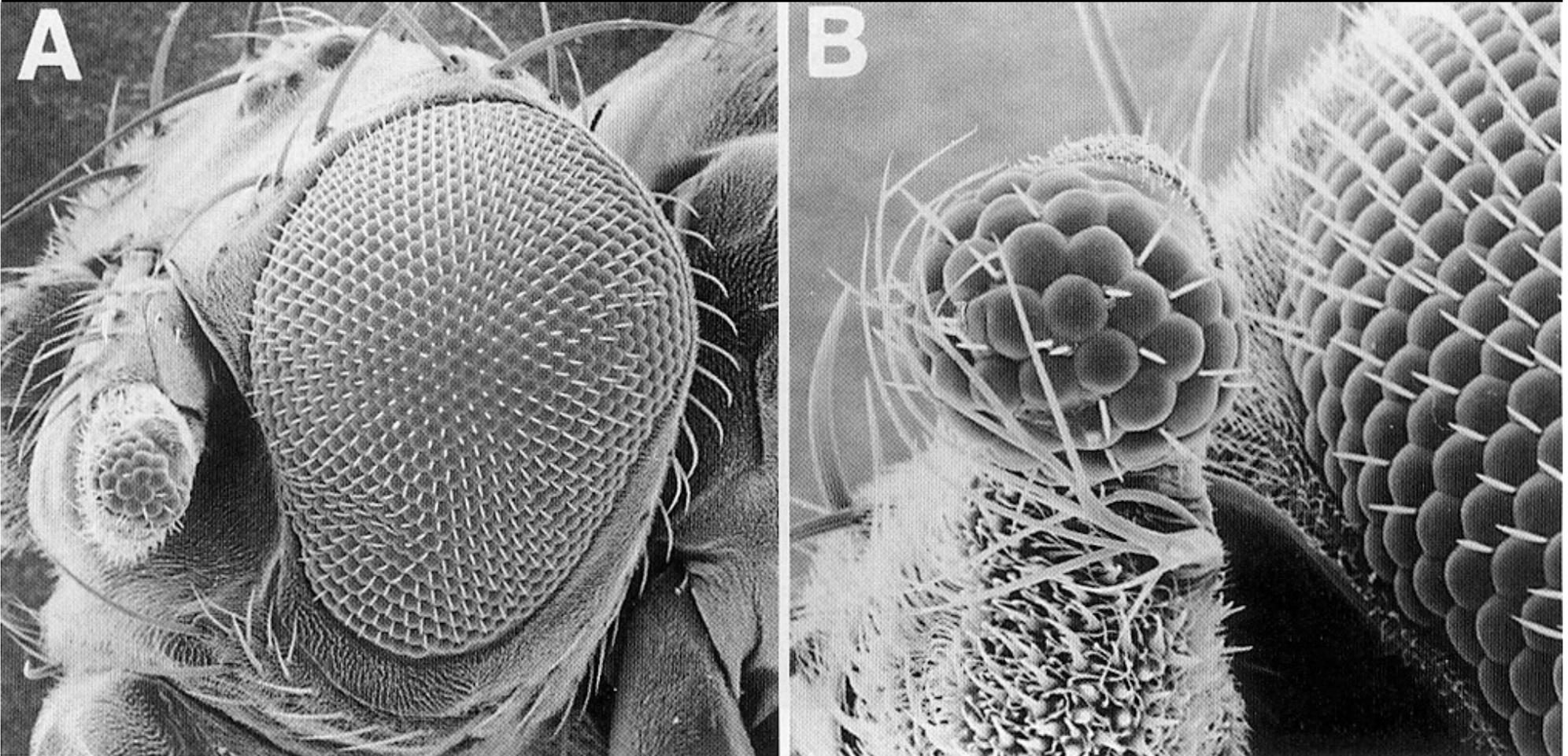


Das Komplexauge von Drosophila

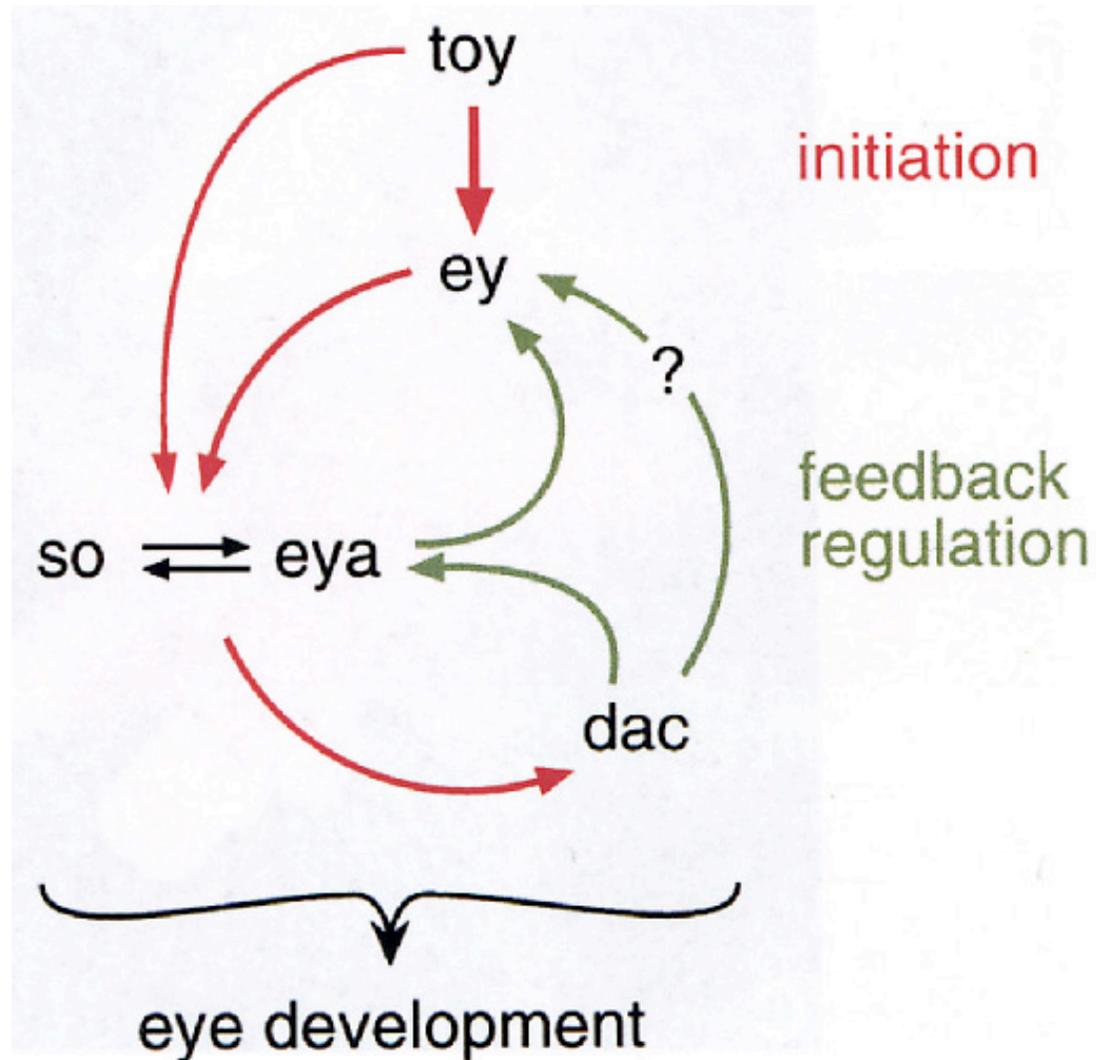
Transformierte Antenne durch die Fehlexpression des *eyeless*-Gens

Ektopisches Antennenaug durch Expression des *Small eye* Gens der Maus

Small eye = *Pax 6* (Vertebratenortholog von *eyeless*)



Master Kontrollgene der Augenentwicklung Bei Drosophila



Das primitive Zwei-Zellauge

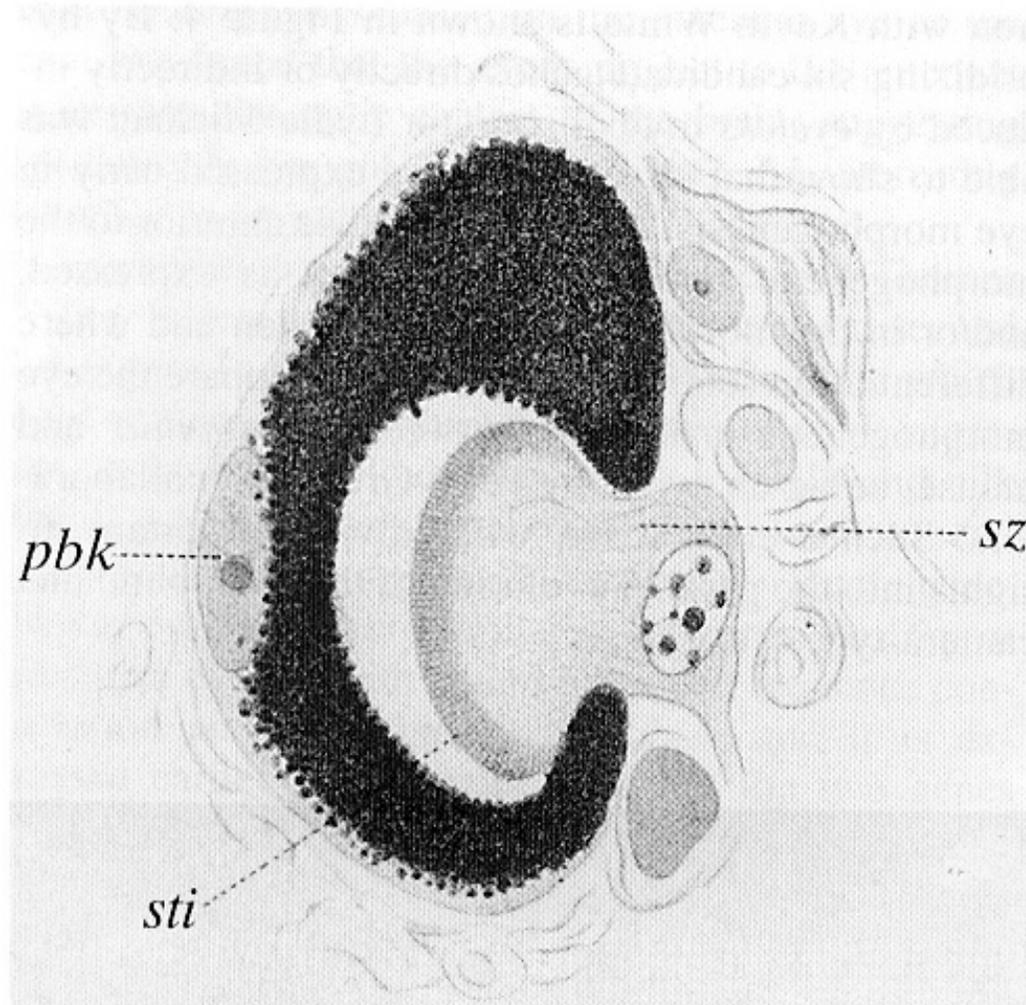
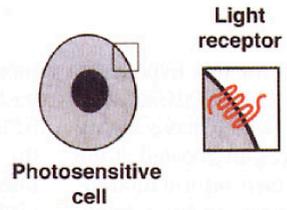
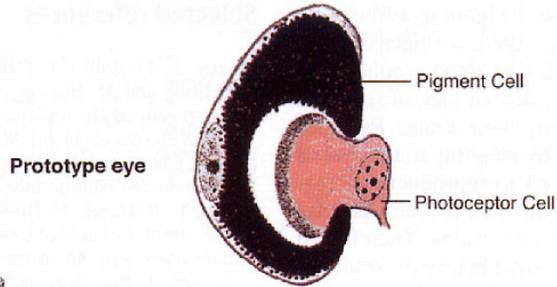


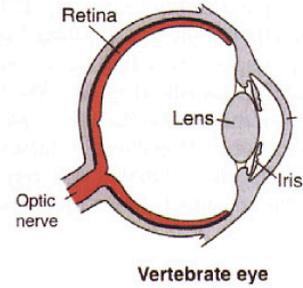
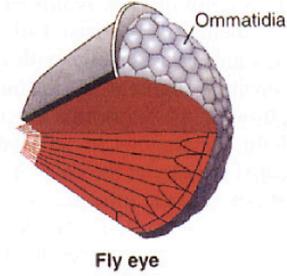
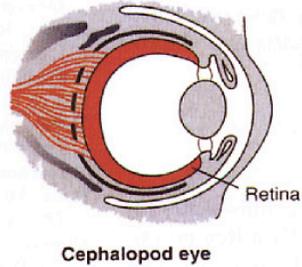
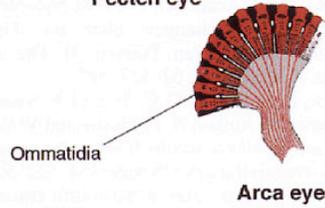
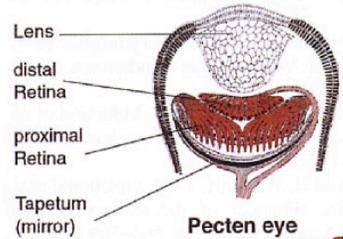
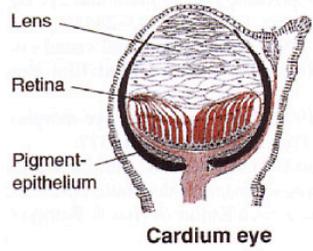
Fig. 5. Histological section across the eye of *Planaria torva* (after Hesse, 1897). sti: microvilli; sz: photoreceptor cell; pbk: pigment cell nucleus. From Hesse (1897).



Pax-6 ?

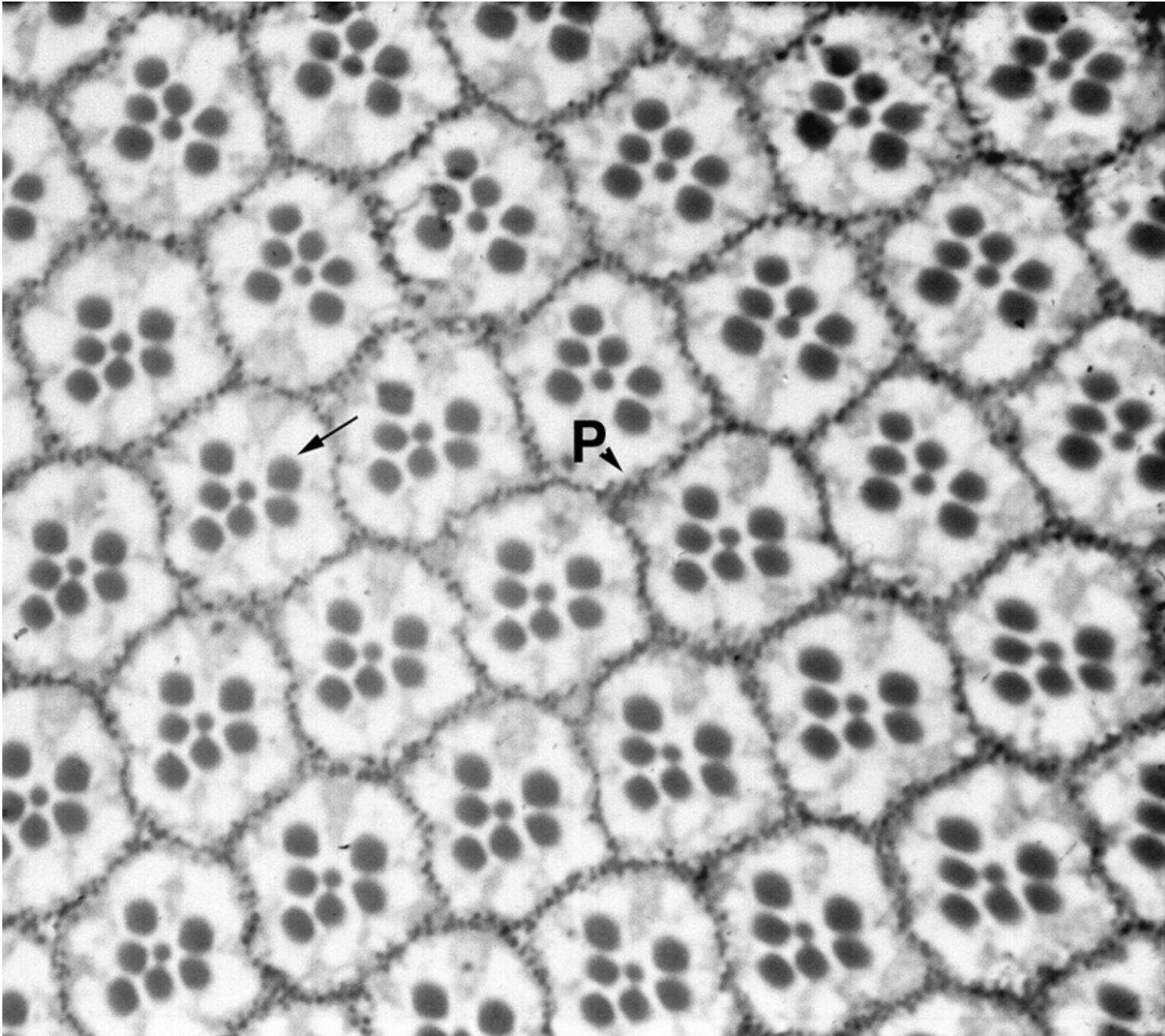


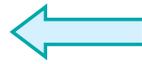
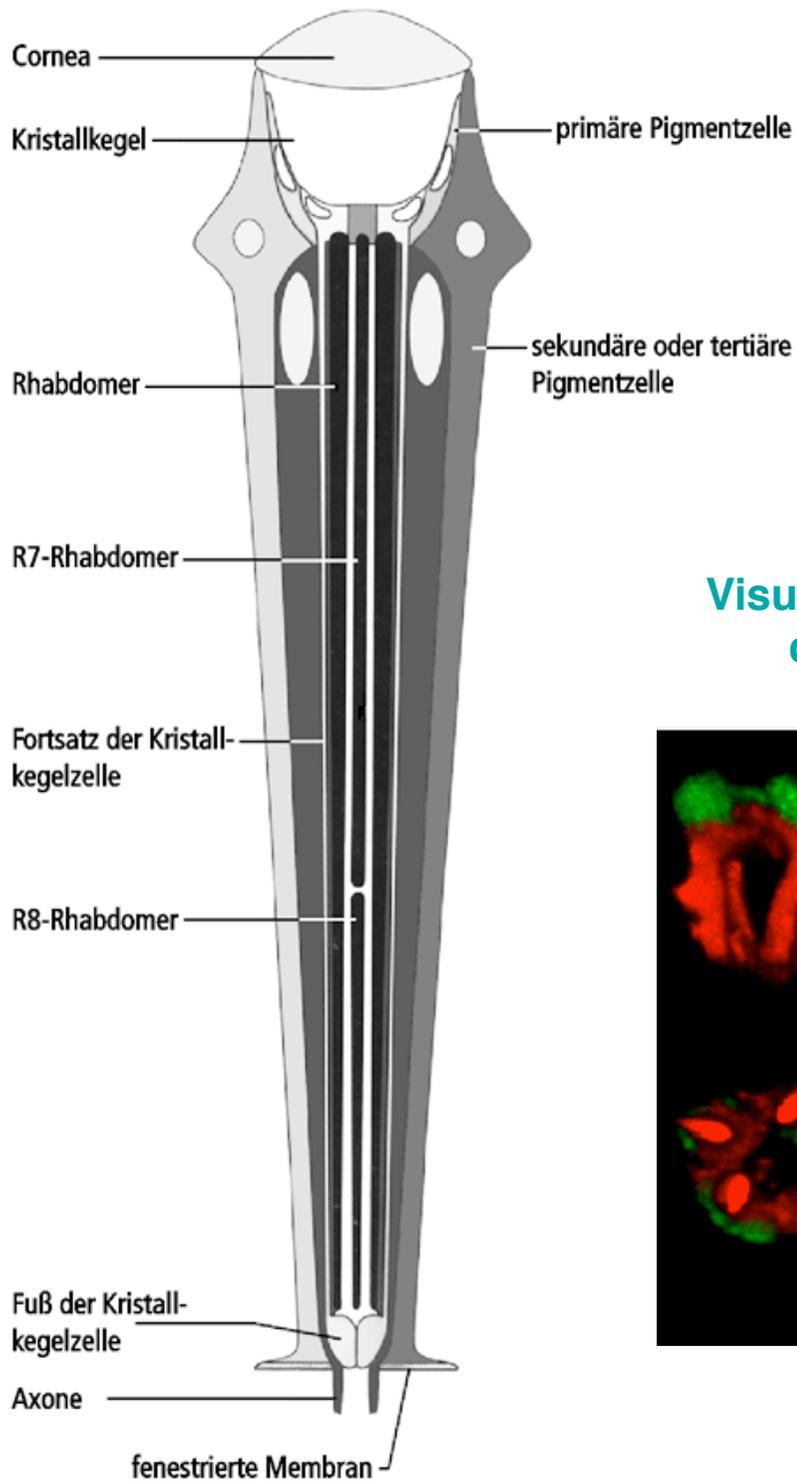
Die Augenevolution ist monophyletisch



Molluscs

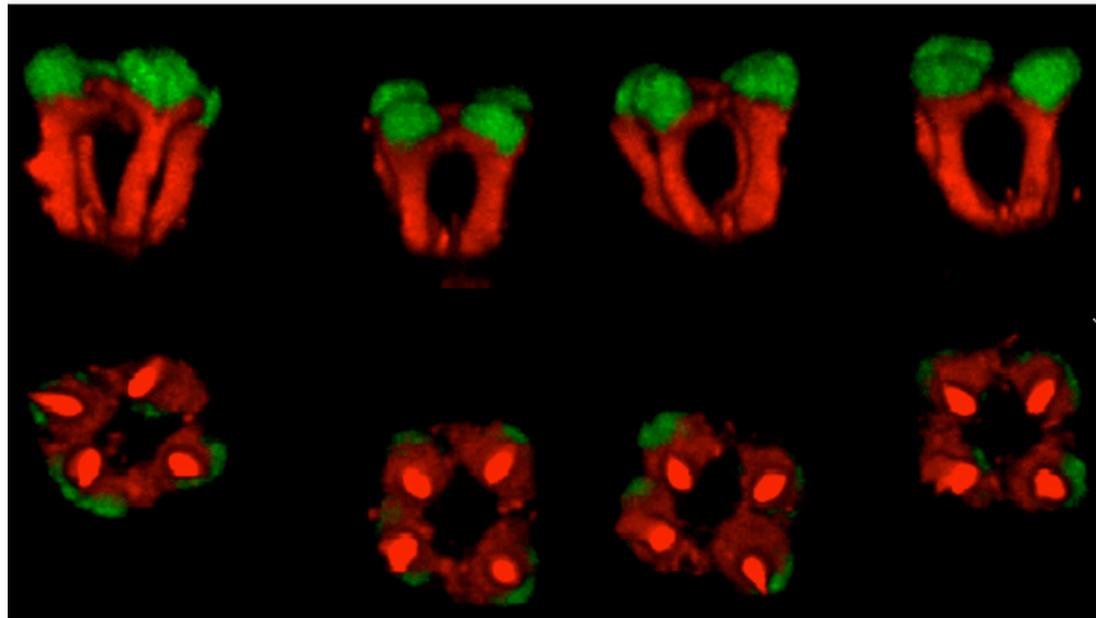
Querschnitt durch das Komplexauges von Drosophila



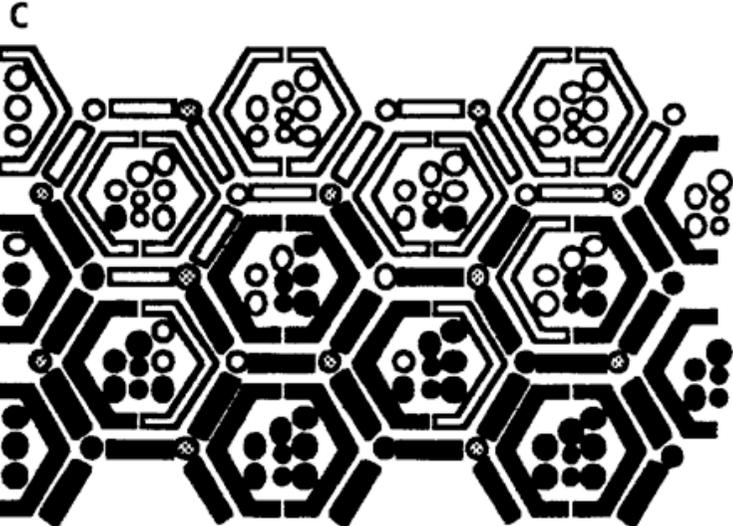
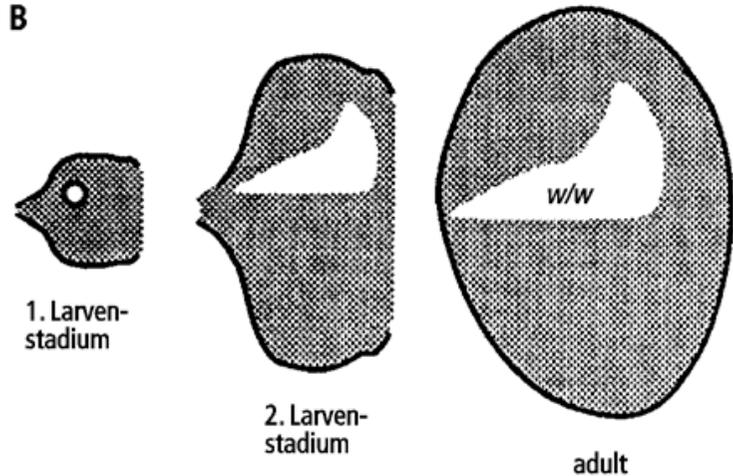
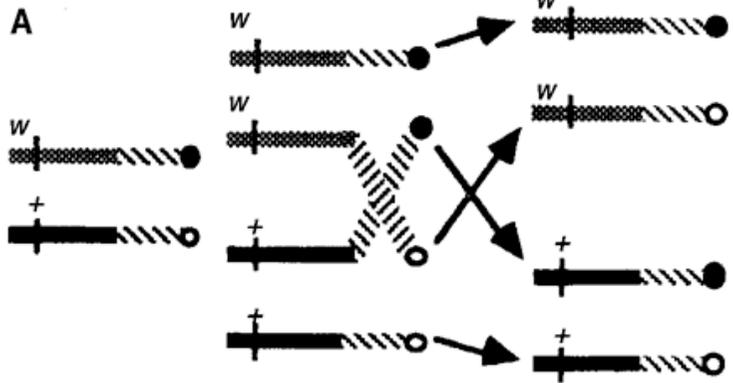


Längsschnitt durch ein adultes Ommatidium von *Drosophila*

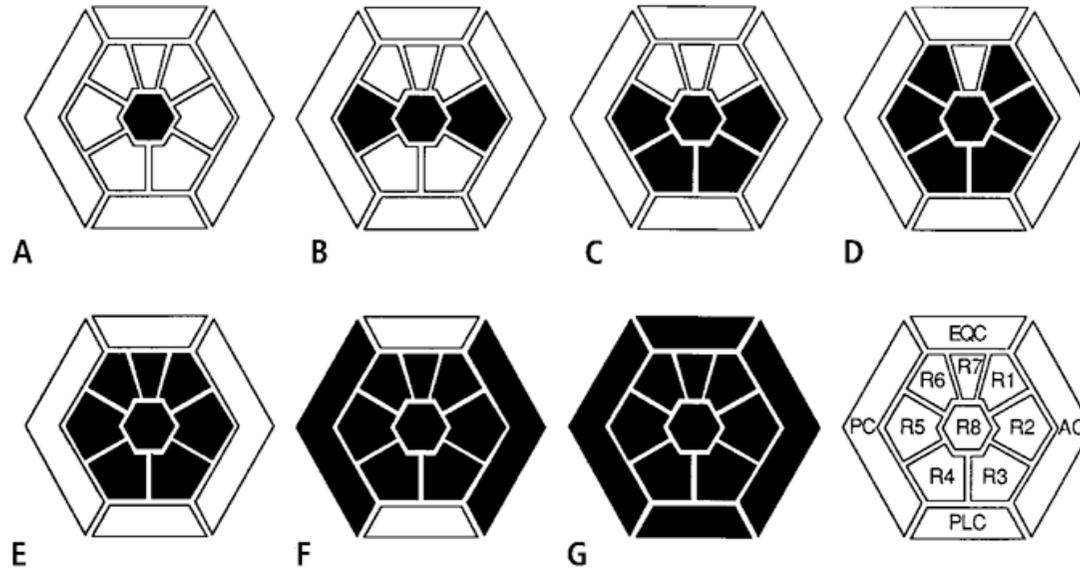
Visualisierung der Kristallkegelzellkerne mit anti-cut und der Kristallkegelzellfüße mit anti-Neuroglian im Puppenstadium



Ein Ommatidium ist kein Klon



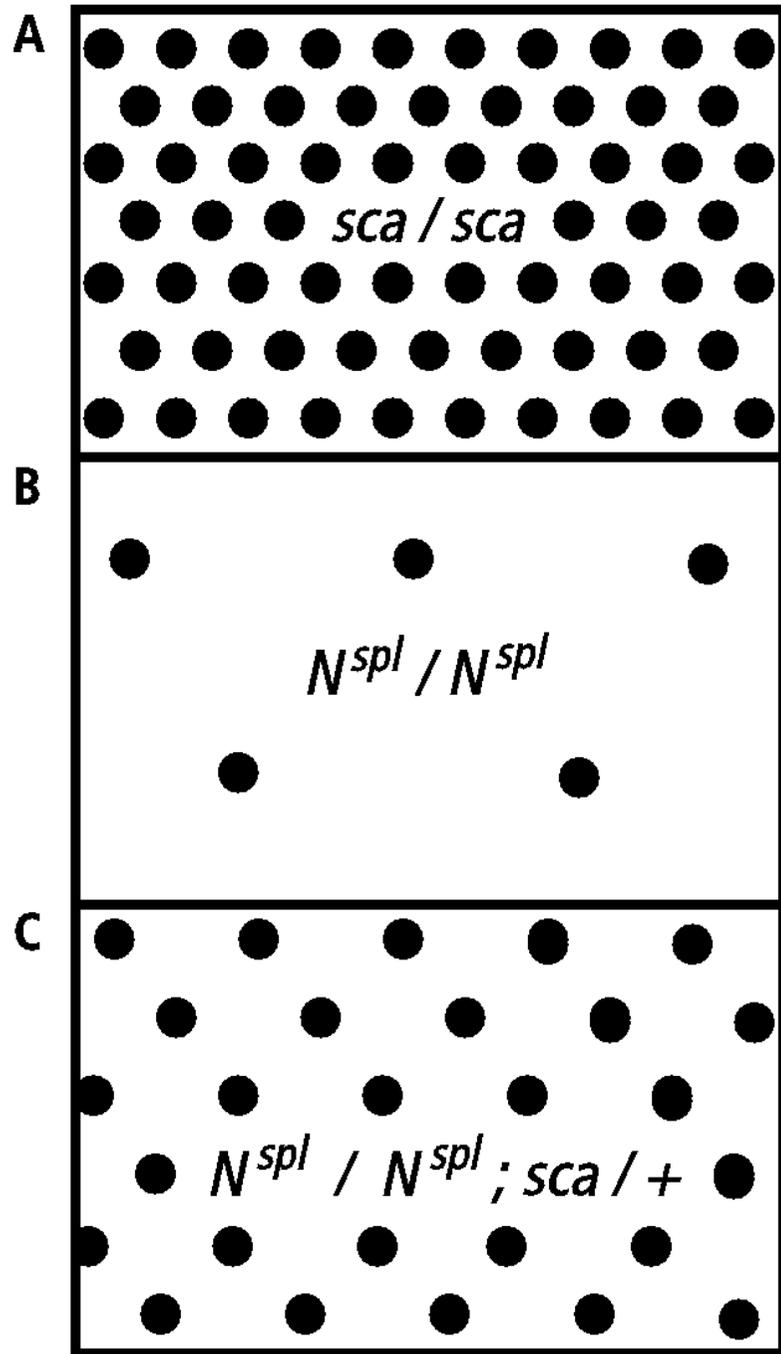
Es gibt eine feste zeitliche Reihenfolge der Zelldifferenzierung



Die R8-Zelle rekrutiert umliegende Zellen
zum Aufbau eines Ommatidiums

R8 -> R2/R5 -> R3/R4 -> R1/R6 -> R7 ->
-> Kristallkegelzellen

Inhibition der Induktion von R8-Zellen.
Variation eines Themas: **laterale Inhibition**



R8-Dichte ist erhöht in
sca-Mutanten



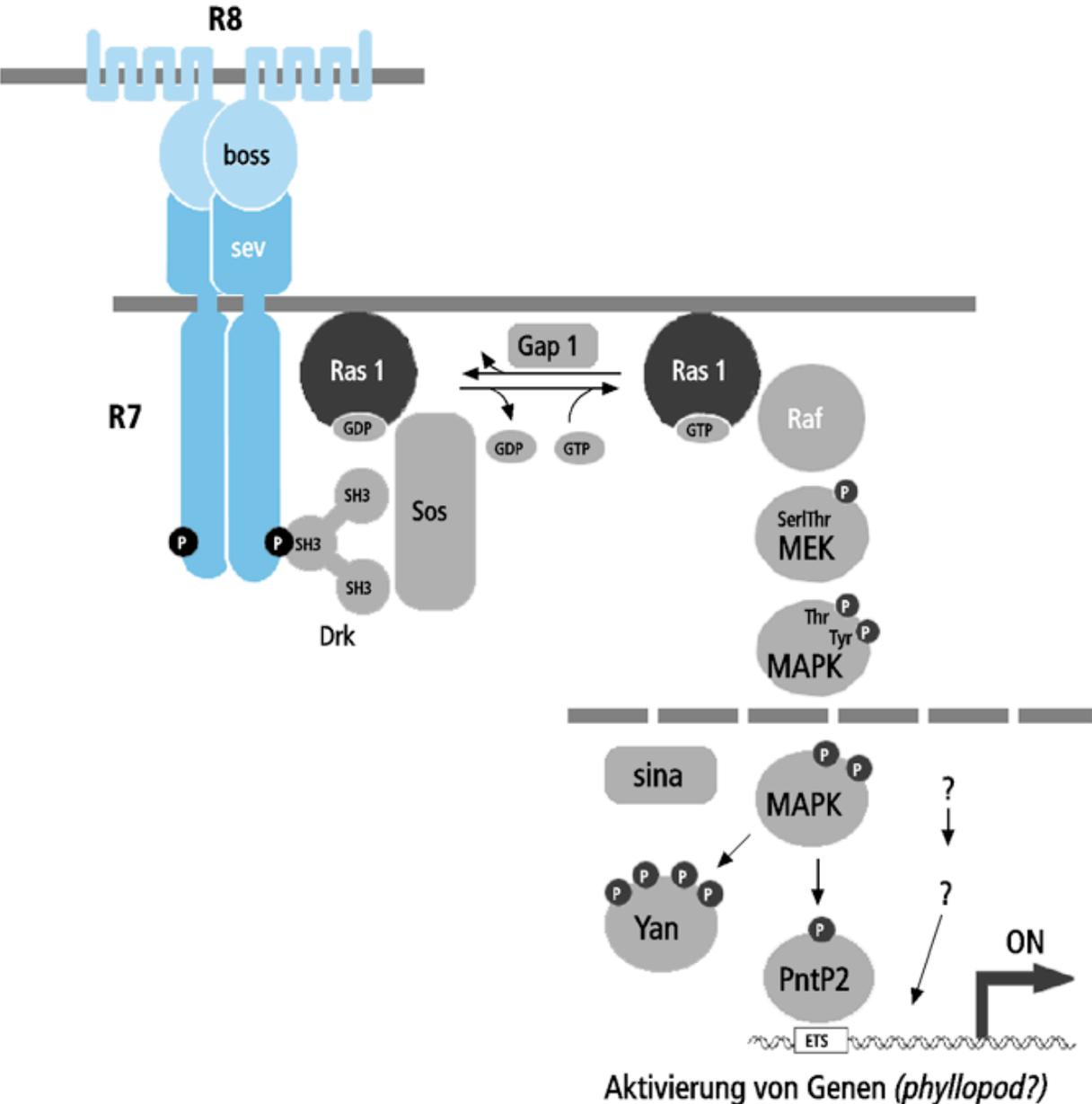
R8-Dichte ist erniedrigt in
gain-of-function *Notch*-
Mutante



sca ist suppressor von
N(*spl*)

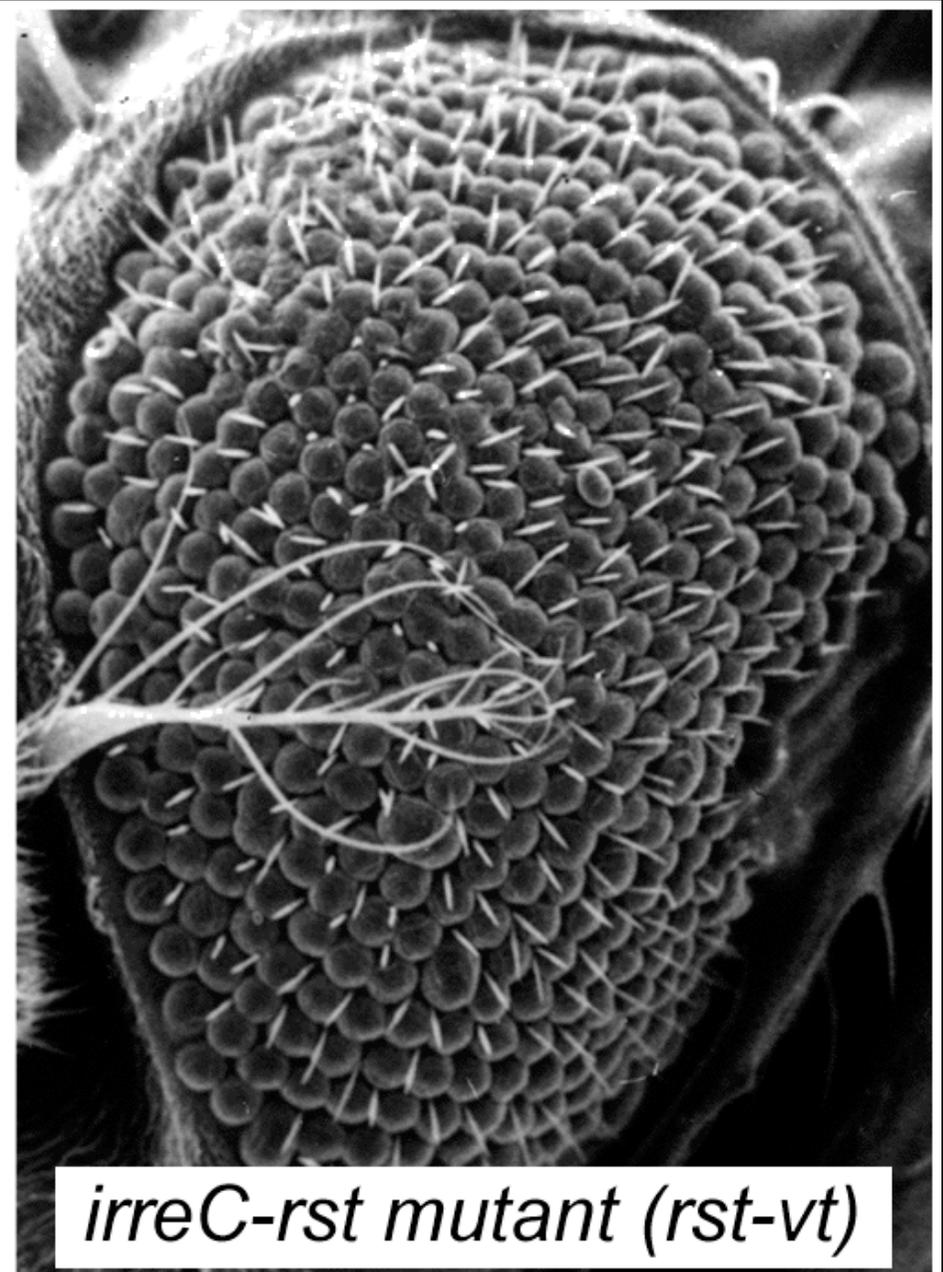
Was sind die Mechanismen der Zellrekrutierungen?

Der *sevenless* Signaltransduktionsweg als Beispiel



neurales Schicksal

IrreC-rst Funktion bei der Augenentwicklung



Entwicklung des Komplexauges von Drosophila

Endphase: Die Bildung des biologischen Kristalls

WISSENSCHAFTLICHE LEITUNG

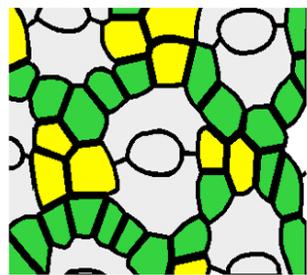
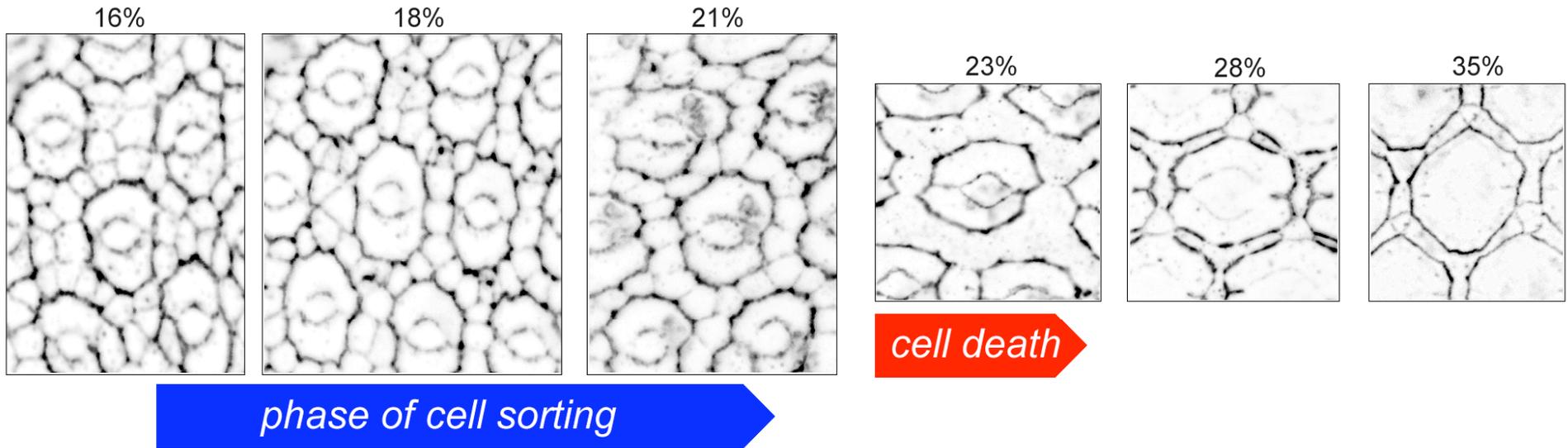
**Prof. Dr. Karl-Friedrich Fischbach
Dipl. Biol. Bernhard Bonengel**

REALISIERUNG

yoose3D.com

Expression of IrreC-rst protein during eye development

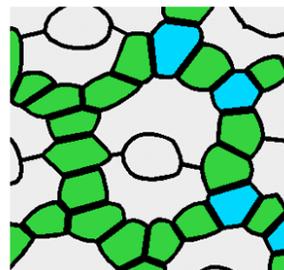
pupal stages p16% - p35%



*repositioning
of cells*

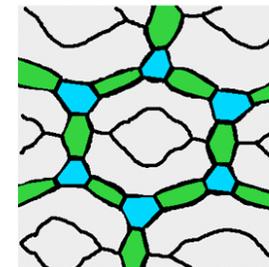


of contacts to
ommatidium



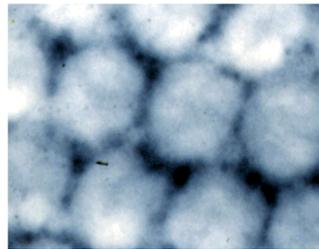
15 x 800 IOC

*reduction of
cell number*

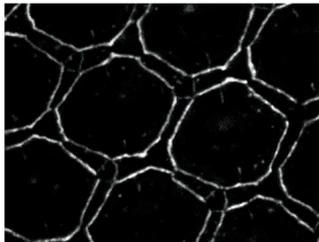


12 x 800 IOC

Evidence for a heterophilic ligand in the eye

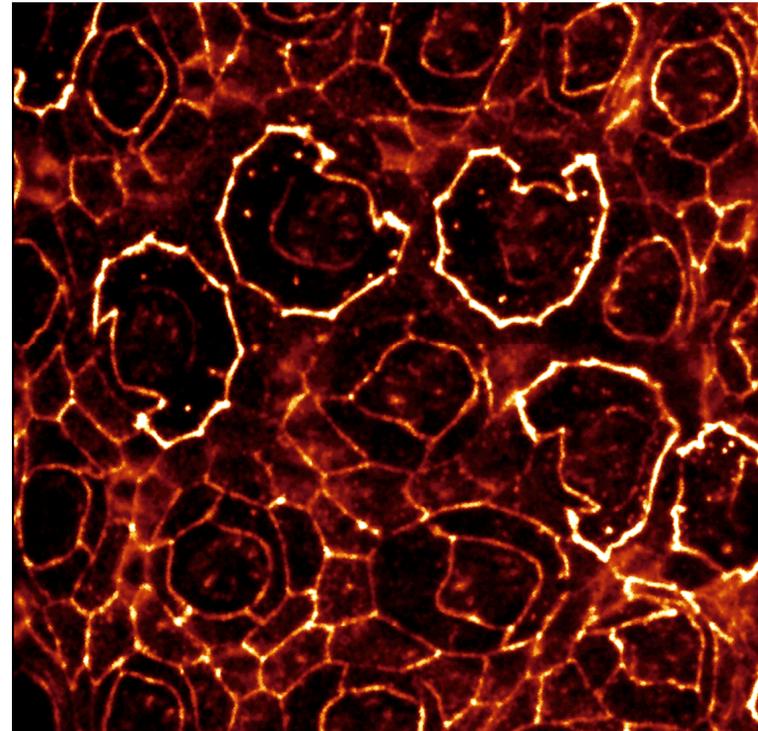


mRNA in situ hybridization in wild type



Accumulation at contacts of expressing and non-expressing cells in wild type

facet-strawberry mutant



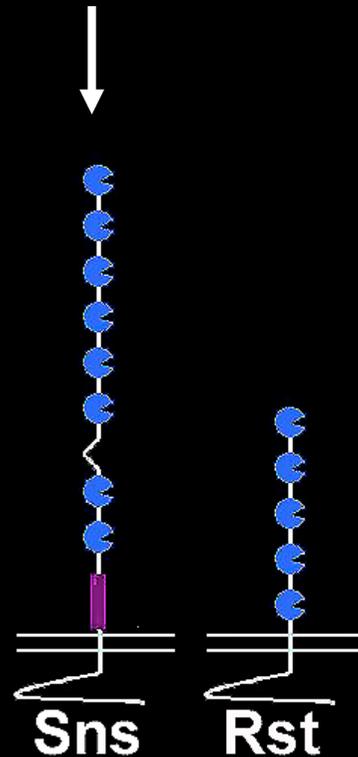
IrreC-rst mRNA is expressed in interommatidial cells. The protein accumulates most strongly at the common border with primary pigment cells (which partially fail to differentiate in *facet-strawberry*).

**Homophile Adhäsion in Zellkultur schließt
heterophile Interaktionen in vivo nicht aus**

The actors:

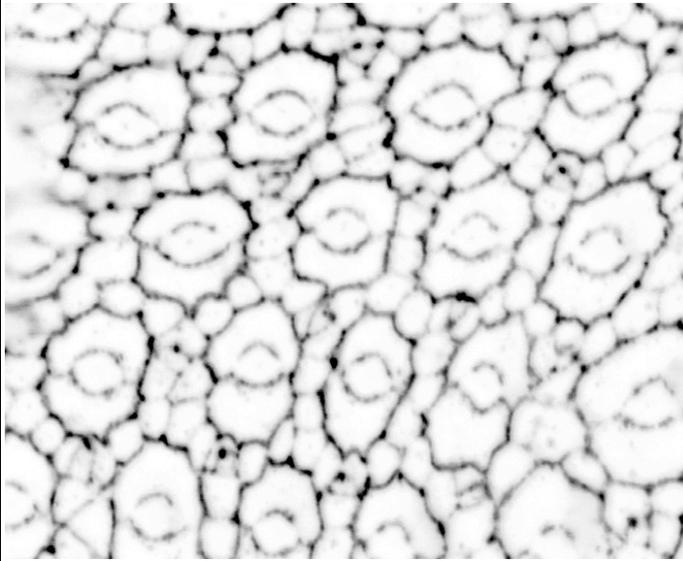
Cell adhesion molecules of the immunoglobulin superfamily

does this protein interact with Rst?

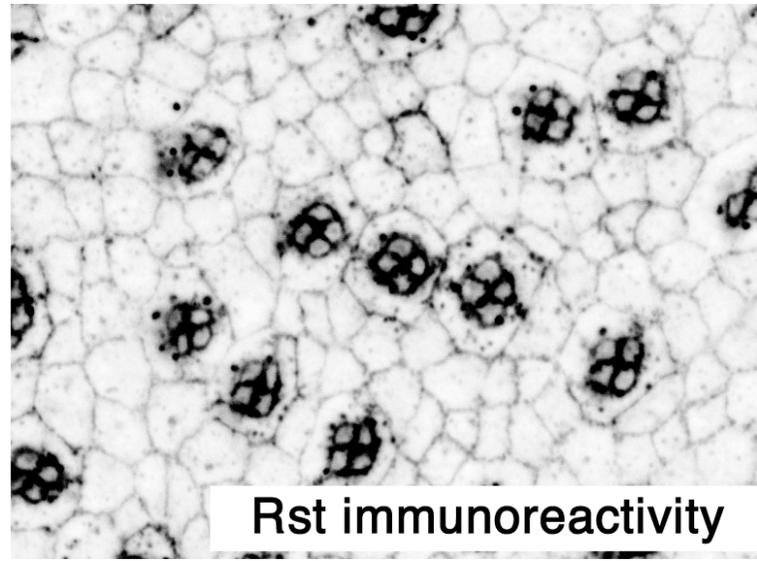


-  fibronectin-like domain
-  immunoglobulin-like domain

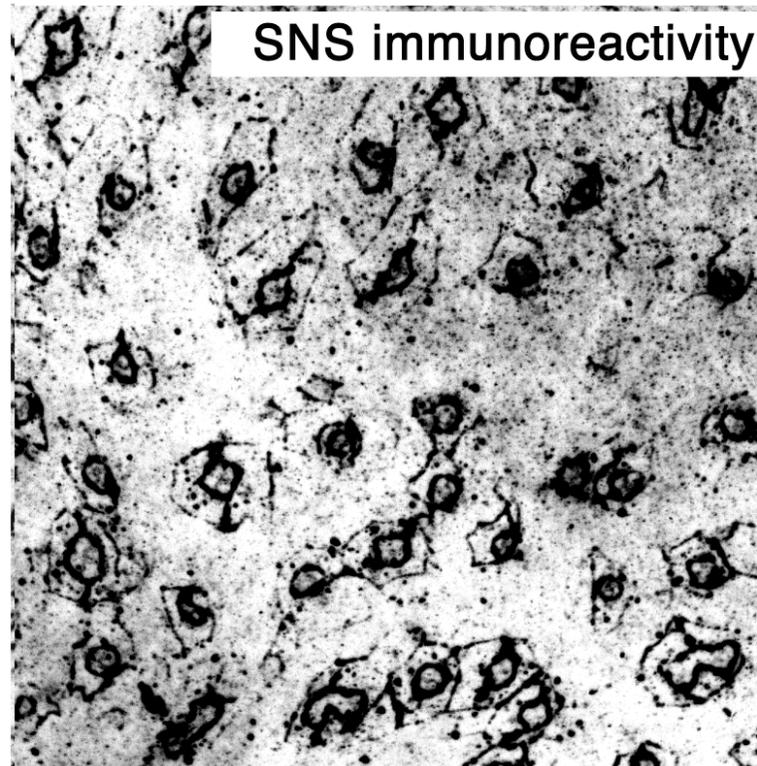
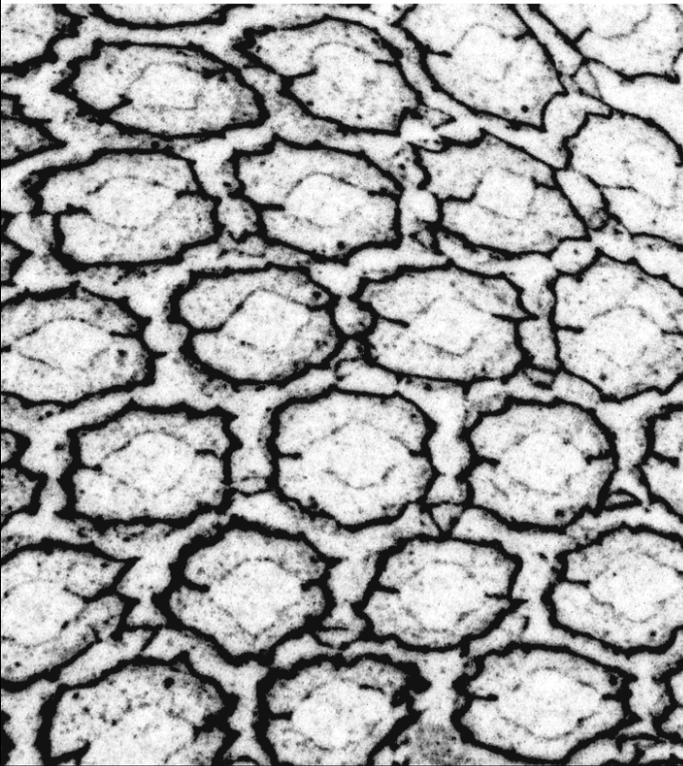
wild type p21%



sev-Gal4/UAS-irreC-rst

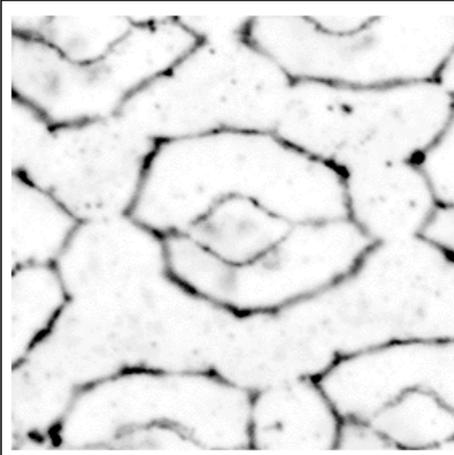


Rst immunoreactivity

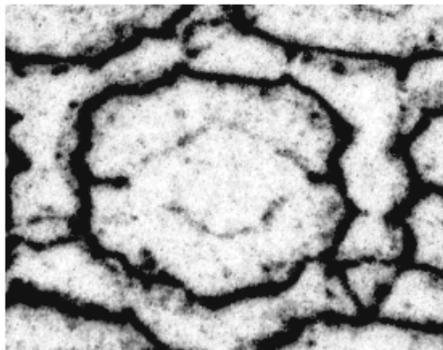
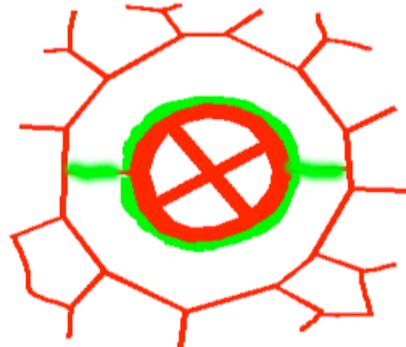
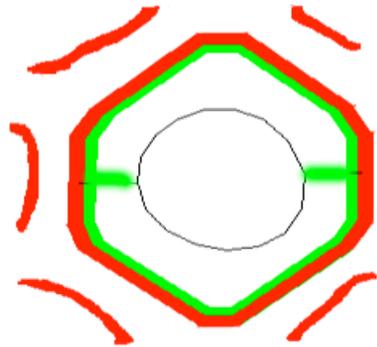
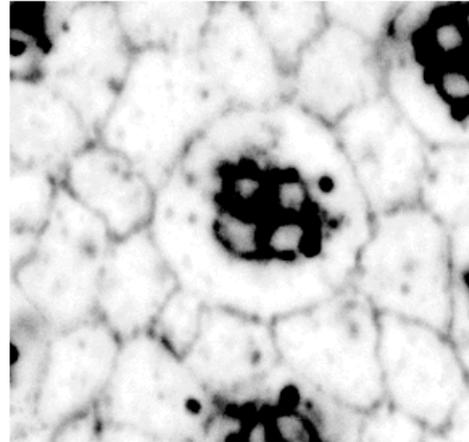


SNS immunoreactivity

wild type



*sev-Gal4/
UAS-irreC-rst*



Rst immunoreactivity

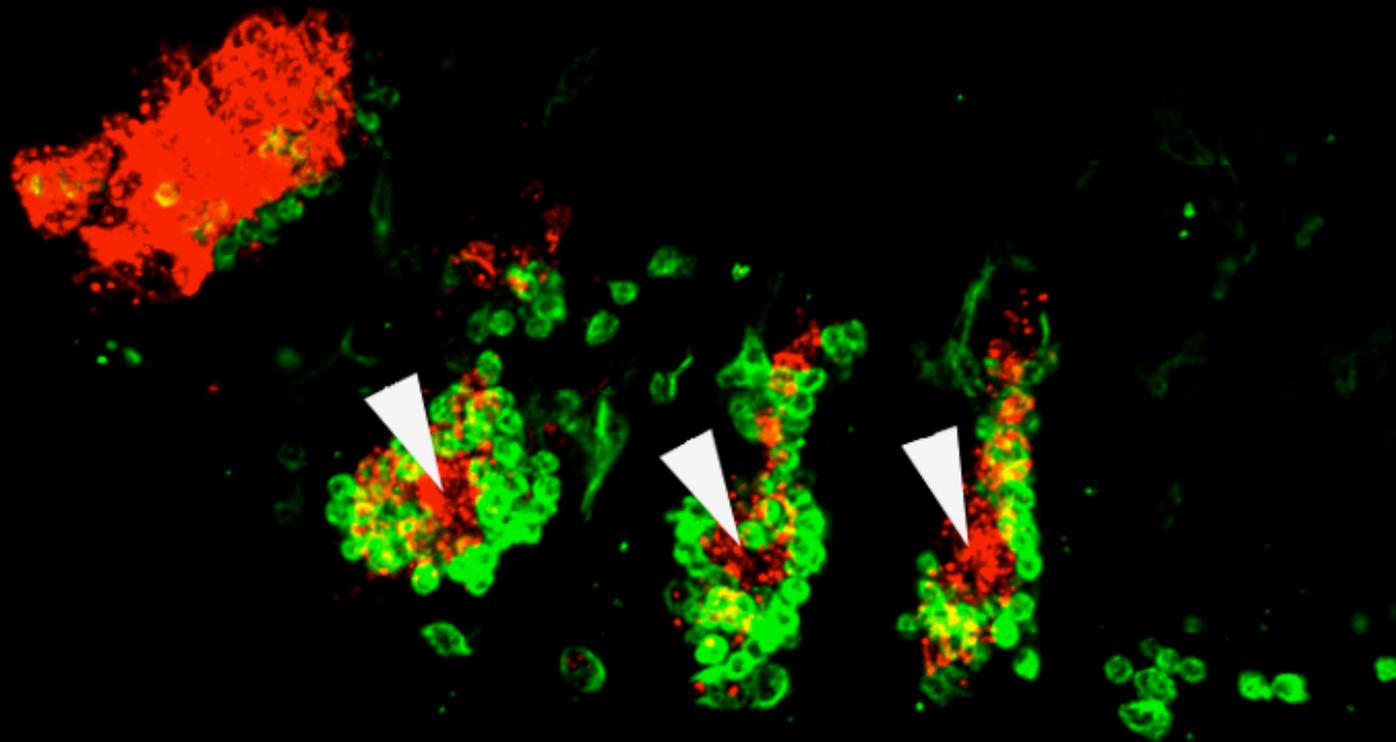
**Predicted redistribution
of a ligand**

SNS immunoreactivity

in vivo (im Embryo) Zelladhäsionsassay

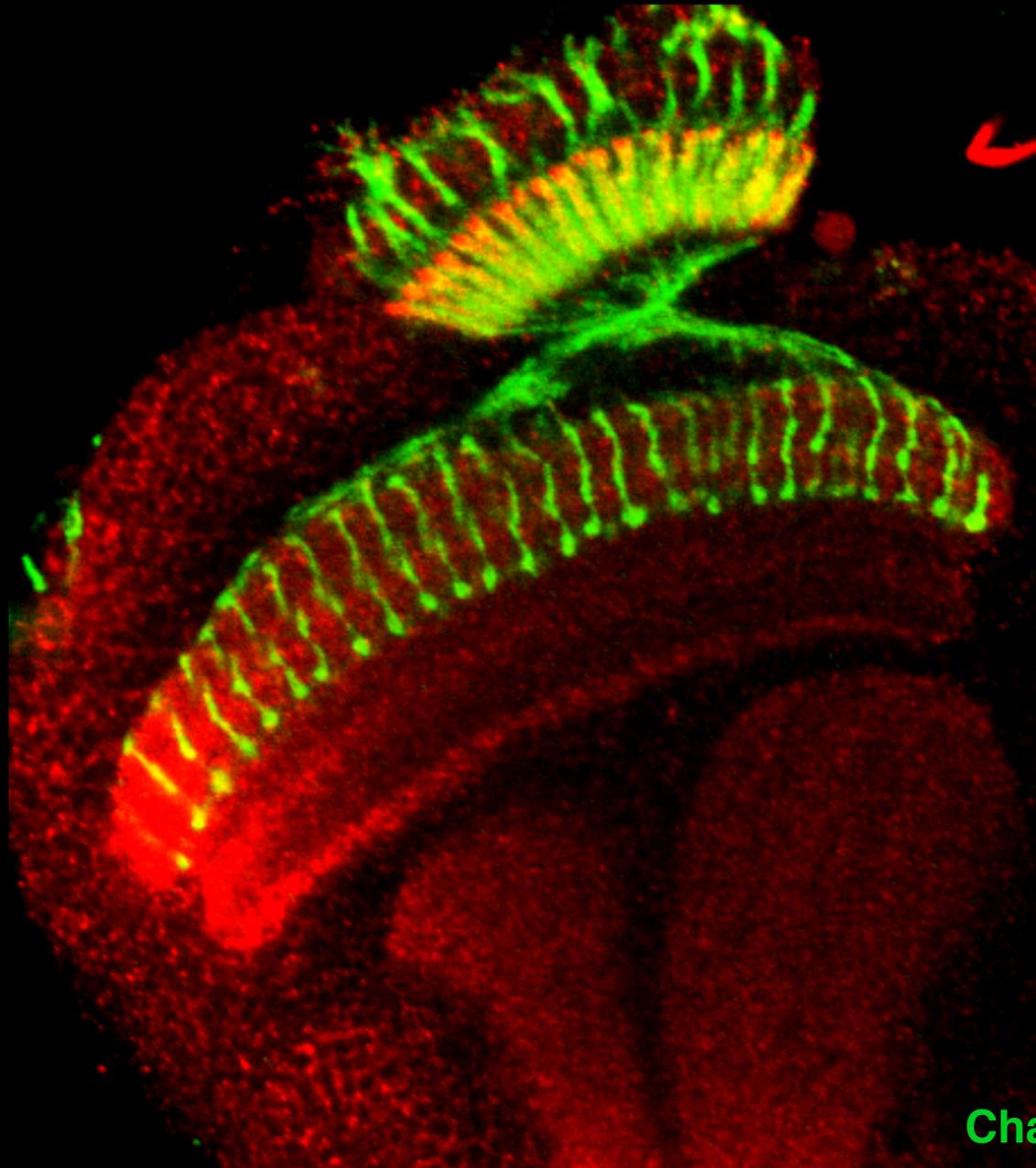
(mit dem Gal4/UAS-System erzeugte Fehlexpression des Gens in Beinanlagen von IrreC-rst-Mutanten)

IrreC-rst Immunfluoreszenz
tubulin Immunfluoreszenz



IrreC-rst ist ein heterophiles Zelladhäsionsmolekül

Selektive Markierung von Photorezeptoren im Gehirn der Fliegenpuppe



Chaoptin-Antikörper