Signaling Themes in Neural Development

Ben Cheyette
office/lab: 2nd floor Rock Hall
bc@ucsf.edu
Office Hours: I'm easy to find.

I

Lateral Inhibition

I'm going to tell you a story about Lateral Inhibition

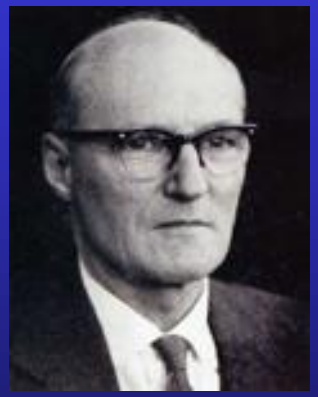
in the sense that Developmental Biologists use it…

Our story begins in pre-World War II England with Vincent B. Wigglesworth…

With a name like "Vincent B. Wigglesworth" you'd become an entomologist too…

Driven by the puerile taunts of his classmates, young Vince retreated into the non-judgemental world of insects – eventually giving himself up entirely to studies of the South American Blood Sucking Bug - *Rhodnius prolixus*
(vector of Chagas disease)

Rhodney in its native habitat (human skin)

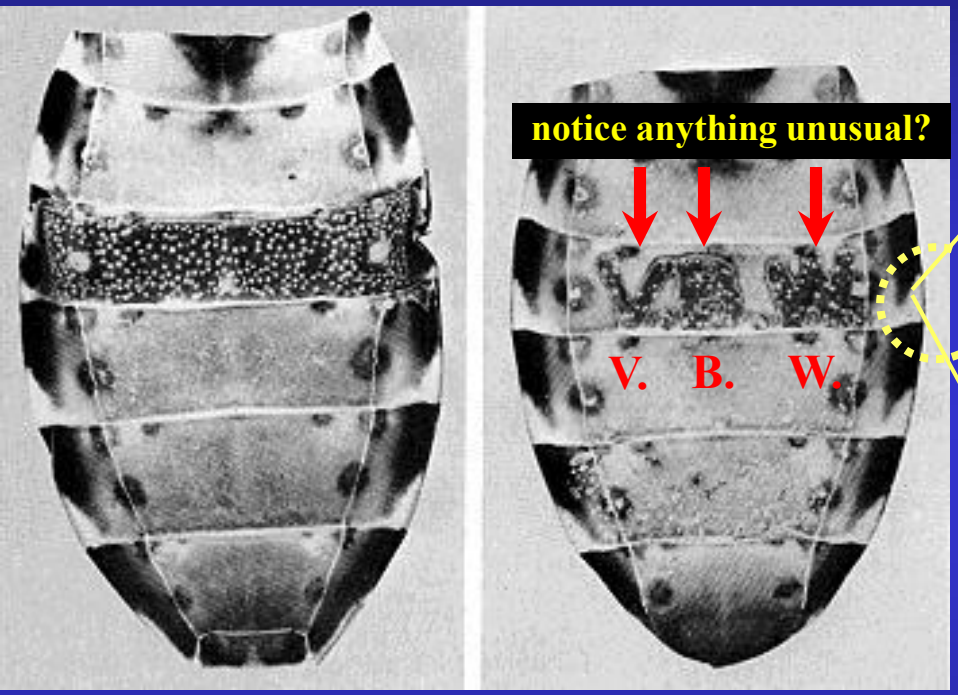


Vincent Wigglesworth
(in one of his lighter moods)

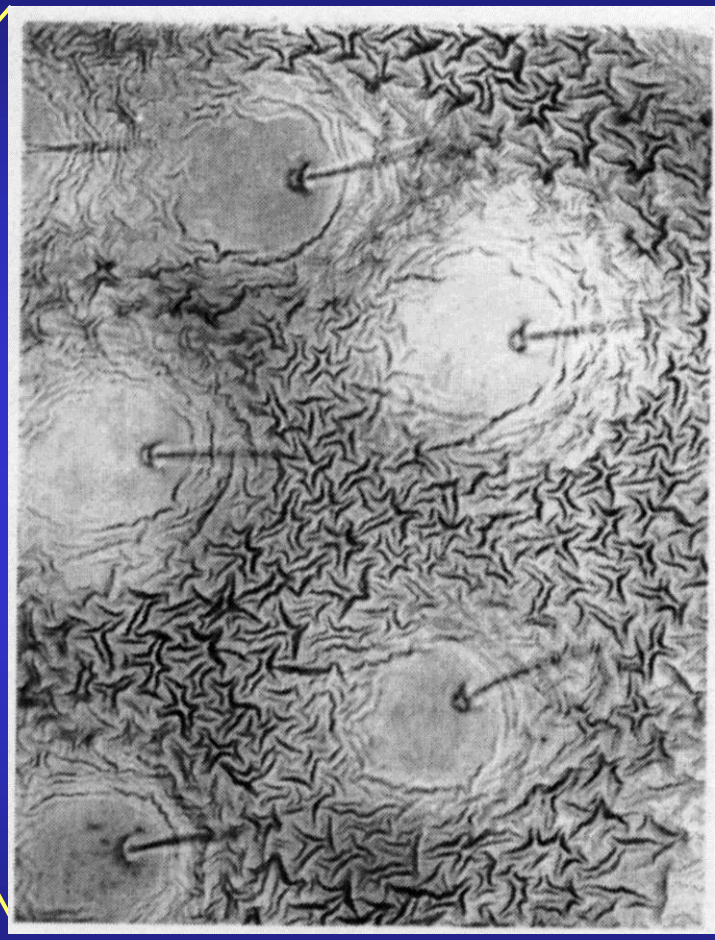
Rhodney after a nice juicy meal

Wigglesworth was *way* ahead of his time. He realized that while traditional British entomology (*i.e.* classifying bugs) was dandy, you could learn some really cool things by conducting *experiments* on them (Egad!) to explore developmental patterning.

He first chose a “simple” system…



Abdomen segments (tergites) of “Wigglesworth’s bug”



Epicuticle bristles of the third tergite

<u>Note:</u> bristles (sensory organs of the peripheral nervous system) are evenly spaced.

<u>Question:</u> How is this accomplished?

One other important fact you need to know about *Rhodnius prolixus* in order to understand Wigglesworth's experiment:

This is a "hemimetabolous" insect

(as opposed to *Drosophila*, which is "holometabolous").

This means *prolixus* goes through several larval instars (stages) as it grows which increasingly resemble the adult, as opposed to a sudden transformation from a larval form to a morphologically distinct adult form (*i.e.* pupation).

This also means that if you injure a region of the larval cuticle (skin) in one instar, you can directly observe it heal over the next several instars.

So here's the experiment:

What happens to the bug if you destroy (*i.e.* burn) its cuticle?

Actually…I can tell you from personal experience that little boys have been doing this experiment ever since the magnifying glass was first invented (I am one of 3 brothers).

But Wigglesworth did refine the experimiental question:

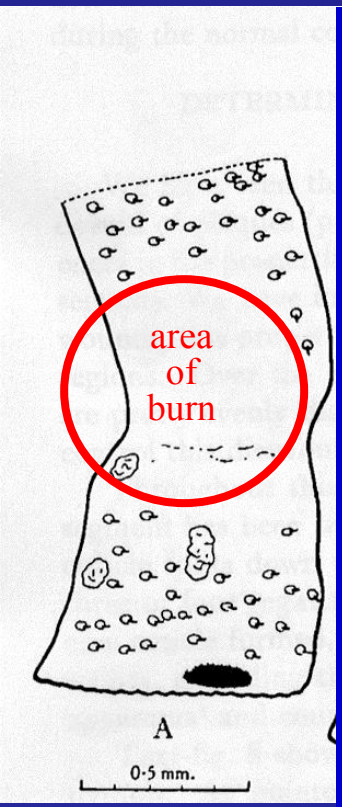
What happens to bristles that grow back after you burn the cuticle?

1. Do they grow back in any observable order?

2. Do they grow back in exactly the same positions as the destroyed bristles?

3. Do they remain evenly spaced, or do they become randomly distributed?

Answers:



1. Bristles first grow back near the edges of the wound.

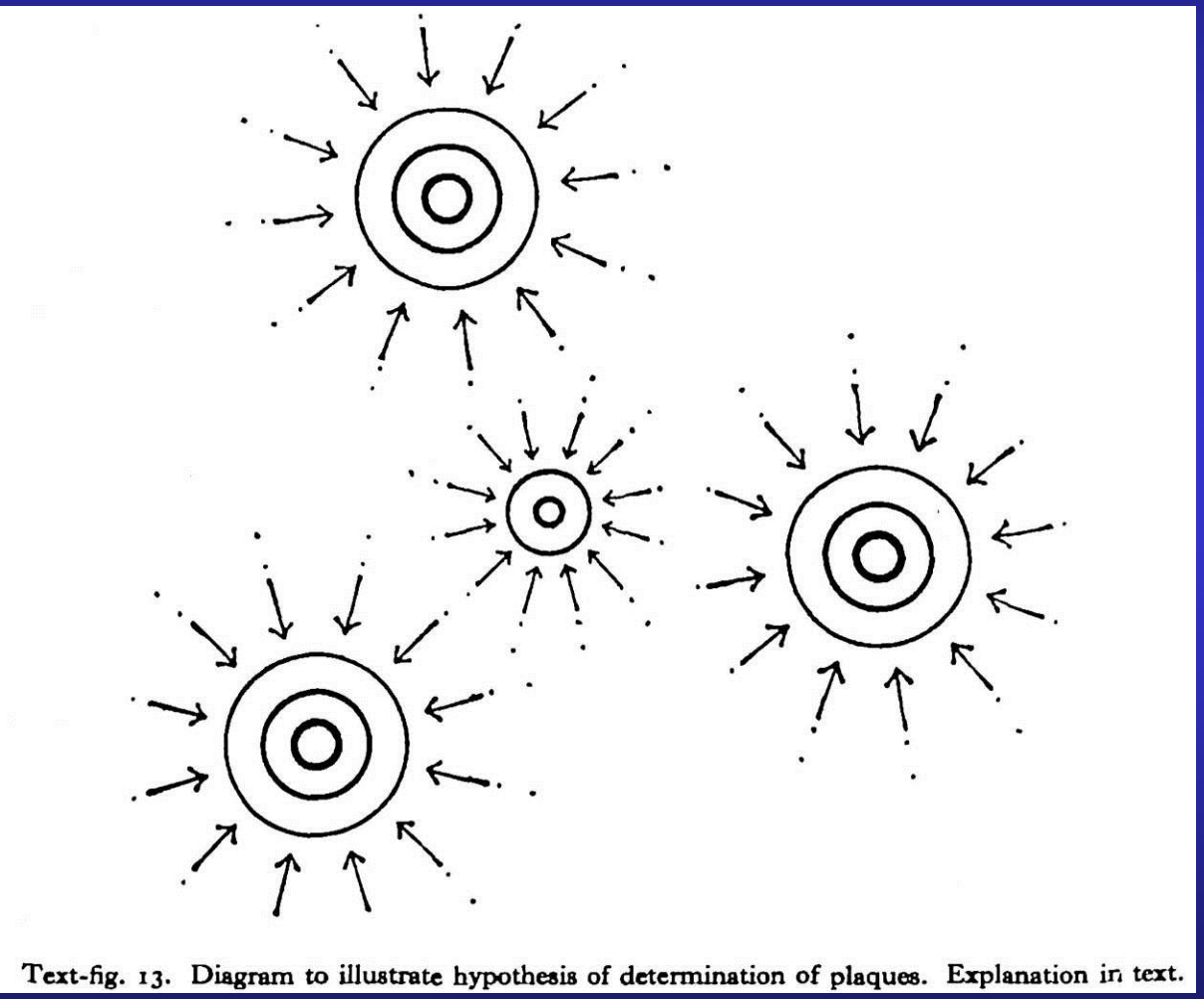
2. The new bristles do NOT grow back in exactly the same positions as the destroyed bristles.

3. The new bristles ARE evenly spaced
(even those that grow back simultaneously).

In fact, Wigglesworth observed that the number of CELLS between bristles remains constant as the animal grows.
(although the absolute linear distance between bristles can vary depending on the feeding and nutritional status of the growing animal)

Based on these experiments, Wigglesworth proposed that:

Each bristle-forming-unit (plaque) "appears to exert an inhibitory influence around it and to prevent the development of new plaques within a certain radius." (lateral inhibition)



Text-fig. 13. Diagram to illustrate hypothesis of determination of plaques. Explanation in text.

He further theorized that adjacent plaques compete for an "essential element" - that is limiting and that they soak up from the surrounding tissue.

Reference: Wigglesworth (1940) Local and General Factors in the Development of "Pattern" in *Rhodnius Prolixus* (Hemiptera), *Journal of Experimental Biology* 17 (2) pp. 180-201.

Riddle of the day

If Wigglesworth were doing these burnt cuticle and bristle regrowth experiments today on blood sucking bugs, what would he call his research?

Answer: Regeneration Medicine.

….Sell your science

For the next chapter of our story,

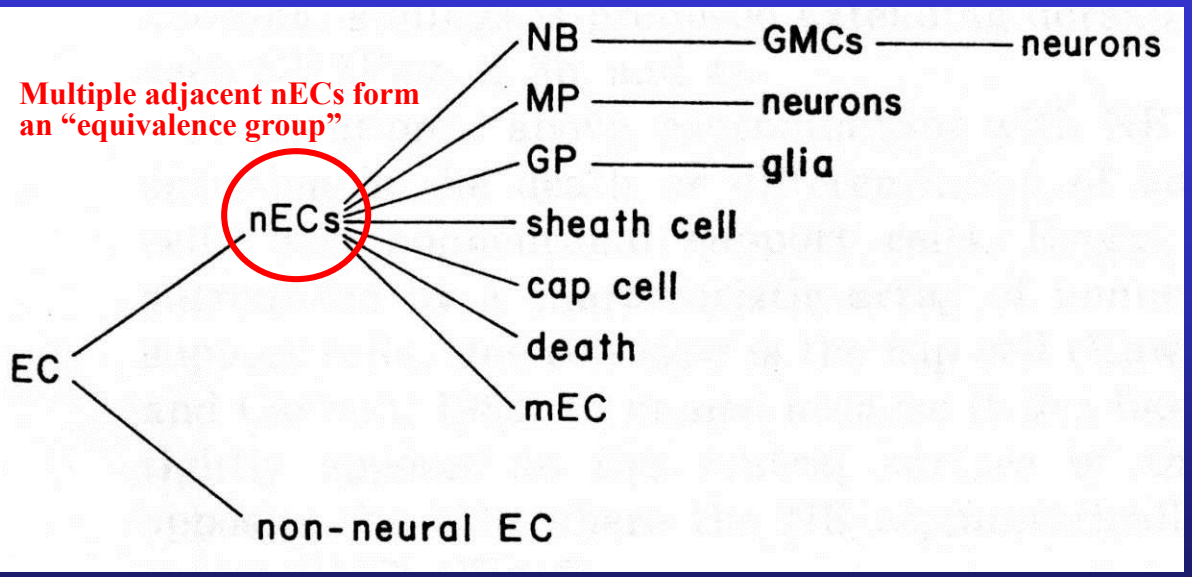
we must *jump*…

"across the pond" to California (Palo Alto)

and ahead 45 years, *c.*1985

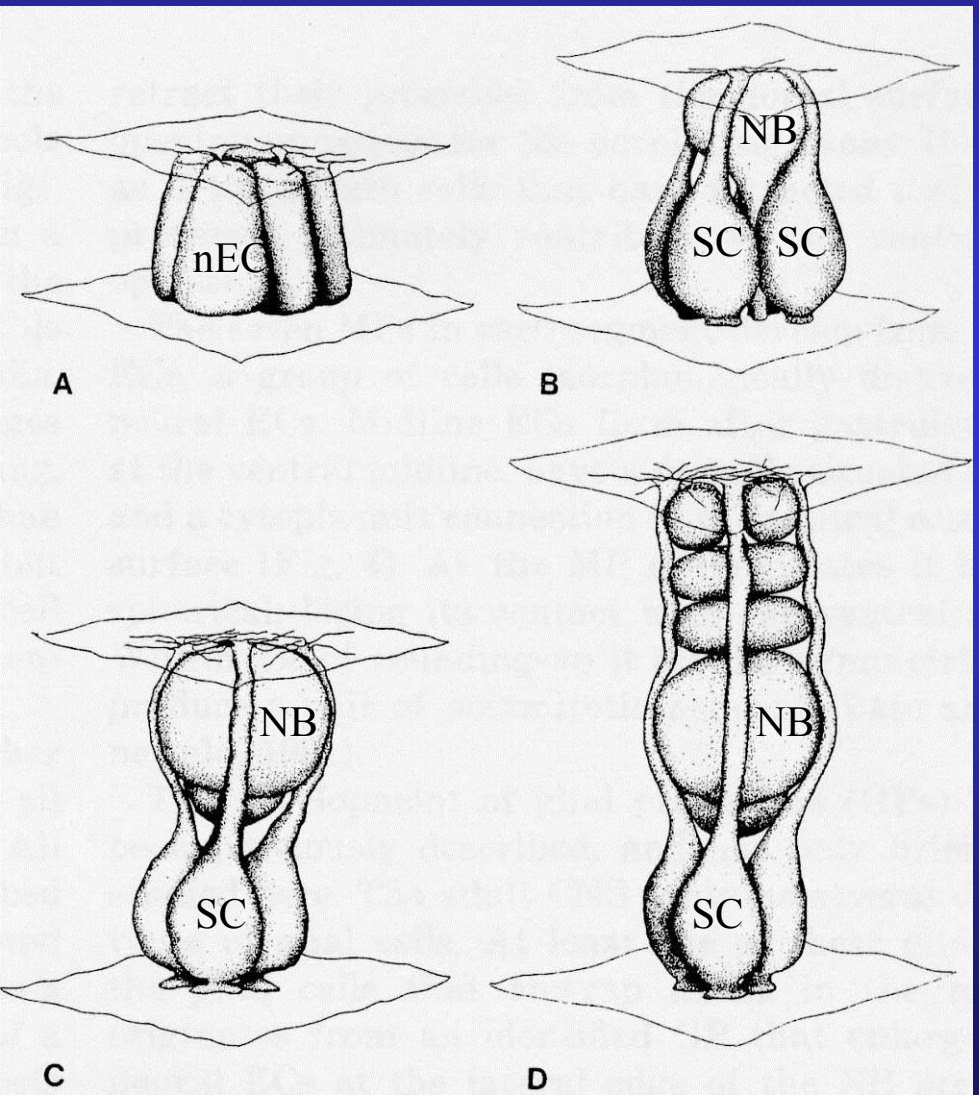
1985: Chris Doe and Corey Goodman analyzed the development of neuronal precursors in the Grasshopper, in a sense extending the work of Wigglesworth at a more detailed cellular level.

Lineage fate map of Ectodermal Cells (ECs), grasshopper larva



Key:
nEC = neural Ectodermal Cell
NB = neuroblast
GMC = Ganglion Mother Cell
MP = Midline Precursor
GP = Glial Precursor
mEC = midline EC

Equivalence groups are clusters of cells with the same developmental potential, but in which only a subset (*e.g.* 1) assumes a particular (*e.g.* neural) fate.

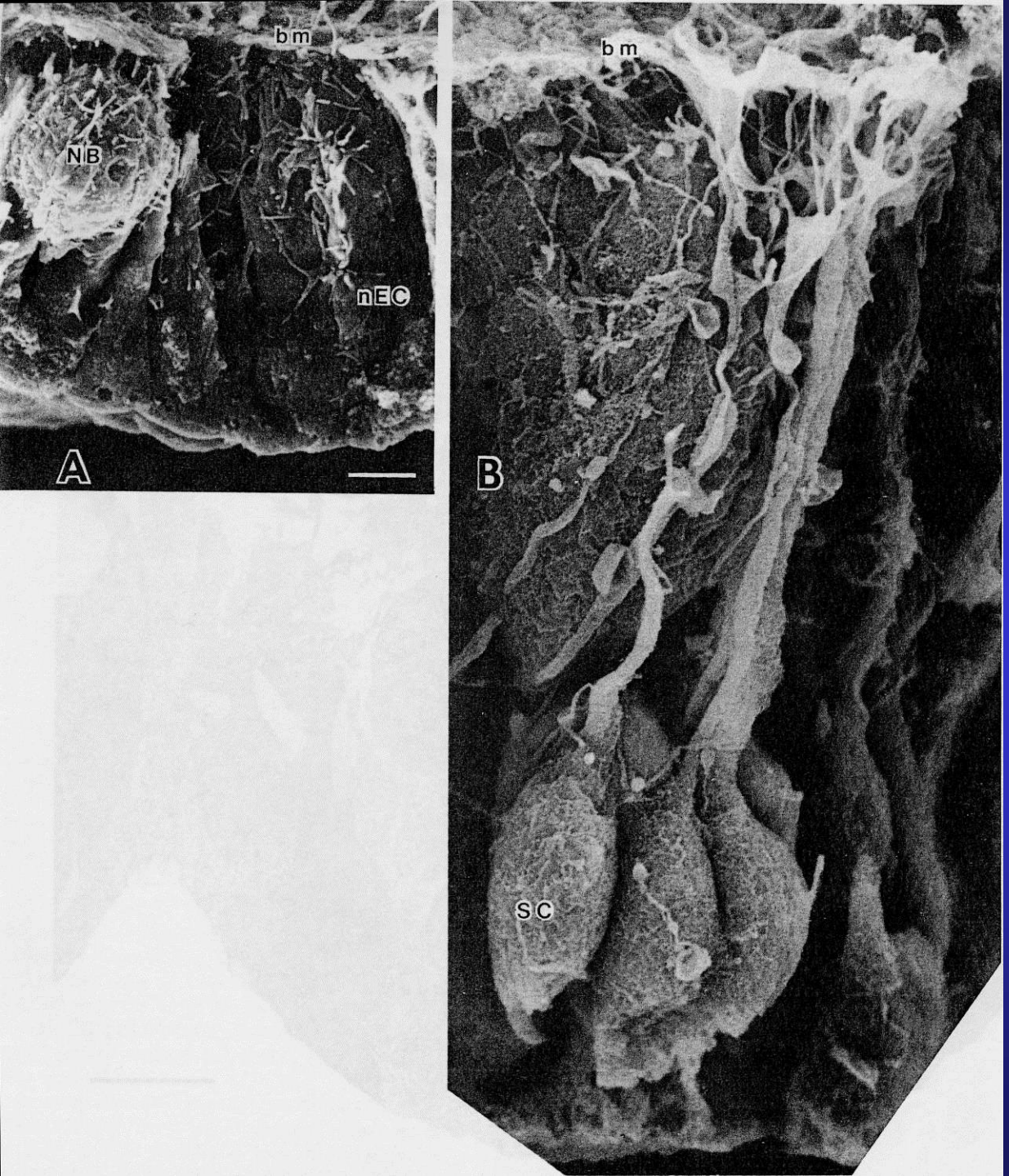


A. Equivalence group of neural Ectodermal cells (nECs) in the larval ectoderm

B. One (central) starts to differentiate as a neuroblast (NB)

C. The neuroblast delaminates from the ventral (apical) membrane, while the other cells in the original equivalence group differentiate as sheath cells (SC).

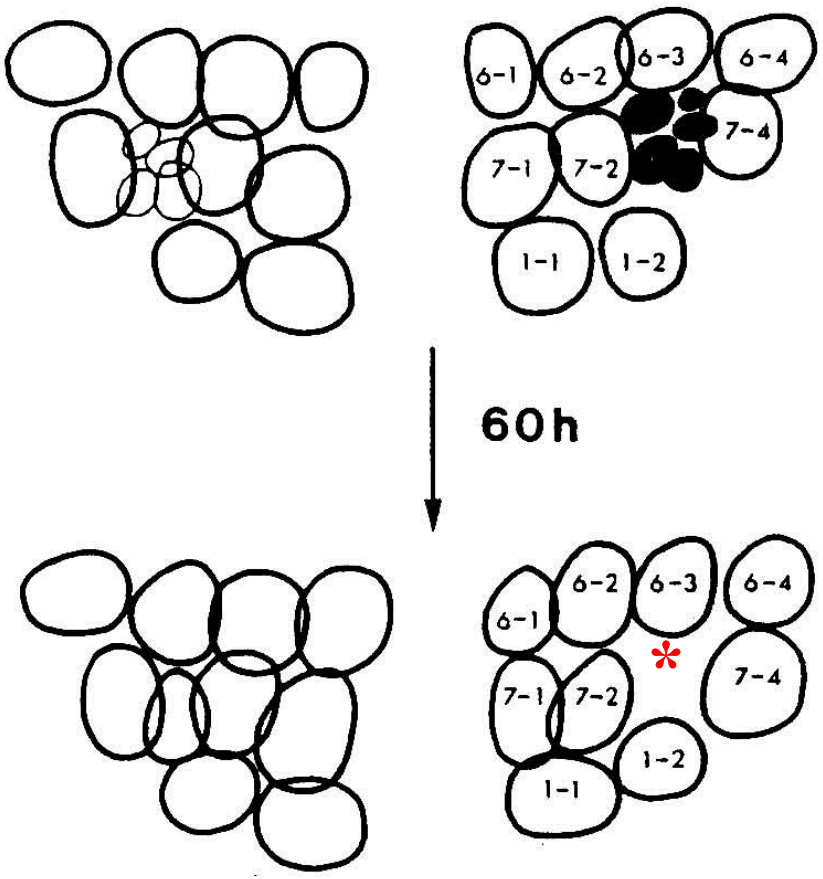
D. The neuroblast divides to produce daughter cells (ganglion mother cells and their post-mitotic neuronal progeny); the sheath cells ensheath all these neuronal cells.



Here's what the system actually looks like…

(by scanning EM)

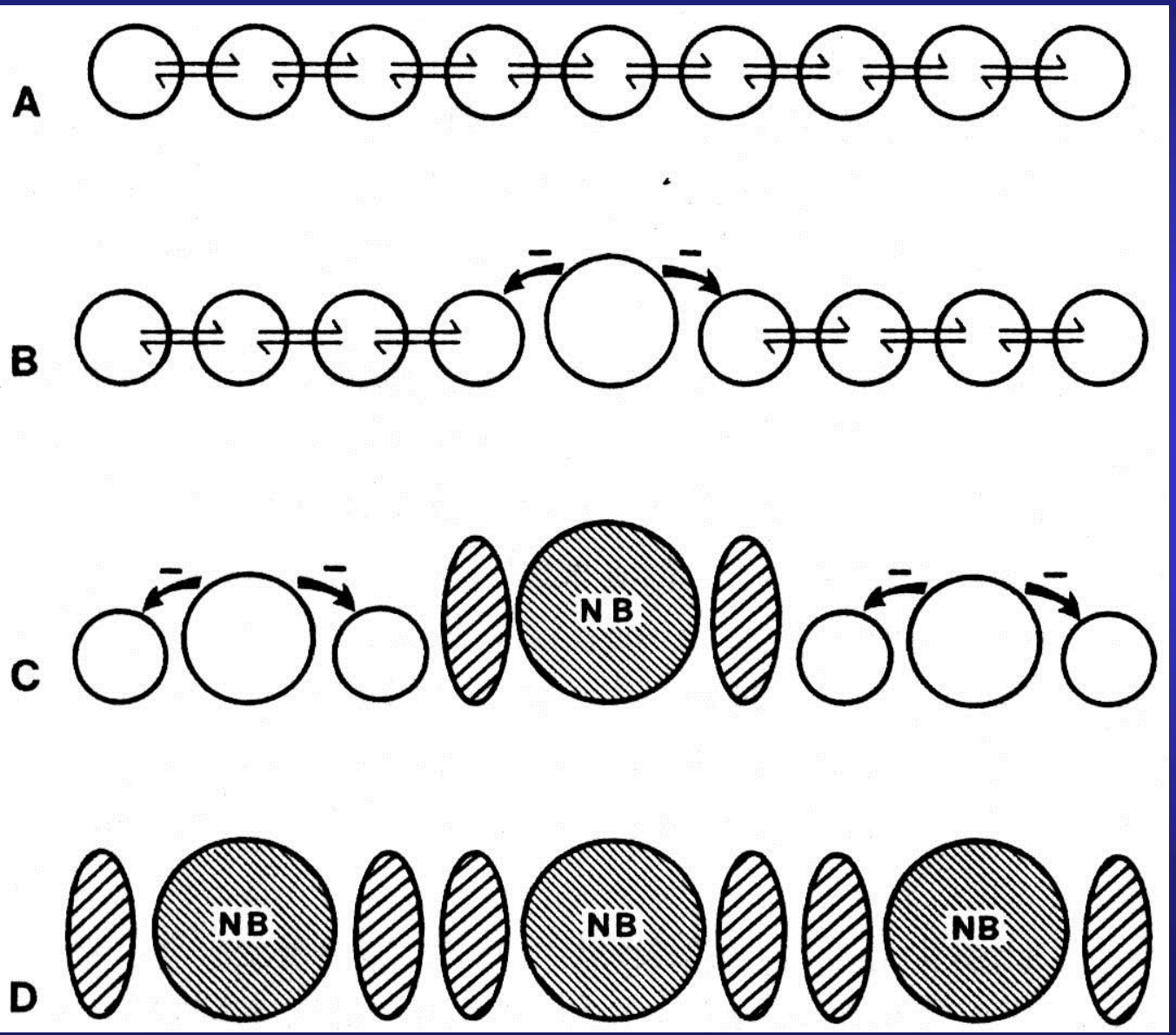
And here's the critical cell ablation experiment



A Laser ablation of all the cells in the equivalence group (black) leads to loss of the resulting neuroblast (* = number 7-3)

Lateral Inhibition Model

Doe & Goodman (1985) *Developmental Biology* 111, pp. 206-219



A. All cells start out equivalent in the larval neuroectoderm, and are in a state of balanced interaction with each other (but the authors have no idea what this means molecularly).

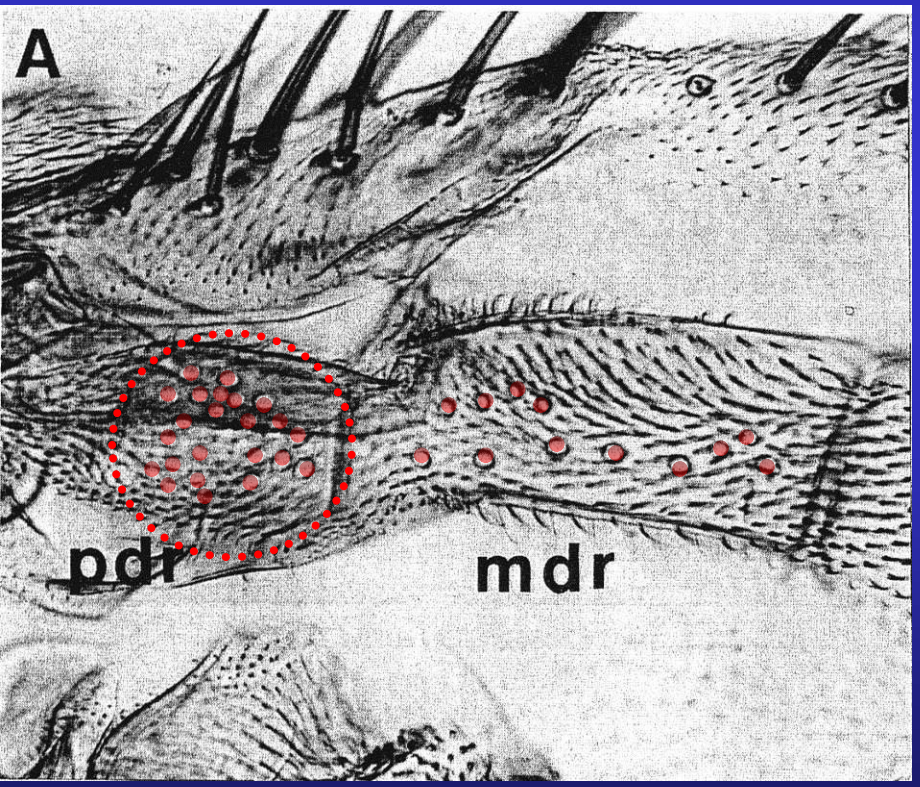
B. One starts to differentiate as a neuroblast (NB) and inhibits its immediate neighbors from doing so (again no molecular mechanism available yet)

C-D. This process is repeated throughout the neuroectoderm to set up evenly spaced neural precursors surrounded by epidermal support cells.

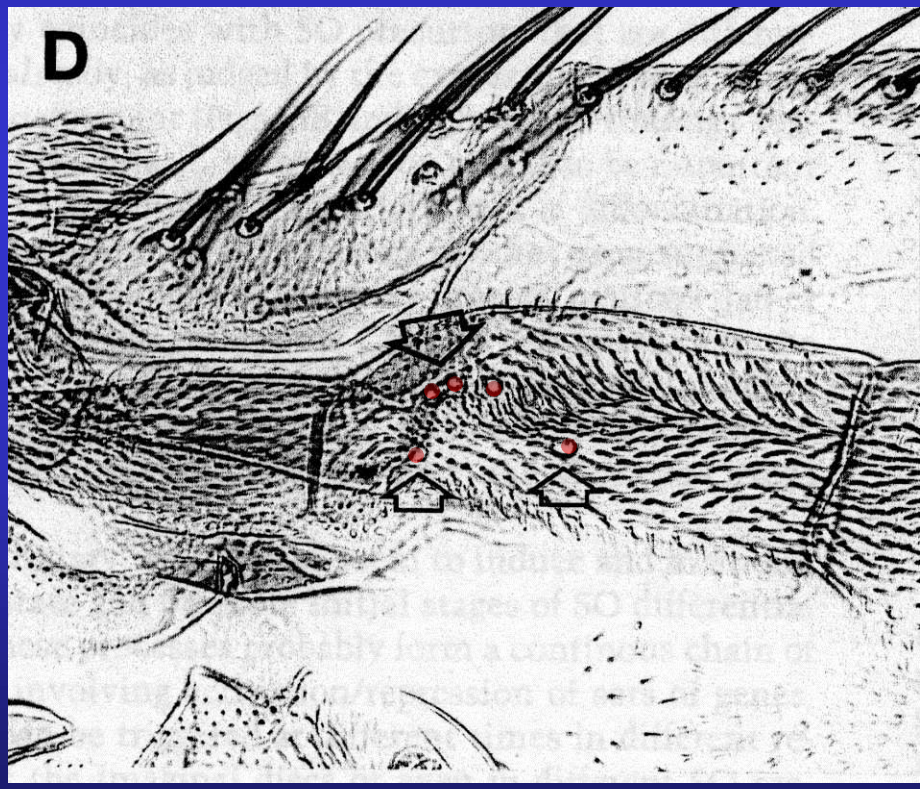
Enter the fruitfly…

…and “the awesome power of (molecular) genetics”

In *Drosophila*, the *achaete-scute* complex was initially defined genetically, as a ~90 kb genomic region comprising several very tightly linked but separable genes necessary for normal bristle development

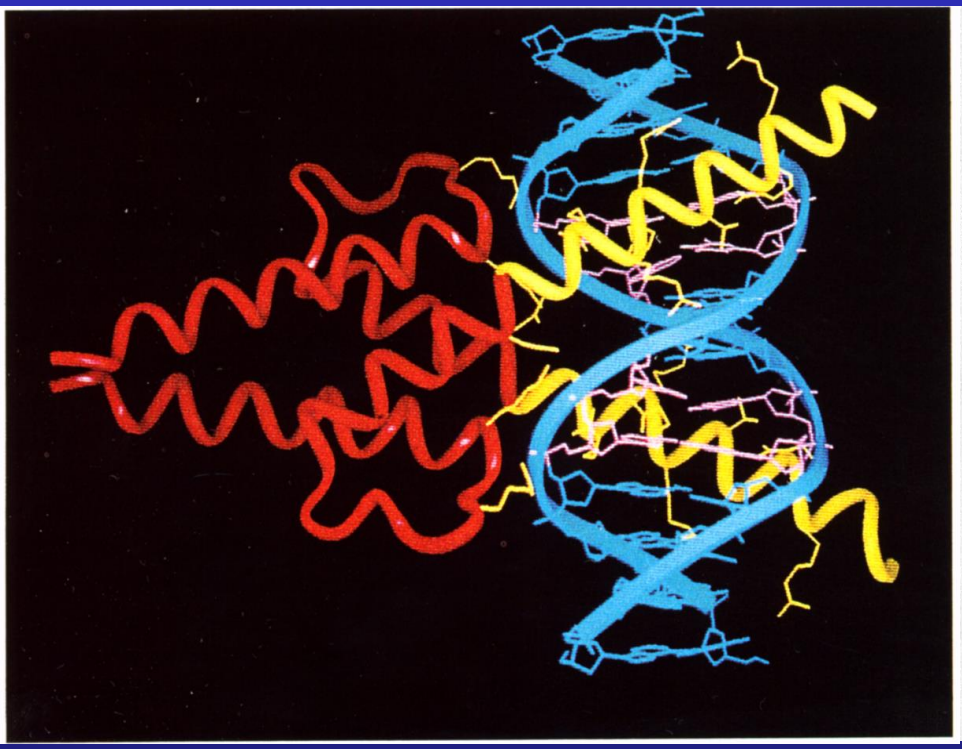


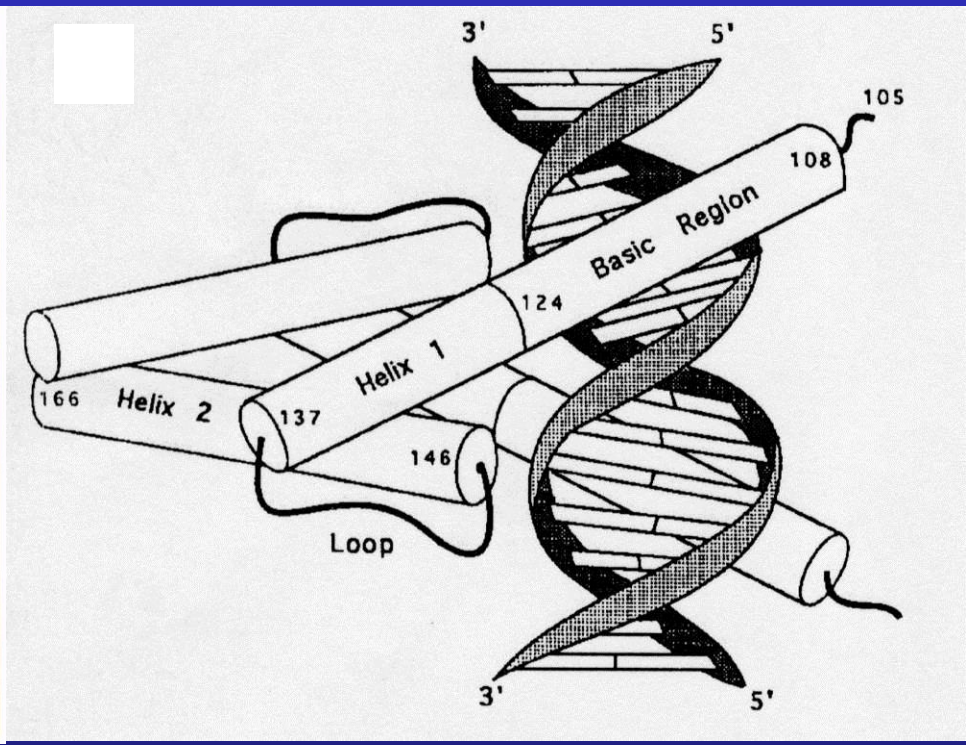
sensilla campaniformia
dorsal wing margin of wild type adult fly



sensilla campaniformia
dorsal wing margin of *scute* mutant

The *achaete-scute* complex contains 4 related transcripts encoding basic-HLH transcription factors with complex genetic interactions: achaete, scute, lethal-of-scute, and asense



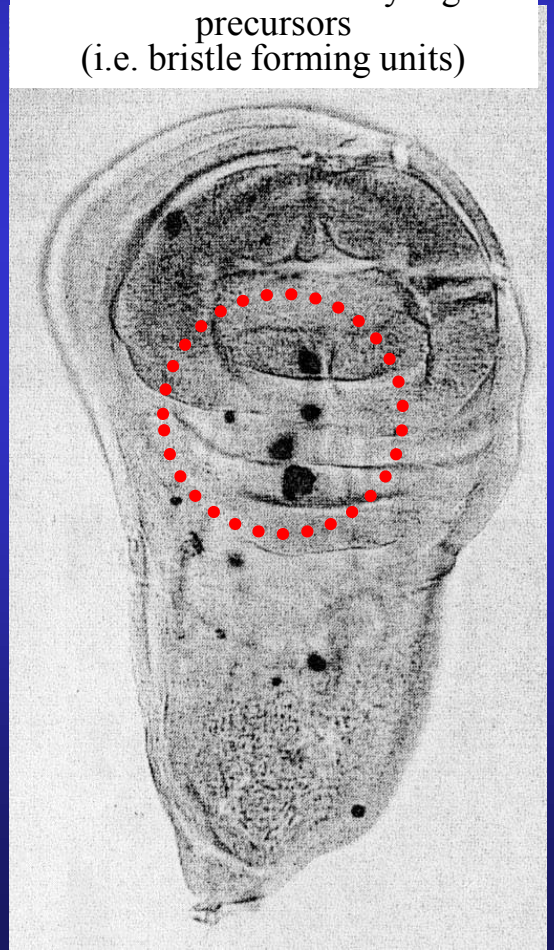


(One explanation for the complex genetic interactions is that in addition to their transcripts being physically linked and co-regulated, their protein products also functionally heteromultimerize.)

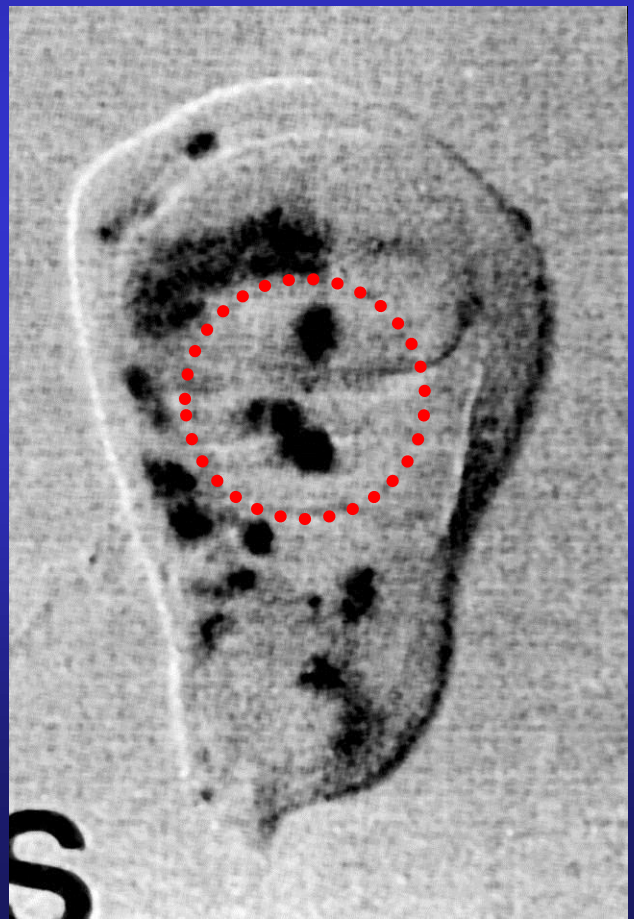
The achaete-scute genes are expressed in proneural clusters (bristle forming units) during development. In larvae mutant for these genes sensory organ precursors fail to differentiate.

Romani et al (1989) Genes & Dev 3: 997-1007; Singson et al (1994) Genes & Dev 8: 2058-2071

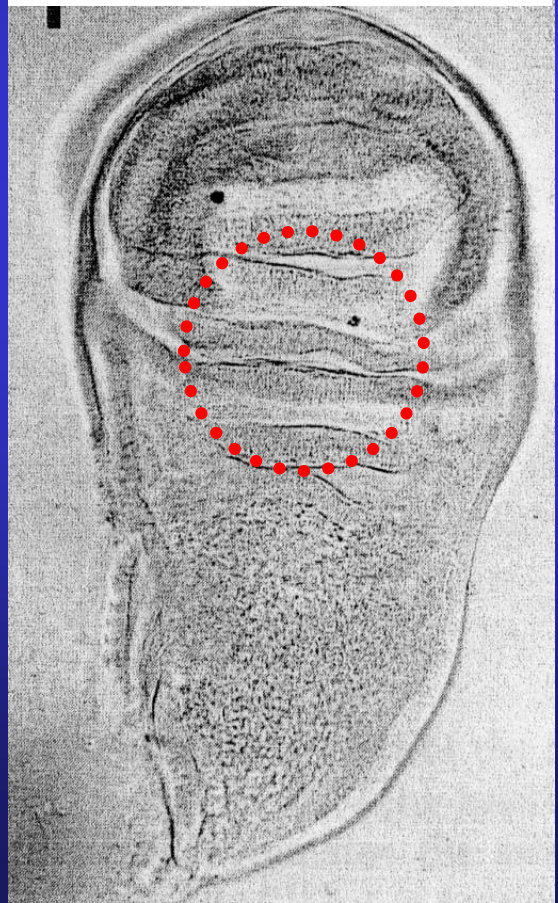
wild type larval imaginal wing disc stained for sensory organ precursors (i.e. bristle forming units)



wild type larval imaginal wing disc stained for expression of *scute* gene



scute mutant larval imaginal wing disc stained for sensory organ precursors



The achaete-scute transcripts are expressed in the precursors of the sensilla (*i.e.* proneural cluster = an equivalence group) and are *cell-autonomously* required for adoption of neural cell fate.

They are therefore often referred to as PRONEURAL genes

Another similar example from outside the achaete-scute complex: *atonal*

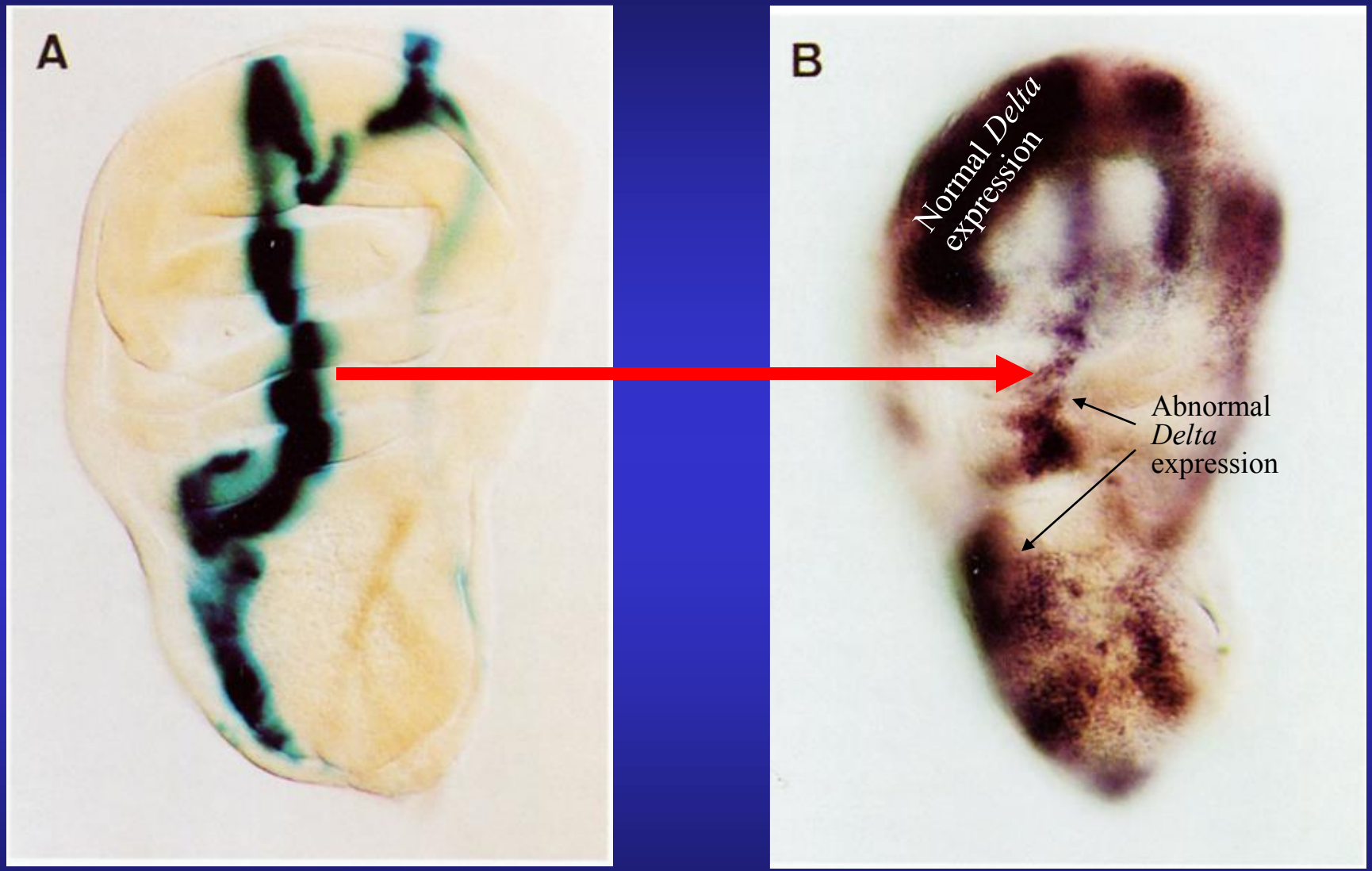
What are the transcriptional targets of the proneural genes?

Among the primary targets of the achaete-scute transcriptional activators is the transmembrane protein:

Delta

Transgenic wing imaginal disc experiment

Hinz et al (1994) Cell *76:*77-87



Ectopic (transgenic) ***lethal-of-scute*** (achaete-scute family member that is NOT normally expressed in the wing imaginal disc).

Resultant pattern of expression of *Delta*. *Delta* is being turned on ectopically wherever lethal-of-scute is expressed.

Delta belongs to another class of fruitfly genes that were also originally defined genetically

(*i.e.* on the basis of contributing to a common phenotype).

In these mutants, all the members of the neuroectodermal equivalence group decide to become neurons.

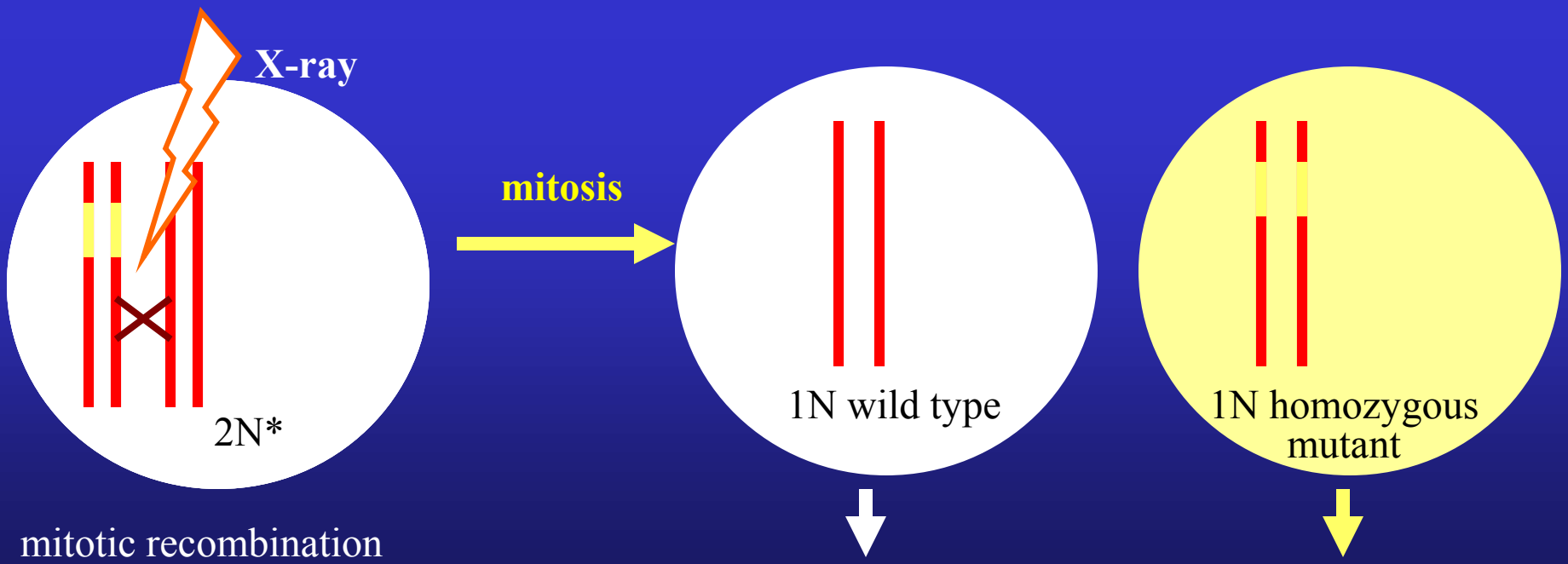
They are therefore referred to as NEUROGENIC genes.

(note: this name can be confusing, since it is based on *the mutant phenotype*; the normal function of the gene is to do the opposite: *i.e.* promote a non-neural fate).

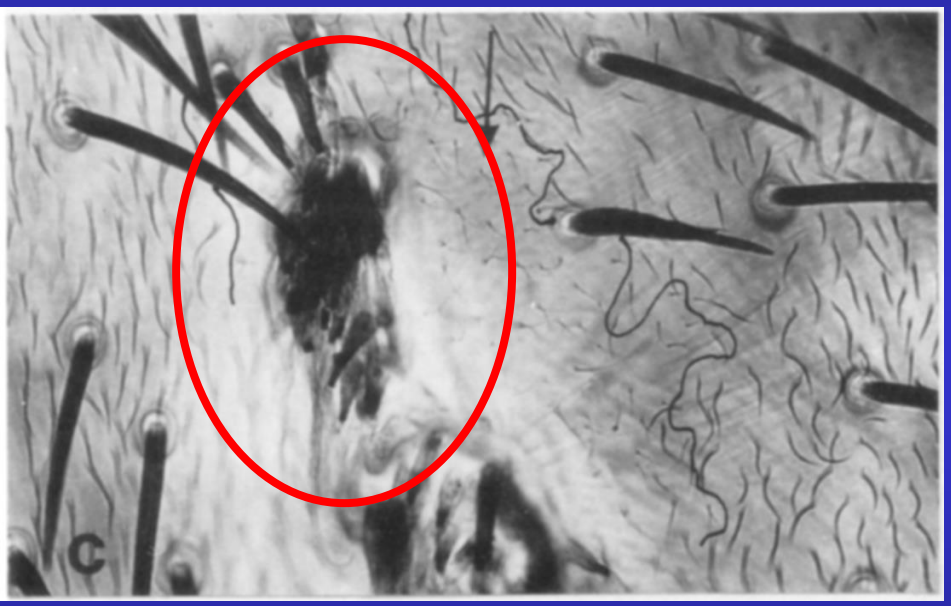
Important neurogenic genes (for this topic):
Delta – transmembrane protein
Notch – transmembrane protein
Suppressor-of-hairless – transcription factor
Enhancer-of-split complex – transcription factors

Because severe mutations of the neurogenic genes cause over-specification of neurons at the expense of ectoderm, they are frequently early embryonic lethal. To study their effects in the PNS, it was helpful to make limited clones of homozygous mutant tissue in an otherwise wild type tissue background.

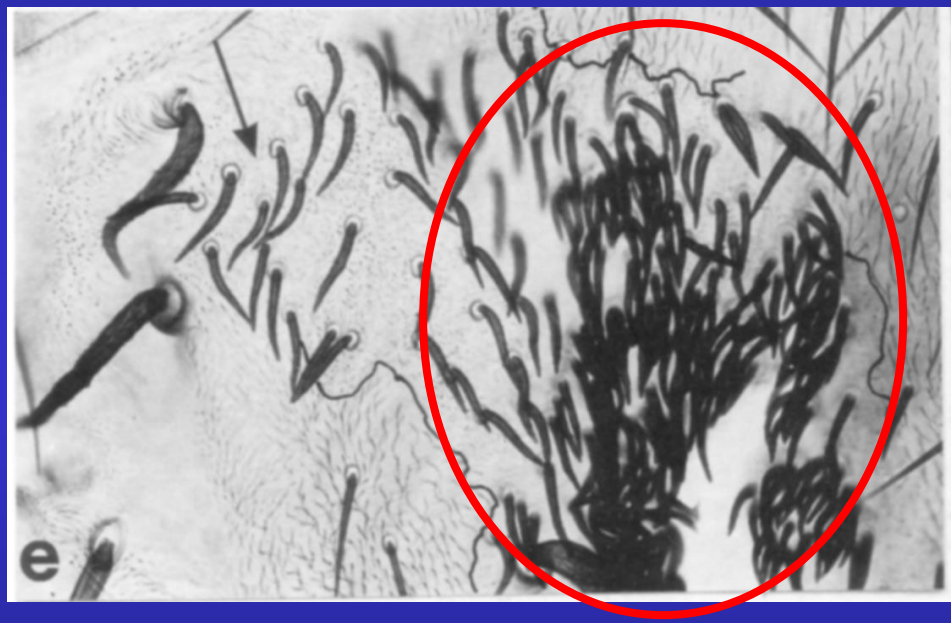
Before recombinases like Cre and Flp came along, this could be accomplished in fruit flies using X-rays to induce mitotic recombination



One "easily-scorable" phenotype for the neurogenic genes occurs in the PNS: an excess of sensory organs (i.e. bristles)



Delta mutant clone in a wild-type background showing abnormal extra bristles

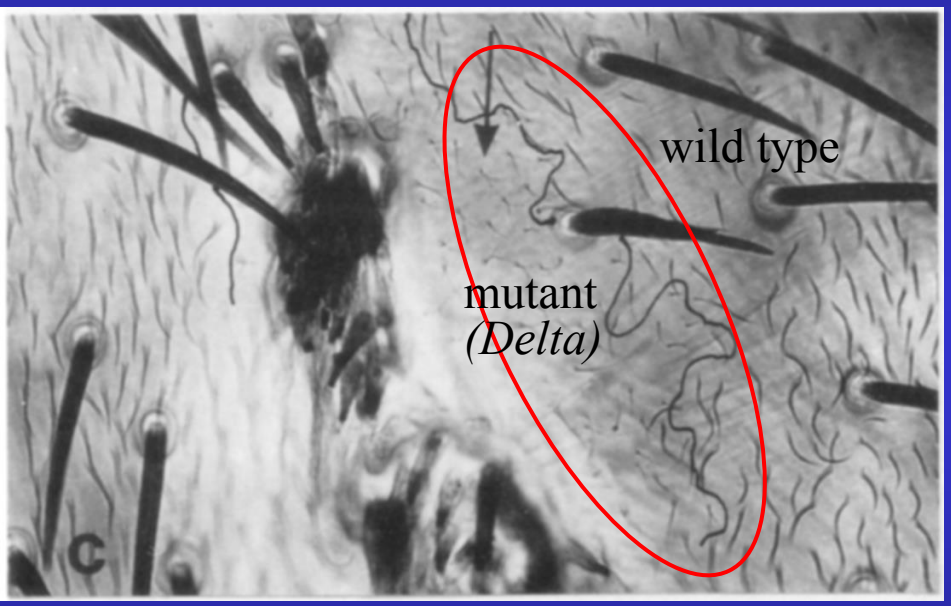


Notch mutant clone in a wild type background showing abnormal extra bristles

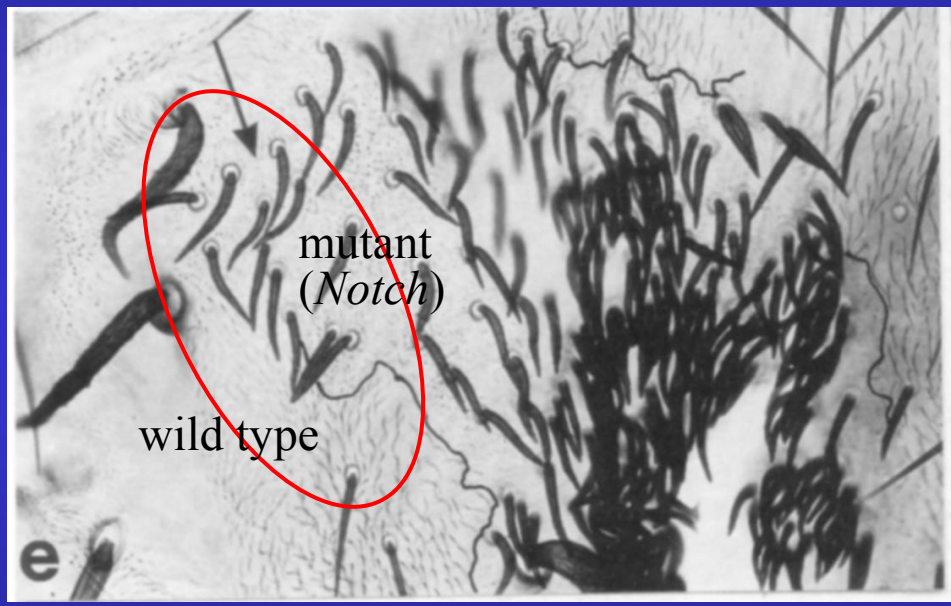
Both *Notch* and *Delta* mutations can lead to over-specification of bristles (PNS)

Heitzler & Simpson (1991) Cell 64: 1083-1092

BUT: Can you detect any differences between these clones?



Delta mutant clone in a wild-type background showing abnormal extra bristles



Notch mutant clone in a wild type background showing abnormal extra bristles

Hint: look at the edges of the clone, where mutant and wild type tissue are adjacent to each other…

Heitzler & Simpson (1991) Cell 64: 1083-1092

Cells at the boundary between mutant and wild type tissue assume opposite fates in *Notch* and *Delta* clones

adapted from: Heitzler & Simpson (1991) Cell 64: 1083-1092

allele	mutant bristle (sensory organ) adjacent to wild type epidermal tissue	wild type bristle adjacent to mutant epidermal tissue
Delta	12 %	88 %
Notch	88 %	12 %

Delta mutant cells next to wild type cells tend to form epidermis, while their wild type neighbors become sensory neurons

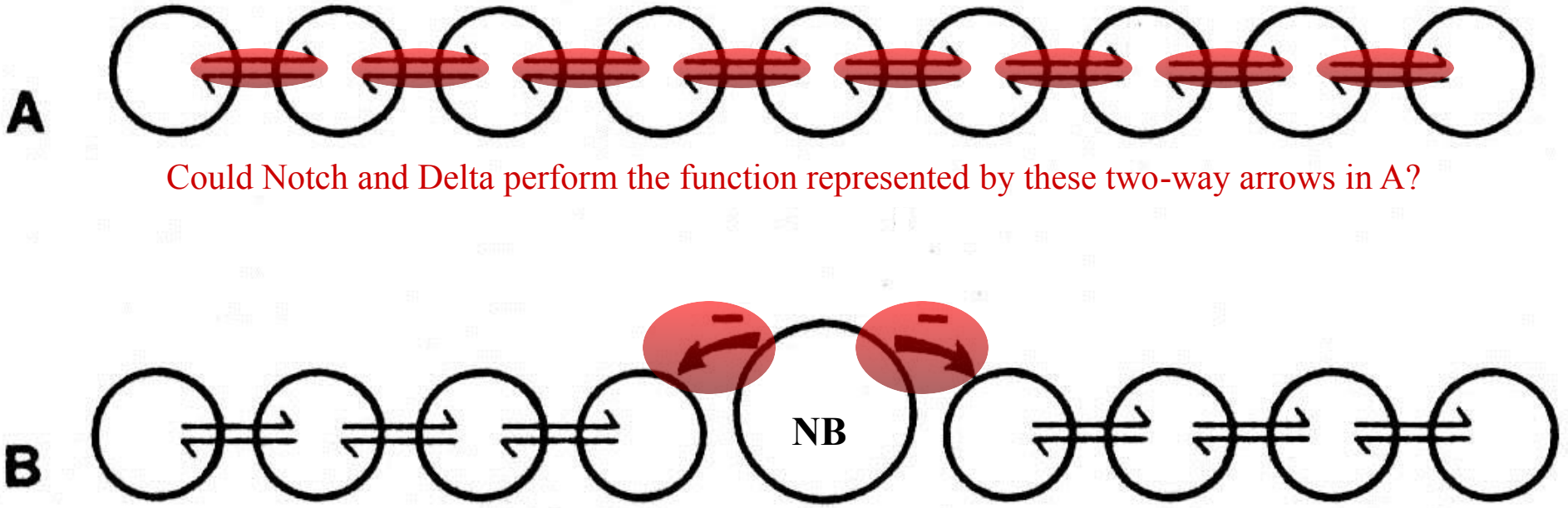
Notch mutant cells next to wild type cells tend to form sensory neurons, while their wild type neighbors become epidermis.

What is going on? Can you explain this by lateral inhibition?

Can we think of this in terms of a molecular model for Lateral Inhibition?

Remember: Notch and Delta are both transmembrane proteins. They are also both widely expressed (at least initially) in these tissues

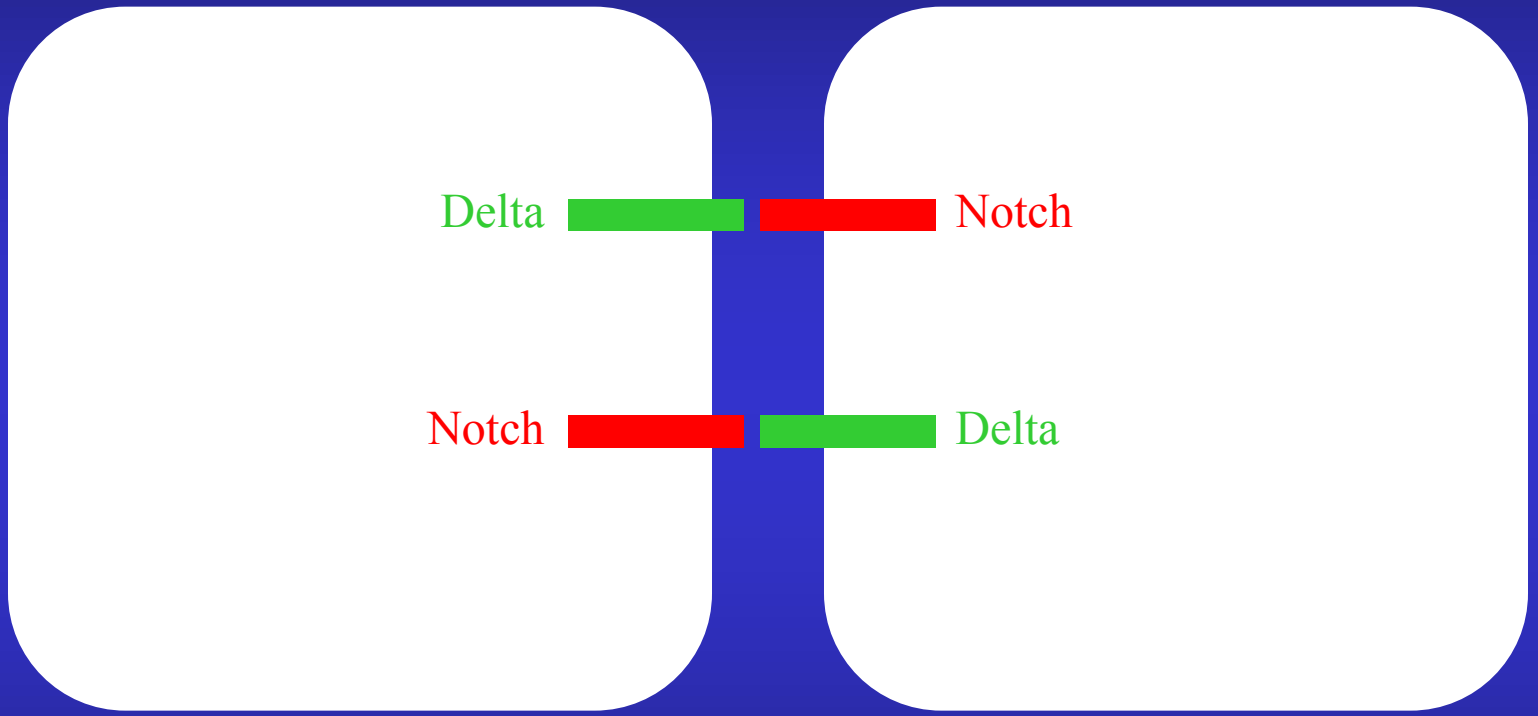
Here's the model from the grasshopper cell ablation studies: Doe & Goodman (1985)



Could Notch and Delta perform the function represented by these two-way arrows in A?

What about these inhibitory arrows in B?

Let's examine this step by step, using the Drosophila X-ray-induced clone data, and the simplest case of only 2 cells.



Since Notch and Delta are both: 1) transmembrane proteins, 2) widely expressed, and 3) their mutants have similar phenotypes - we'll start with an educated guess that they probably function together in the same process (i.e. by binding to each other.)

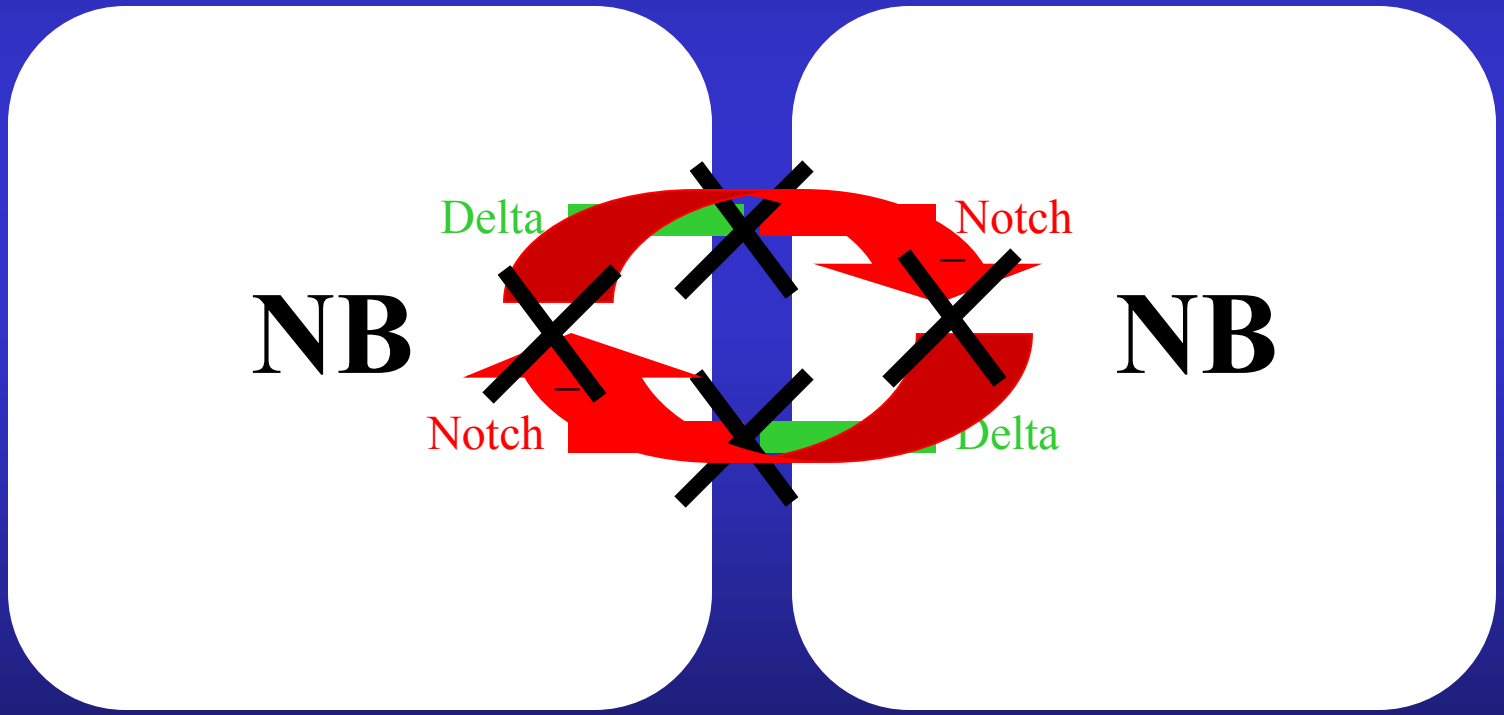
Since we're focusing on lateral inhibition as a model, we'll further take as a starting supposition that these proteins might communicate across adjacent cells

Let's examine this step by step:

1) using *Drosophila* mitotic recombination clone data

2) and the simplest case of only 2 cells.

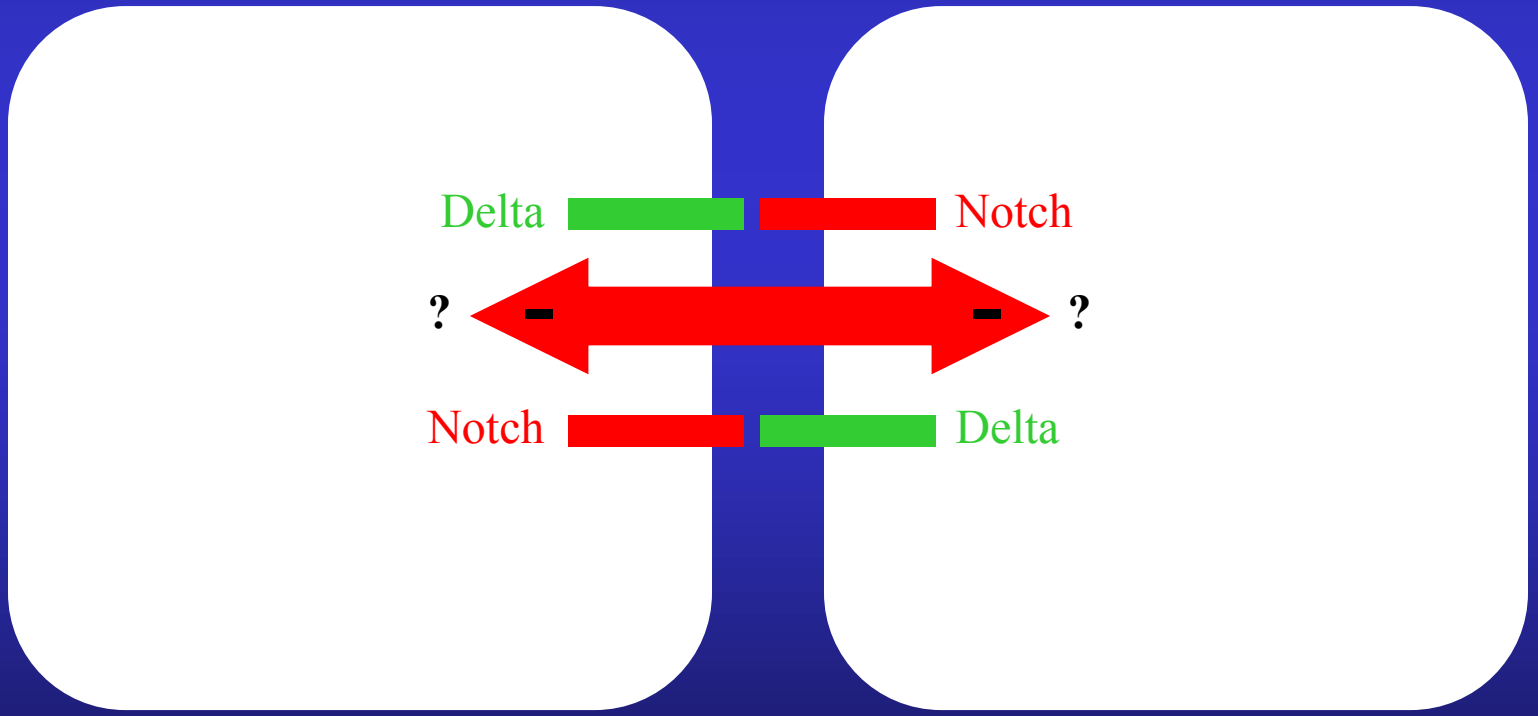
If we eliminate either *Notch* or *Delta* (*e.g.* by mutation)…



….then both cells differentiate as neurons (the "Neurogenic phenotype"). This supports the hypothesis that these proteins are indeed acting in lateral inhibition

Let's examine this step by step, using the Drosophila X-ray-induced clone data, and the simplest case of only 2 cells.

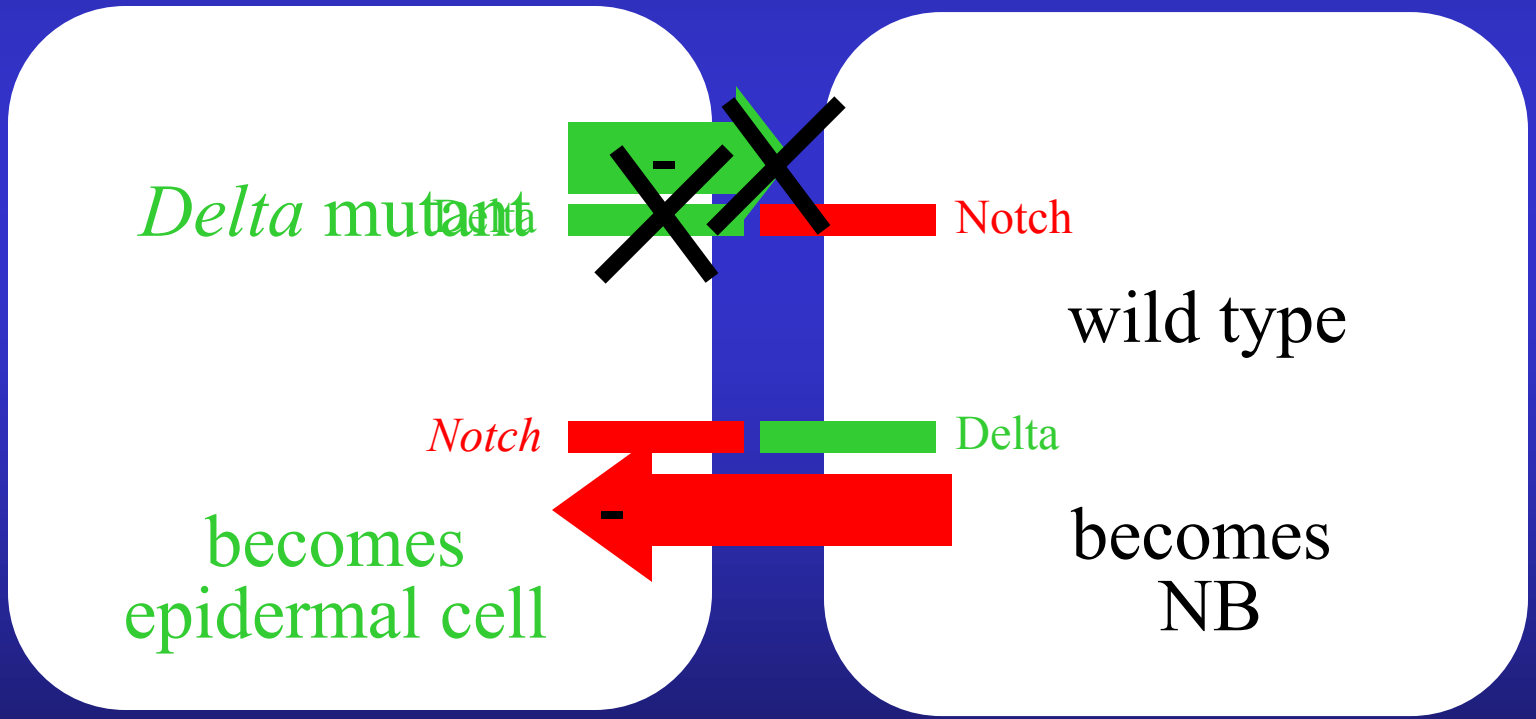
But *in which direction* does this lateral inhibition signaling go?



Notch could be signaling through Delta, or the other way around…

Let's examine this step by step, using the Drosophila X-ray-induced clone data, and the simplest case of only 2 cells.

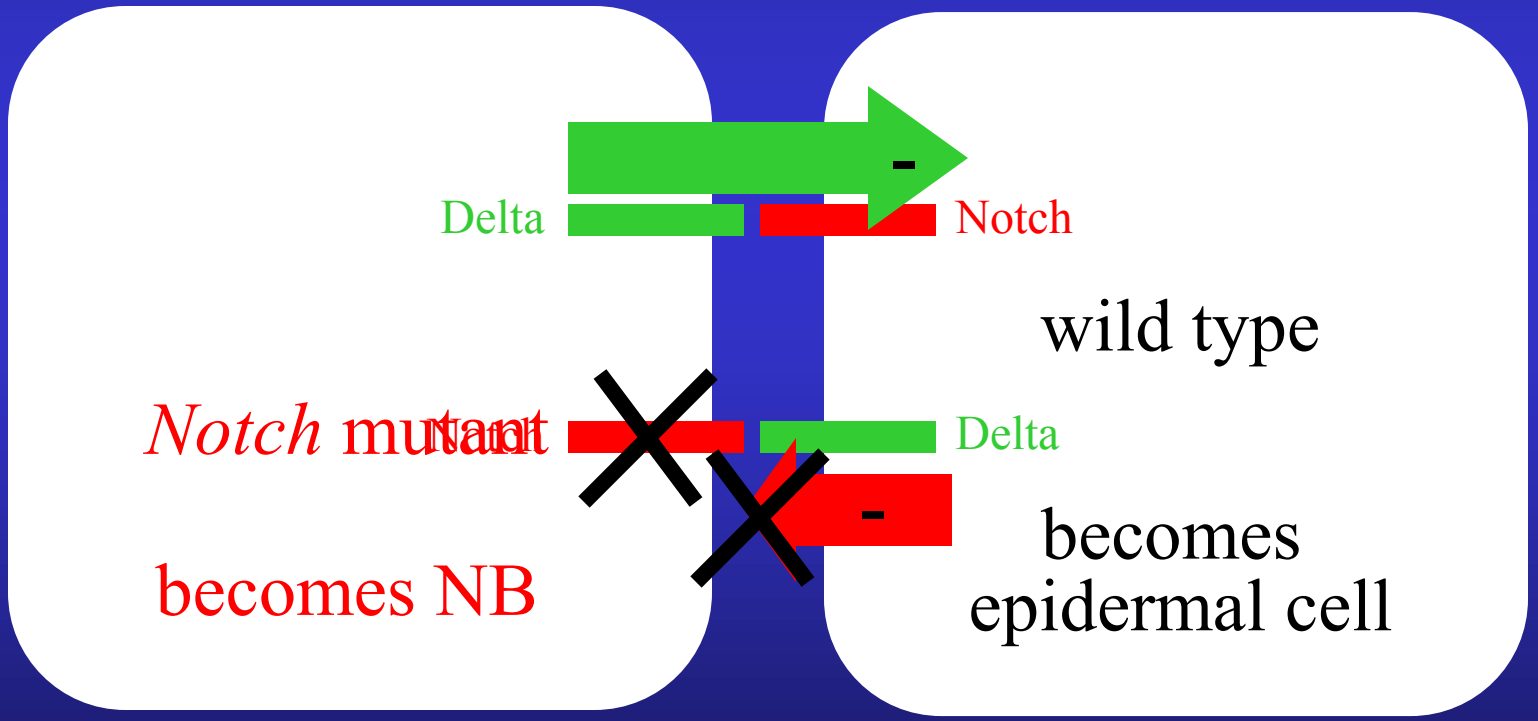
In the mitotic recombination experiments of Heitzler & Simpson, we learned that if a *Delta* mutant cell is next to a wild type cell, the *Delta* mutant cell tends to assume an epidermal fate (88% of the time), while the adjacent wild type cell tends to assume the neural fate.



If lateral inhibition is the working model, this suggests that Delta is responsible for SENDING the negative signal from the presumptive neuroblast to its neighbors (so the *Delta* mutant cell can't inhibit its neighbor and always become epidermal)

Let's examine this step by step, using the Drosophila X-ray-induced clone data, and the simplest case of only 2 cells.

In the mitotic recombination experiments of Heitzler & Simpson, we learned that if a *Notch* mutant cell is next to a wild type cell, the *Notch* mutant cell tends to assume a neural fate (88% of the time), while the adjacent wild type cell tends to assume the epidermal fate.



This suggests that Notch is responsible for <u>RECEIVING</u> the negative signal from an adjacent cell (so the *Notch* mutant cell can't be inhibited and always become neural…
…forcing the adjacent cell to become epidermal)

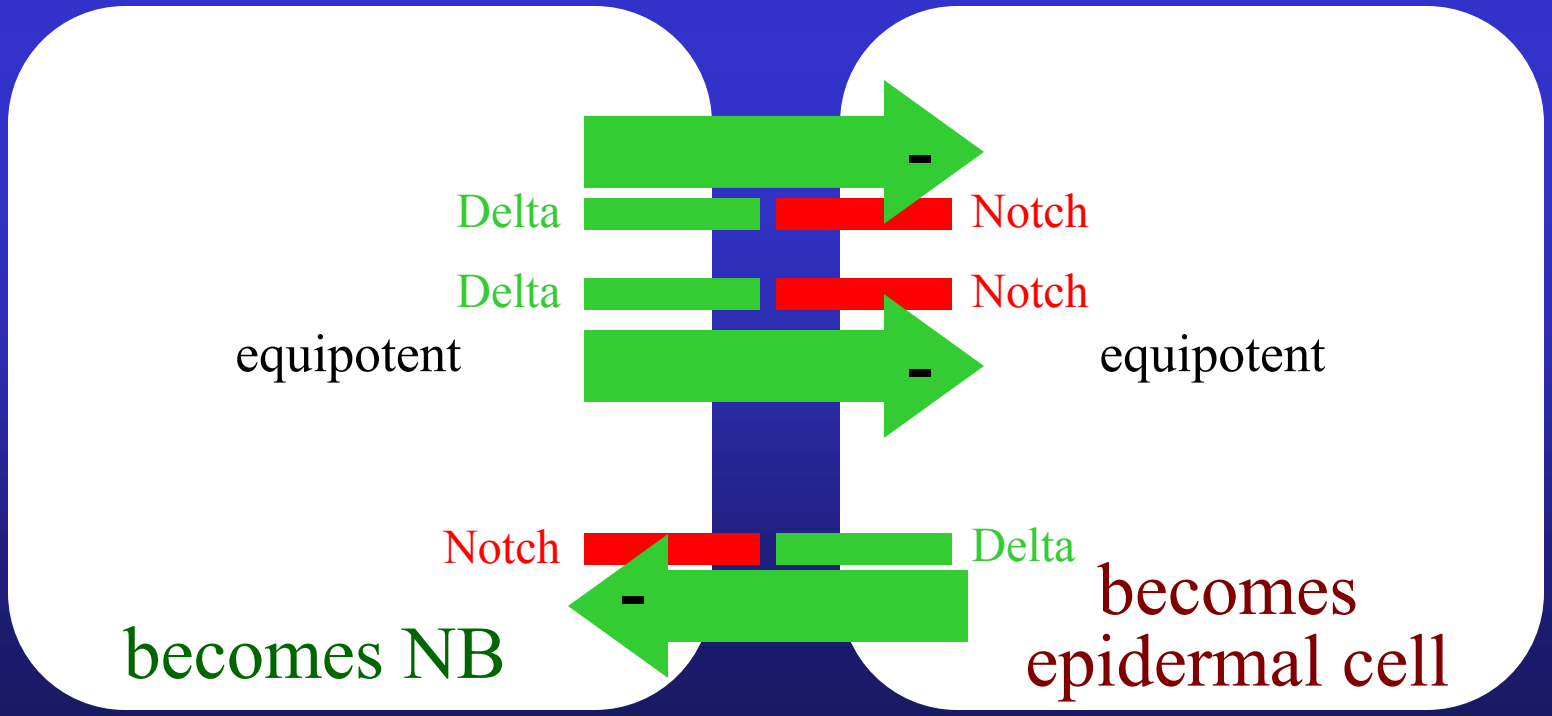
Here's the model as we now have it in the wild type situation; only including Notch and Delta.

Cells in the equivalence group (neuroectoderm) start out with equal potential.

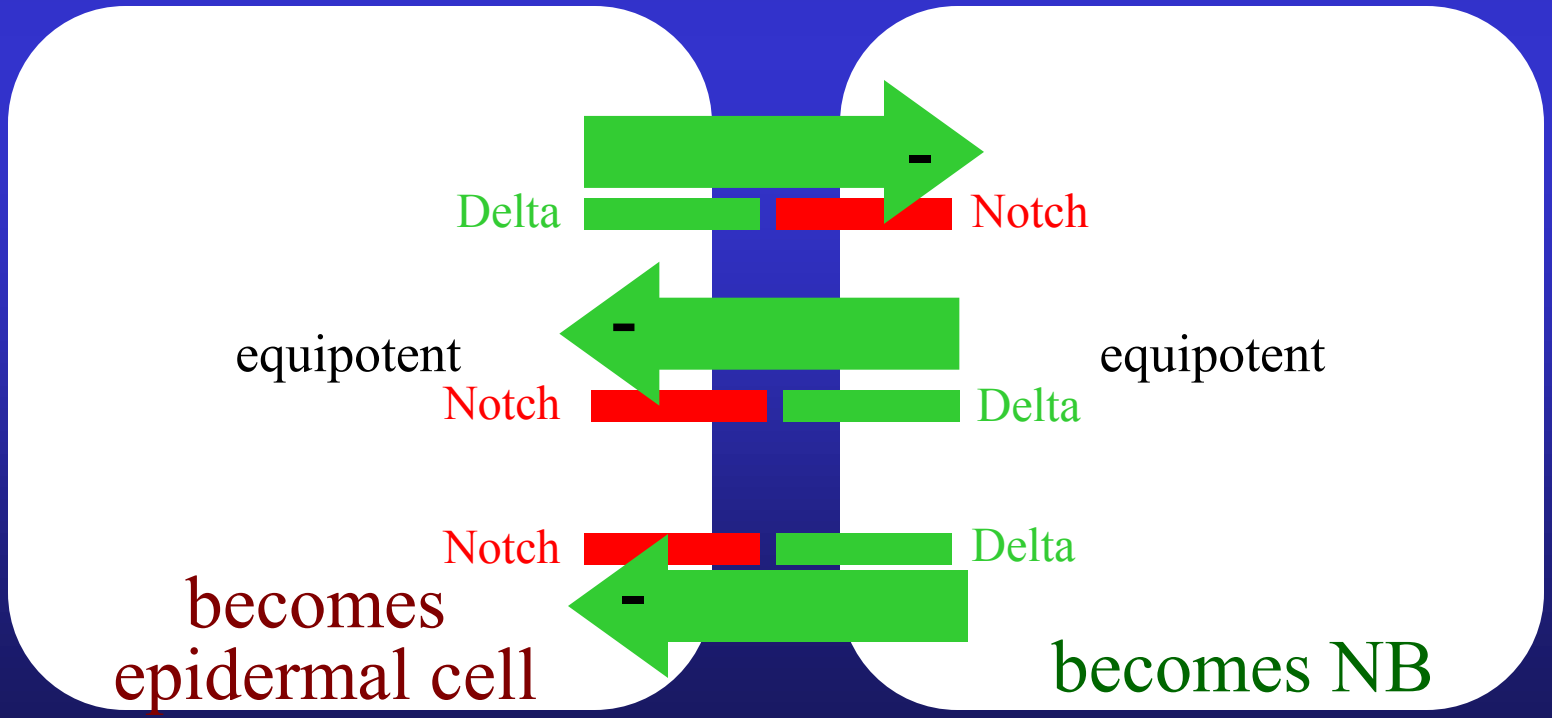
Both express equal levels of Notch and Delta initially, and so are in a state of competitive (unstable) equilibrium.

Through some process [stochastic? local cytoarchitecture? (remember that in reality there are more than 2 cells participating) Other molecular (signaling) influences?] one starts to express a bit more Delta than the other, thus increasing lateral inhibition on its neighbor.

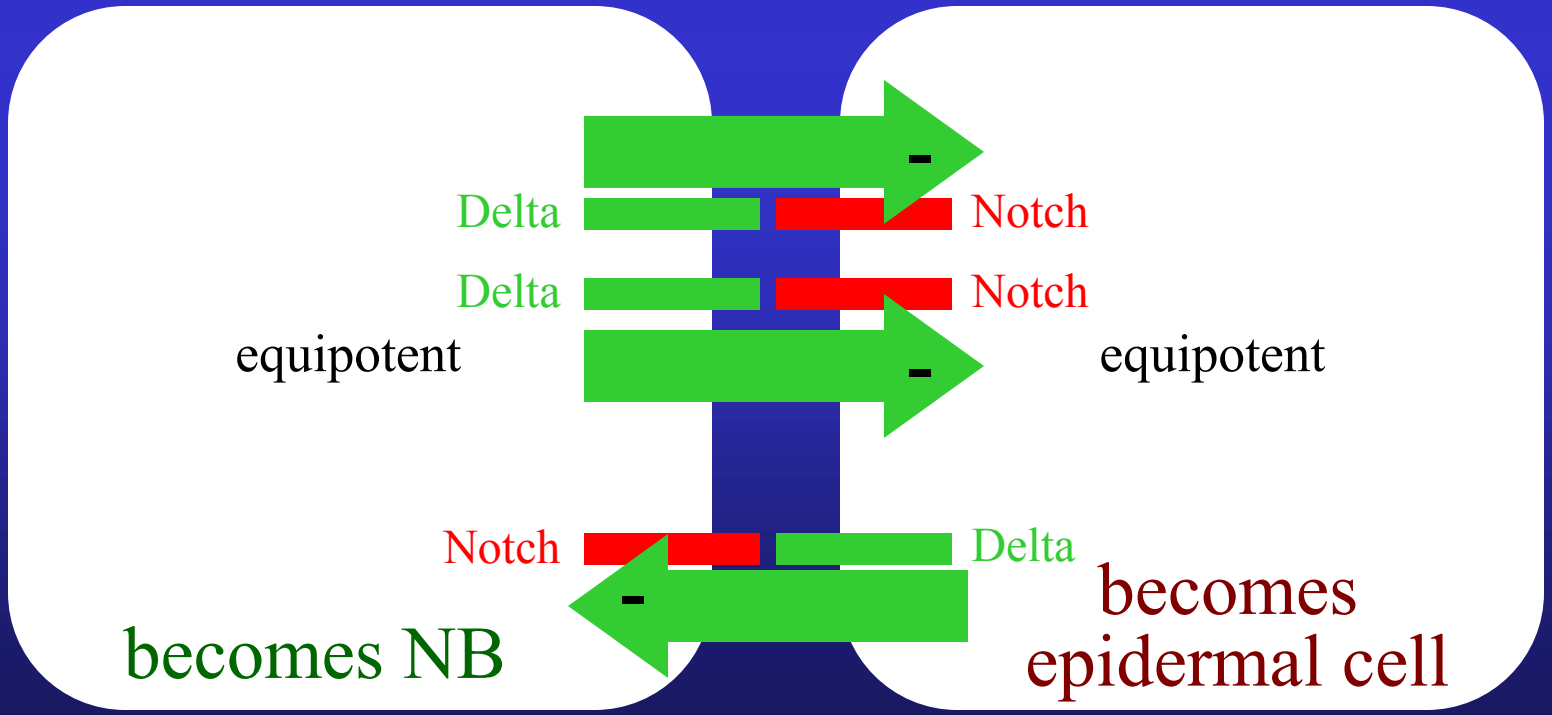
This immediately drives the system on a run-away course away from equilibrium, so that one cell adopts a neural fate, and ensures that its neighbor does not.



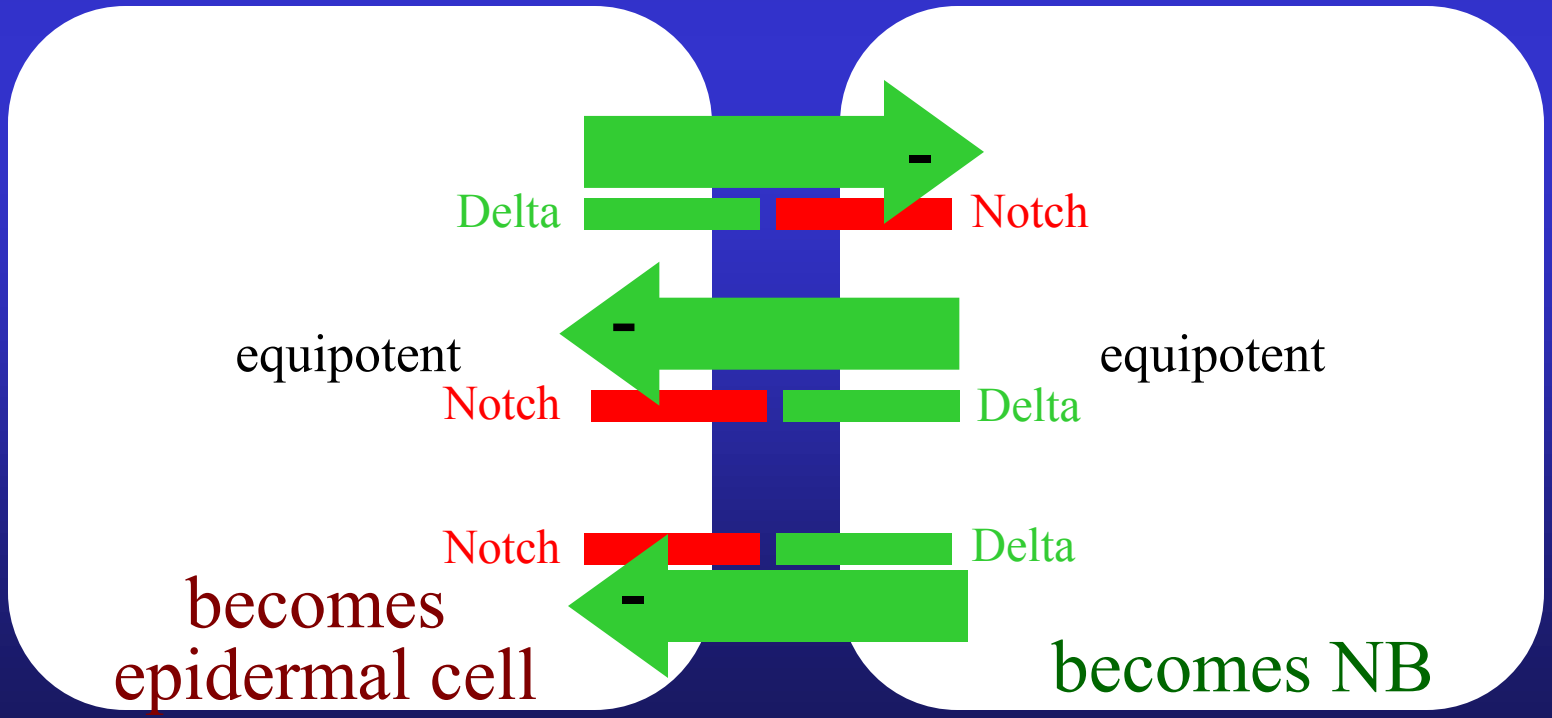
Either cell can become the NB or the epidermal cell, depending on initial conditions, chance, or how the system is experimentally manipulated (i.e. ablating one cell, making one cell mutant for *Notch* or *Delta*, etc.)



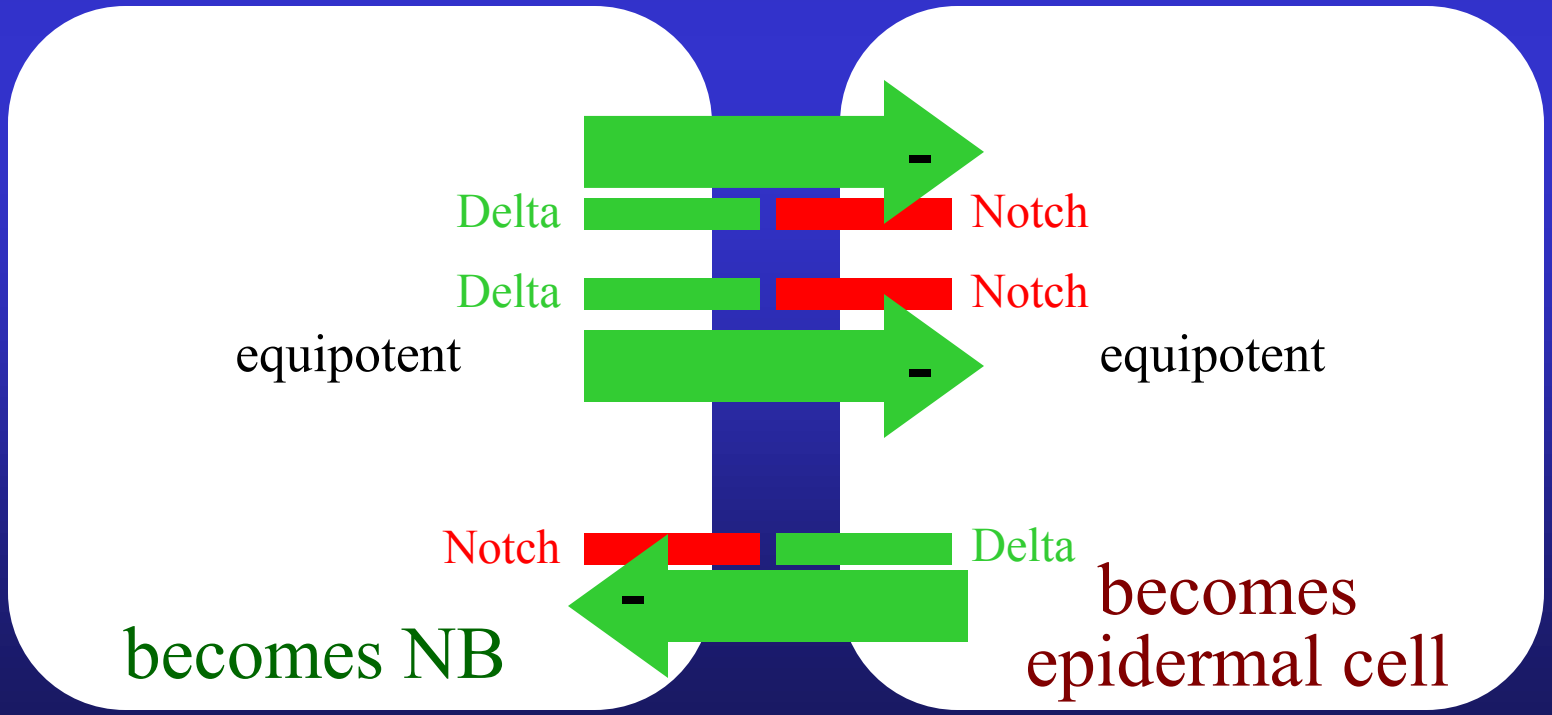
Either cell can become the NB or the epidermal cell, depending on initial conditions, chance, or how the system is experimentally manipulated (i.e. ablating one cell, making one cell mutant for *Notch* or *Delta*, etc.)



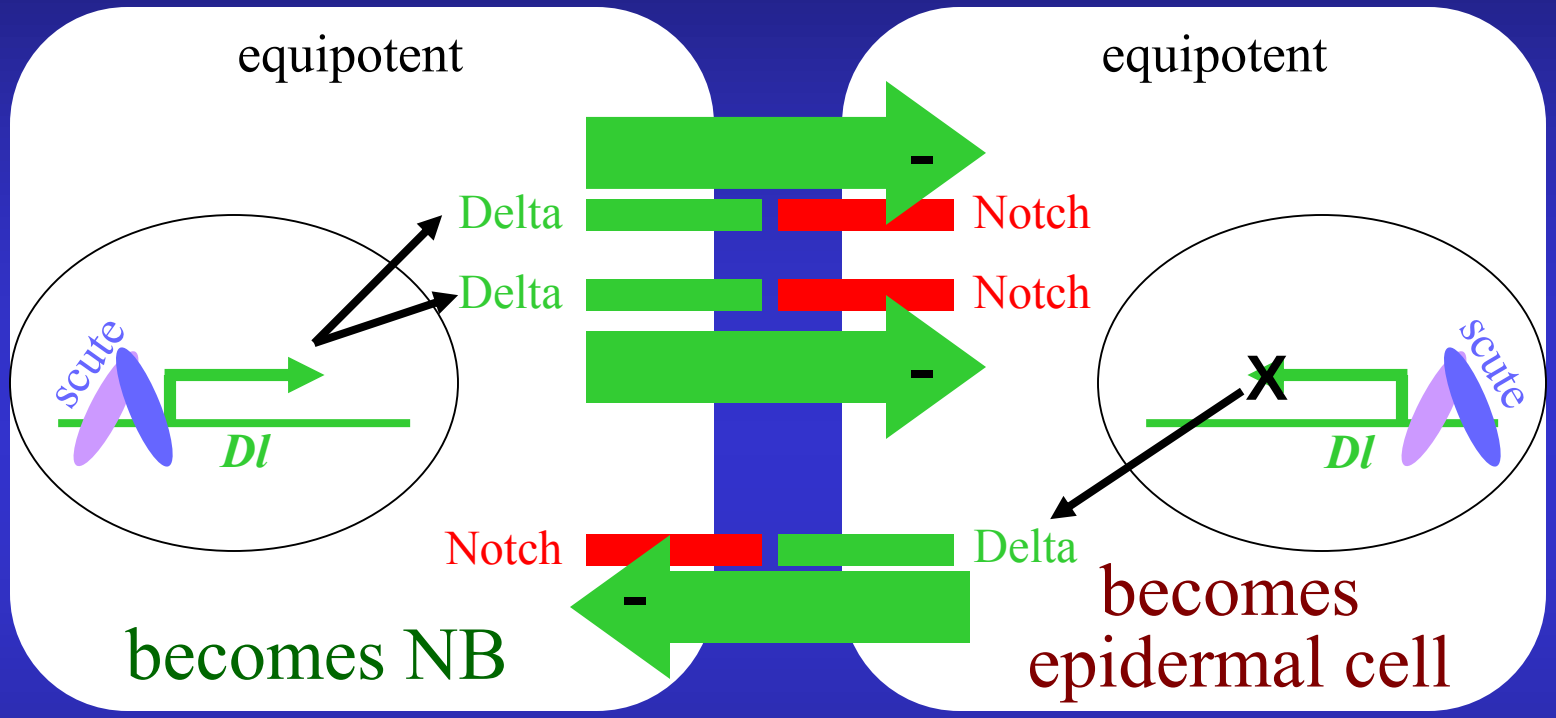
Either cell can become the NB or the epidermal cell, depending on initial conditions, chance, or how the system is experimentally manipulated (i.e. ablating one cell, making one cell mutant for *Notch* or *Delta*, etc.)



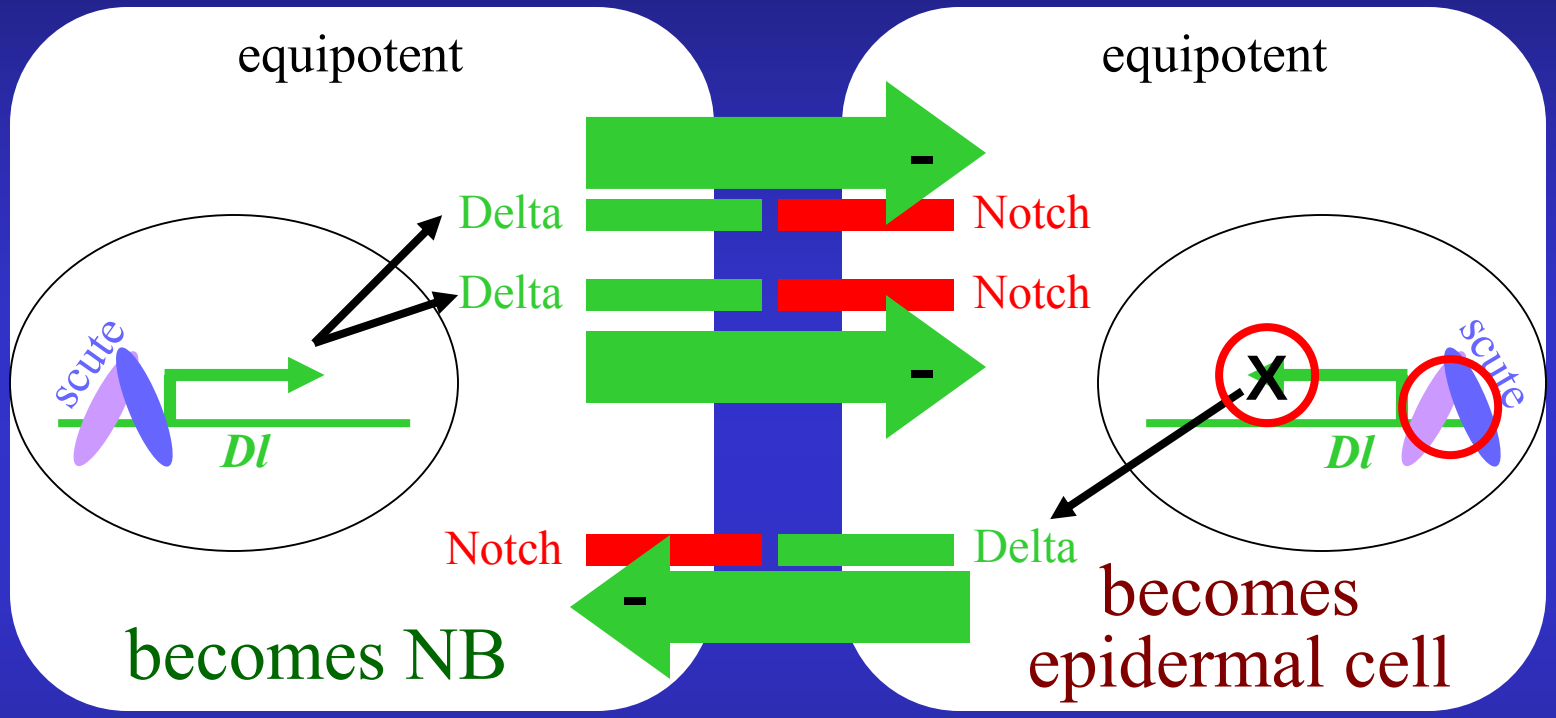
Either cell can become the NB or the epidermal cell, depending on initial conditions, chance, or how the system is experimentally manipulated (i.e. ablating one cell, making one cell mutant for *Notch* or *Delta*, etc.)



Knowing that the proneural transcription factors encoded by the *achaete-scute* complex positively regulate *Delta* expression, we can add this nuclear component into the model.



Knowing that the proneural transcription factors encoded by the *achaete-scute* complex positively regulate *Delta* expression, we can add this nuclear component into the model.



Notice: I've kind of gotten ahead of myself. Our model would work a lot better if there was something to **TURN OFF** expression of *scute* (and *Delta*) in the presumptive epidermal cell.

Which signaling molecule is in position to do this?

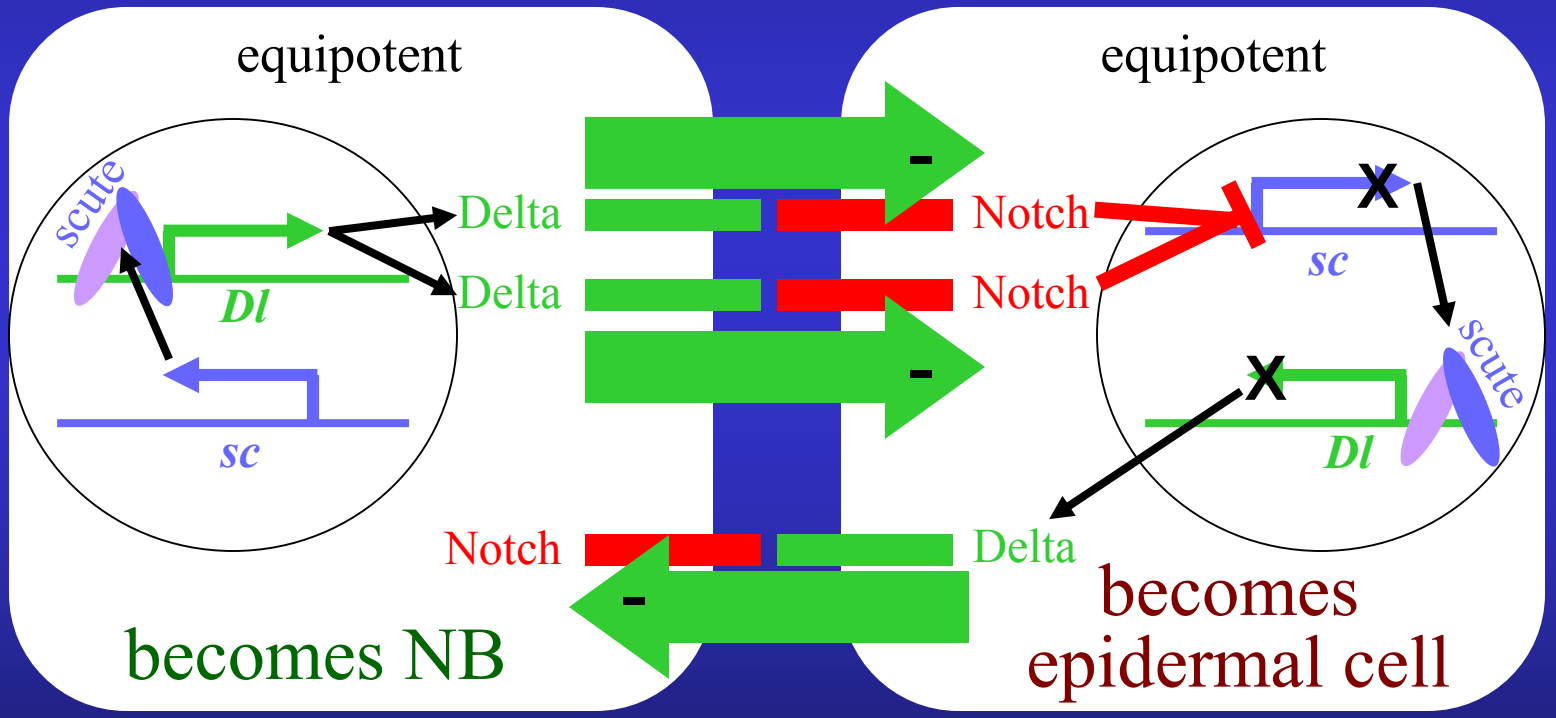
….I'm taking suggestions from the audience.

Of course, the answer is Notch!

1. Initially: the *proneural* transcription factors (*e.g. scute*) are turning on the *Delta* gene in both cells; the Delta and Notch proteins are laterally signaling to both cells, which are therefore in a state of unstable equilibrium.

2. One cell expresses *Delta* a bit more than the other
(perhaps it's getting less lateral inhibitory signals from its neighbors due to the shape of its cell contacts)

3. This stimulates the Notch signaling cascade in its neighbor. Notch signaling TURNS OFF the *achaete-scute* complex. Consequently, this cell stops expressing *Delta*.

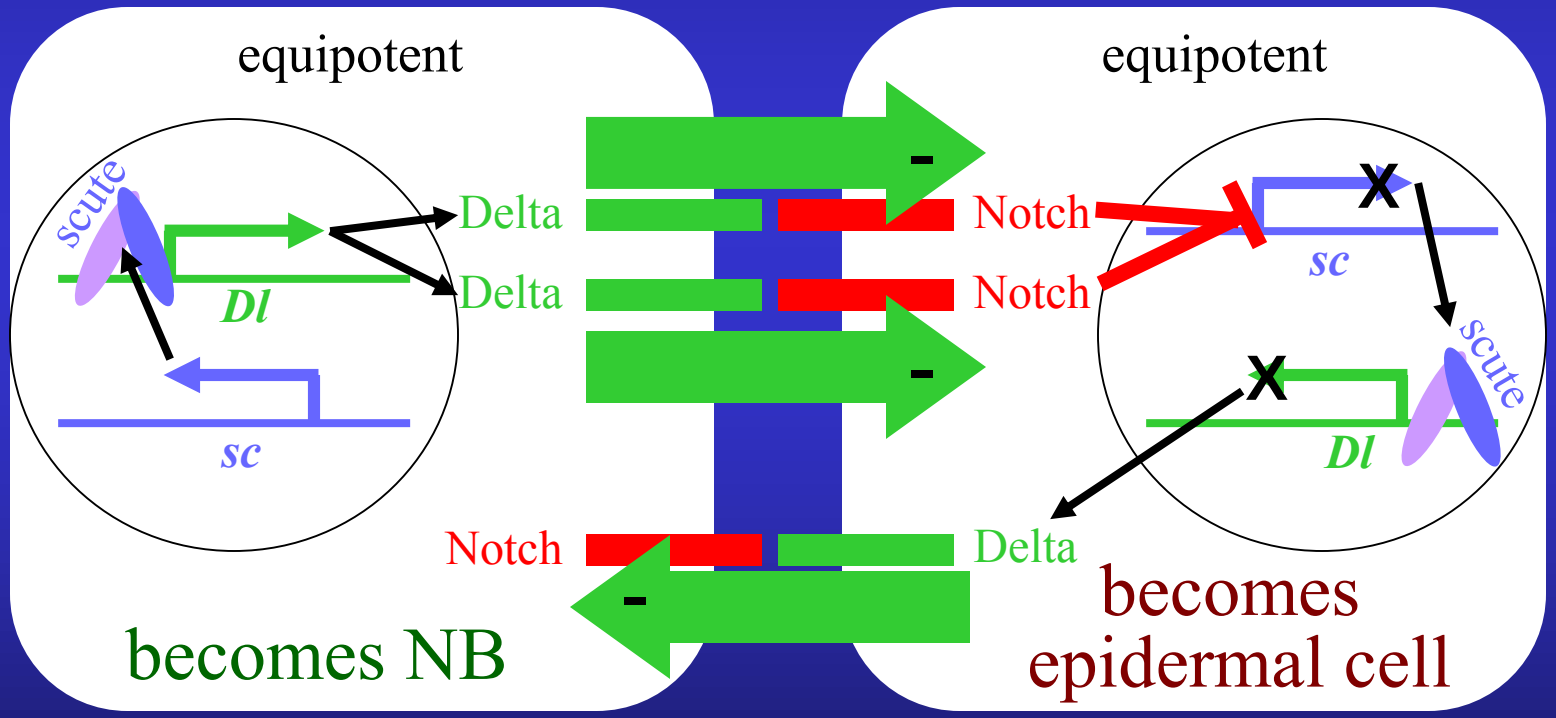


The net result is that one cell has the *achaete-scute* complex ON and makes lots of Delta protein. This cell differentiates down the neural lineage. The other cell, responding through Notch signaling to the high Delta levels in its neighbor, turns the *achaete-scute* complex OFF. This cell stops making Delta, and differentiates down the epidermal lineage.

I thought I'd better run through that one more time…

1. Initially: the *proneural* transcription factors (*e.g. scute*) are turning on the *Delta* gene in both cells; the Delta and Notch proteins are laterally signaling to both cells, which are therefore in a state of unstable equilibrium.
2. One cell expresses *Delta* a bit more than the other
(perhaps it's getting less lateral inhibitory signals from its neighbors due to the shape of its cell contacts)

3. This stimulates the Notch signaling cascade in its neighbor. Notch signaling TURNS OFF the *achaete-scute* complex. Consequently, this cell stops expressing *Delta*.



The net result is that one cell has the *achaete-scute* complex ON and makes lots of Delta protein. This cell differentiates down the neural lineage. The other cell, responding through Notch signaling to the high Delta levels in its neighbor, turns the *achaete-scute* complex OFF. This cell stops making Delta, and differentiates down the epidermal lineage.

There is one more component that I mentioned earlier, that we should add in to complete our basic model of Lateral Inhibition

Remember, several slides back (when our model was just getting started) I listed some major Neurogenic genes in *Drosophila*, all of which result (when mutated) in making too many neurons and not enough epithelial cells.

In other words, in these mutants too many cells in the equivalence group decide to become neurons, as though Lateral Inhibition were not working properly.

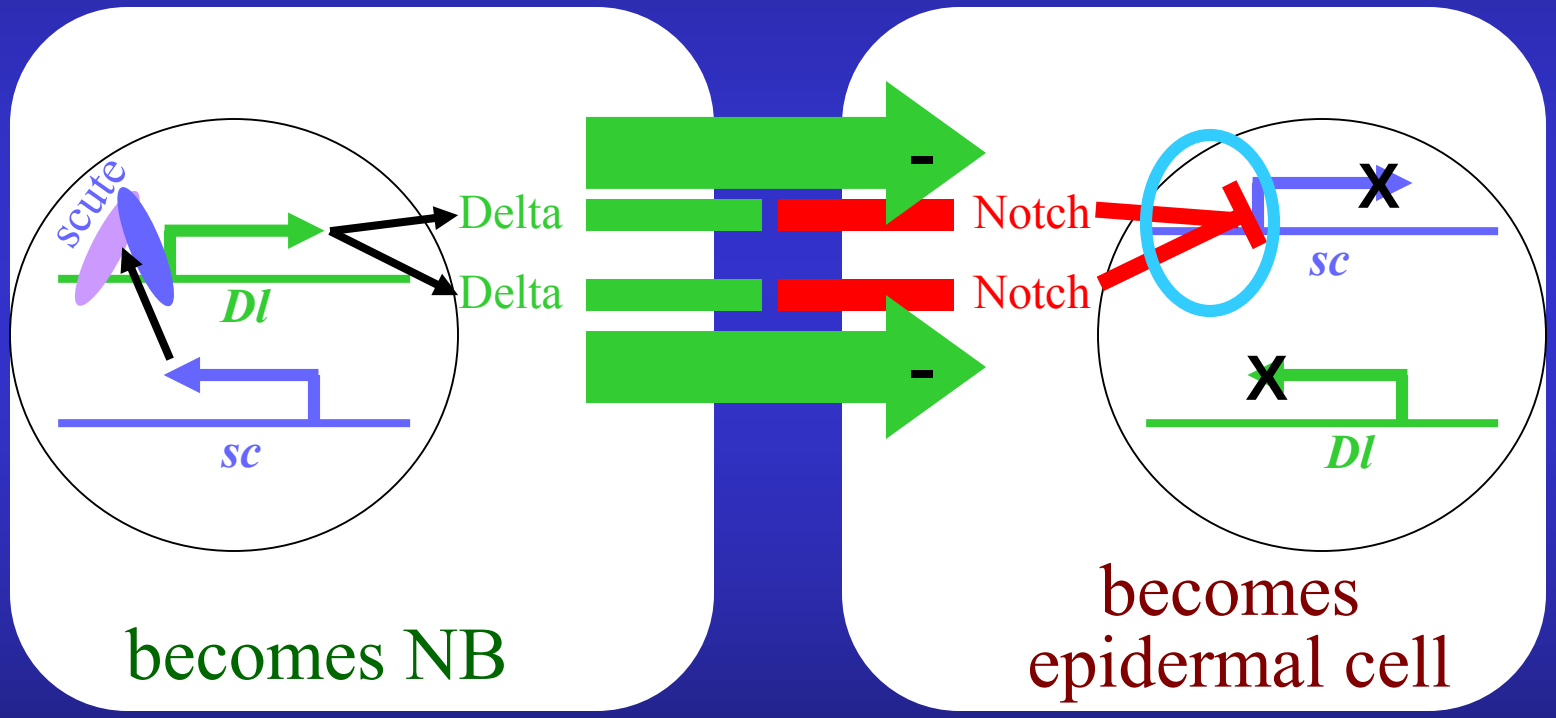
Here's that list again…

Important neurogenic genes (for this topic):
Delta – transmembrane protein
Notch – transmembrane protein
Suppressor-of-hairless – transcription factor
Enhancer-of-split complex – transcription factors

So where do you think the *Suppressor-of-Hairless* and *Enhancer-of-split* genes fit into all this?

Key points to consider:

1. These are transcription factors just like the *achaete-scute* gene products
2. Based on their mutant phenotype, they are required to make epithelial cells (*Su(H)* and *E(spl)* mutants have too many neurons, not enough epithelium)

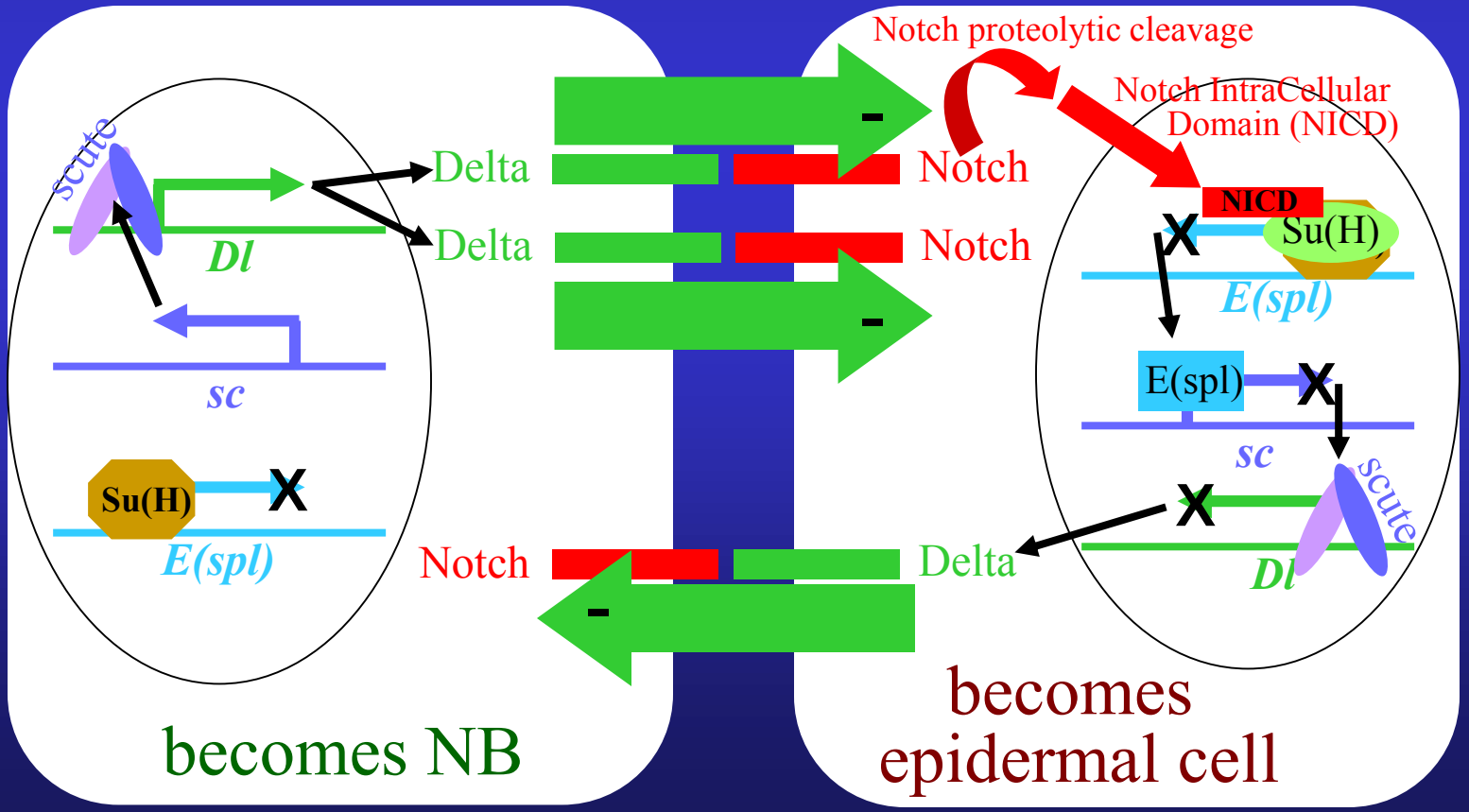


Any suggestions?

Hint: What is downstream of Notch?

Notch signaling works through a cascade of transcriptional repressors, exemplified by the Su(H) and E(spl) gene products

1. In the absence of a Notch signal, the Su(H) protein acts as a transcriptional repressor at *E(spl)*
2. Delta binding causes intramembrane proteolytic cleavage of the Notch protein, releasing the Notch IntraCellular Domain (NICD) into the cytoplasm.
3. The NICD traffics to the nucleus where it binds to Su(H) protein;
   Together, NICD+Su(H) act as transcriptional co-activators at the *E(spl)* locus, turning it ON.
4. E(spl), in turn, acts as a transcriptional repressor at the *achaete-scute* complex, turning this off and committing the Notch-receiving cell to an epidermal fate.



Got complicated pretty fast didn't it?

Notch signaling *is* very complicated

(a lot more than what I've presented)

…but also very important.

It's highly conserved across species and is used pretty much wherever equipotent cells in an equivalence group need to choose their fate cooperatively (or competitively, depending on how you look at it).

It is especially prevalent in neural development

This is why *all* of you should be at least acquainted with it.

Drosophila and vertebrate CNS
Drosophila and vertebrate PNS
Drosophila and vertebrate eye (retina)
Many other organ systems…

Major take home points

(what I want you to remember after you've forgotten every thing else I said)

- Lateral inhibition (also called lateral specification) is the process whereby neighboring cells in an equivalence group communicate about cell fate decisions

("You go this way, I'll go that way".)

- This was worked out in insects, first through relatively crude tissue ablation experiments, but then refined with increasingly sophisticated genetic and molecular approaches in *Drosophila melanogaster*.
- It almost invariably involves Notch signaling, which is highly conserved among other animals.
- It is very important particularly during development of the nervous system – including in the CNS of vertebrates

you *will* encounter it again.

Clinical Correlations

CADASIL ("Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy") is the most common form of hereditary stroke disorder, and is thought to be caused by mutations of the *Notch 3* gene on human chromosome 19

Alzheimer's Disease: γ-Secretase is a protease that cleaves Notch, and also cleaves APP (Amyloid Precursor Protein)…

Some other names for Delta-Notch family members

(underlined ones are probably worth *recognizing*)

Delta, Serrate (flies);
Delta-like (Dll), Jagged (vertebrates)

Lag-2 (worms)

all have extracellular EGF repeats

Notch (flies and vertebrates)

Lin-12 (worms)

lots of extracellular EGF repeats and a cleavable NICD

Presenilin (gamma-secretase that clips Notch into NECD & NICD

also important in Alzheimer's Disease

Suppressor of Hairless:

CBF1 (human), Lag-1 (worms)

Hairy, Enhancer of split (flies), Hes1 (vertebrates)

Ref-1 (worms)

Tomorrow we'll talk about a different type of signaling which is fundamentally different from Delta-Notch in that the signaling molecules involved are not transmembrane proteins, but are secreted from their cellular source.

For today and tonight, please think a bit about the likely properties of such secreted signaling molecules. What might they be good for when compared to the Delta-Notch signaling pathway? How are their signaling mechanisms and cellular roles likely to differ?

Lateral Inhibition:

Which one will become Barbie?

Either way, the other will then become…something else