Neuronal Determination and Differentiation

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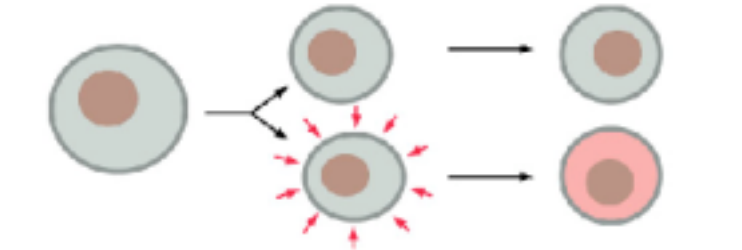
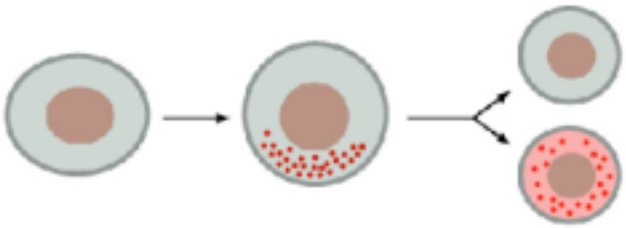
7.68J/9.013J

Cell differentiation strategies

- Cell differentiation achieved through differential gene expression
- Strategies for setting up differential gene expression:
  - Symmetric division-cell:cell signaling
    - Receive extrinsic determinants (signals)
  - Asymmetric division
    - Inherit intrinsic determinants



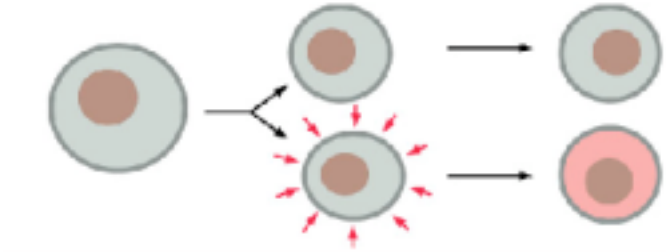
symmetric division : sister cells become different as result of influences acting on them after their birth



asymmetric division : sister cells born different

Extrinsic determinants

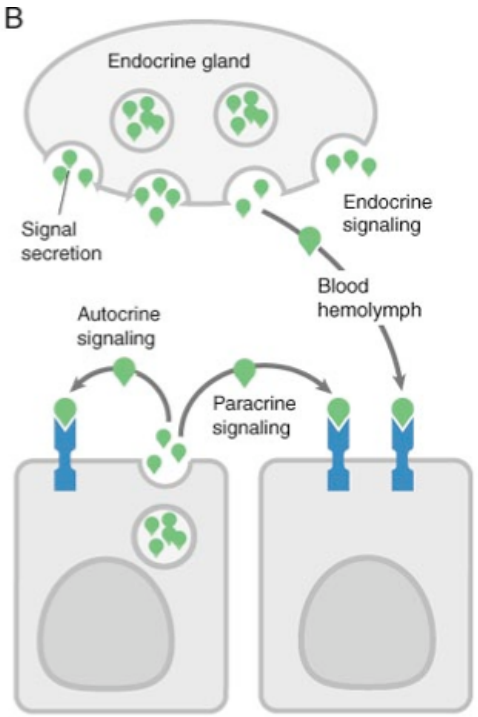
- **Extrinsic determinants external signals**



symmetric division : sister cells become different as result of influences acting on them after their birth

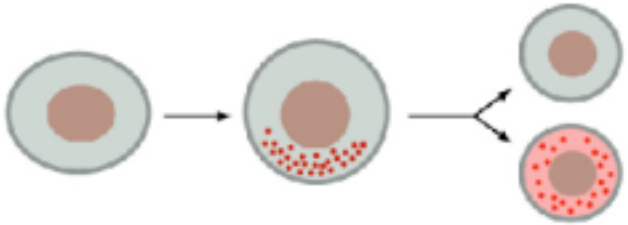
Extrinsic determinants

- **Sources of external signals:**
  - **Distant tissue: endocrine signal**
  - **Nearby cell: paracrine signal**
  - **Self: autocrine signal**
- **Common signals:**
  - **Secreted/cell-surface proteins**
  - **Hormones**

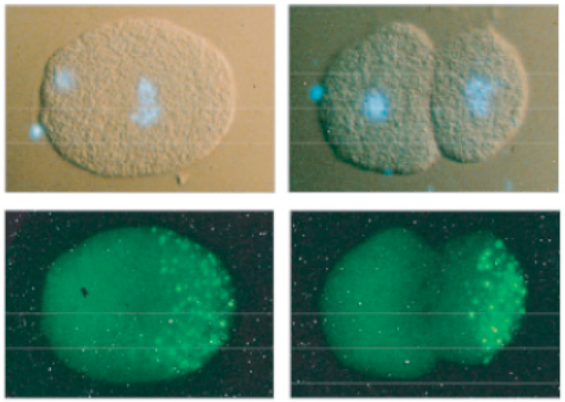


Intrinsic determinants

- **Differentially inherited factors**



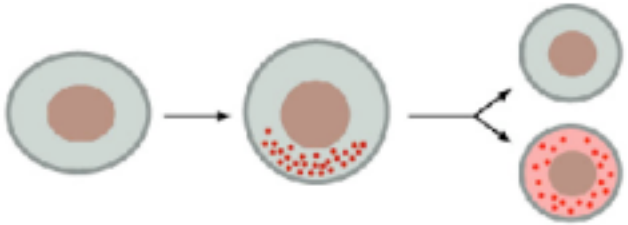
asymmetric division : sister cells born different



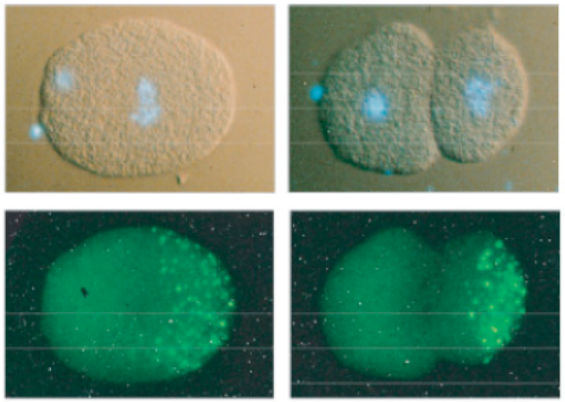
C. elegans embryo -- P-granule

Intrinsic determinants

- **Examples of intrinsic determinants:**
- **Protein: eg.,**
  - **Transcriptional regulator**
  - **Signal transduction regulator**
- **RNA: eg.,**
  - **mRNA for transcriptional regulator**
  - **mRNA for signal transduction regulator**



asymmetric division : sister cells born different

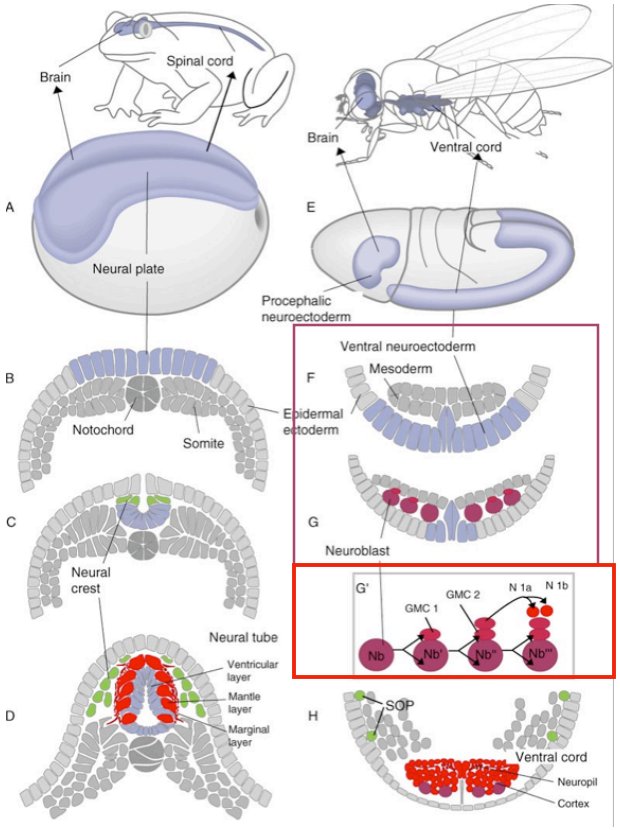


C. elegans embryo -- P-granule

Common terms

- **Extrinsic determinants: external signals**
- **Intrinsic determinant: resides within cell from its birth**
- **Induction: action of external signal to promote cell fate**
- **Competence: ability of cell to respond to inductive signal**
- **Equivalence group: cells of equal competence**

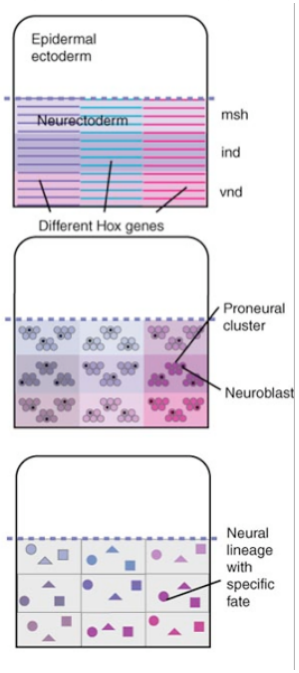
Neural development in amphibians and insects



Neuroblast determination:
Lateral inhibition

Neurons and glia from neuroblasts

Generation of neurons and glia in insects: example of key mechanisms



Patterning of neurectoderm

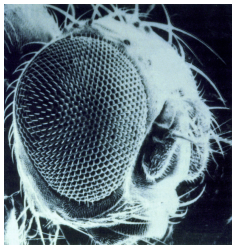
Early patterning sets competence

Neuroblast selection

Lateral inhibition

Specification of neuroblast progeny

Intrinsic determinants

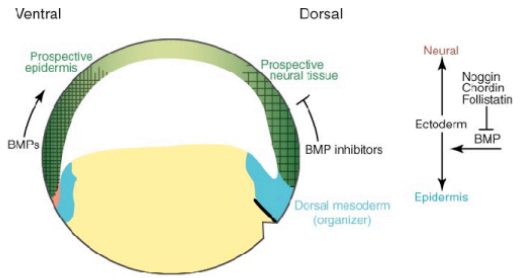


Retinal development

Competence, Equivalence group, Induction

Neural Induction (review)

Vertebrates:
Inhibition of BMP signaling promotes neural induction



Inhibition of BMP signaling is also involved in neural induction in invertebrates

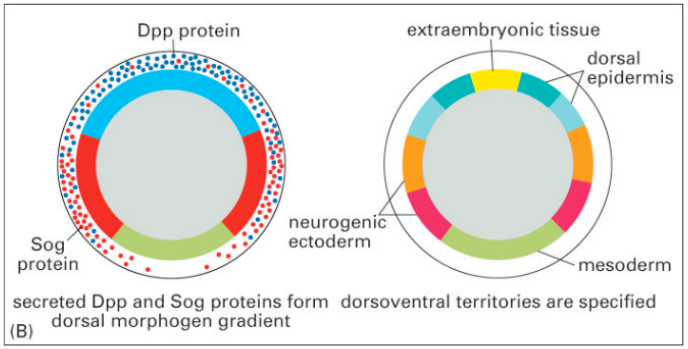
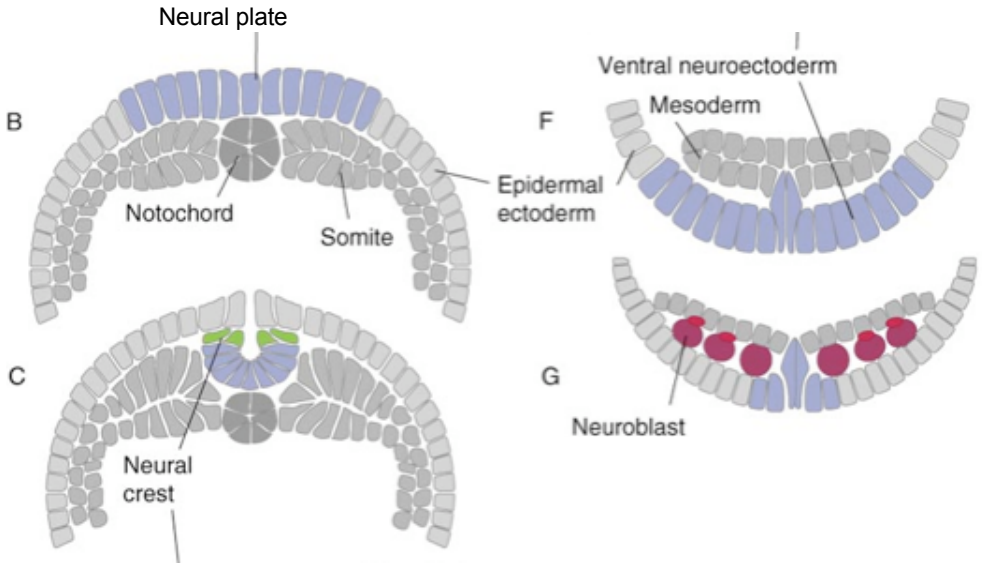


Figure 21–33 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Generation of neural stem cells

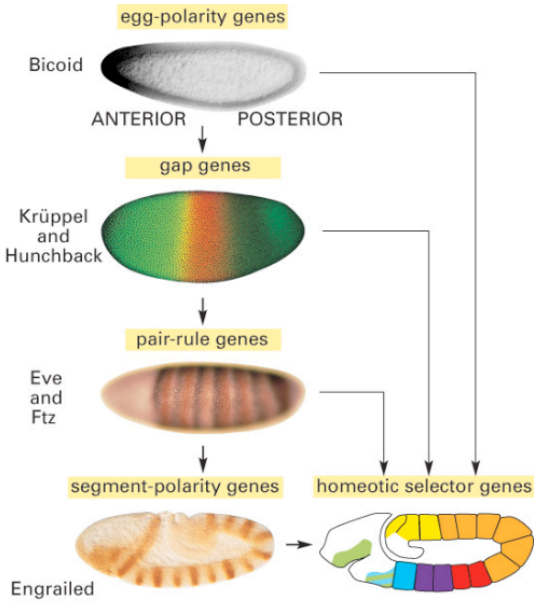
<u>Amphibians</u>:
All dorsal neurectoderm cell appear to become neural stem cells

<u>Insects</u>:
Subset of neurectoderm cells become neural stem cells: Neuroblasts



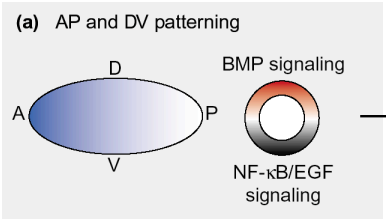
Early AP patterning in *Drosophila* : Progressive subdivision of embryo

- Different levels of Bicoid activate different "Gap genes" in different regions along AP axis
- These gap genes cross-regulate one another to set up sharper boundaries
- Gap genes act in combination to regulate downstream pair-rule genes --- which are expressed in narrower regions
- Segment-polarity genes are targets of pair-rule genes --- yielding even finer regional regulation
- Sets up pattern of homeodomain-containing homeotic selector genes

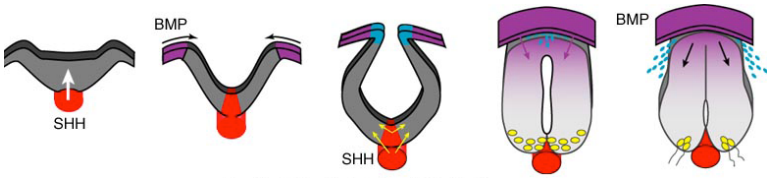


Neurectoderm patterning in insects: medio-lateral system

- Set up by graded BMP and EGF signaling:
  - BMP signaling highest in Dorsal regions
  - EGF signaling (EGF -- protein ligand/EGF-receptor is RTK) highest in Ventral regions
- Analogous to opposing gradients of BMP/Shh in vertebrates

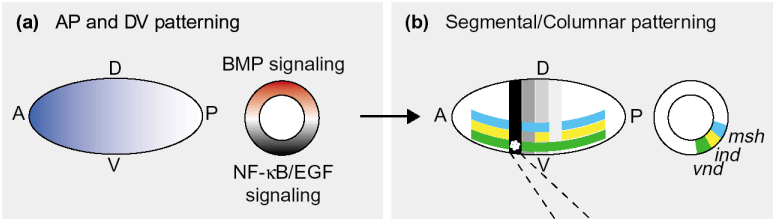


Skeath and Thor (2003) Curr. Opin. Neurobiol. 13:8.



Neurectoderm patterning in insects: medio-lateral system

- **BMP/EGF signaling sets up stripes of "columnar genes"**
- **Homeodomain-containing transcription factors:**
  - **Vnd (ventral nervous system defective)**
  - **Ind (intermediate neuroblasts defective)**
  - **Msh**



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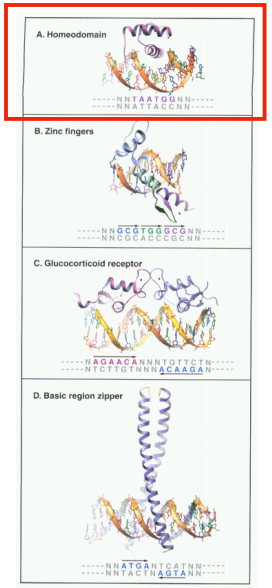
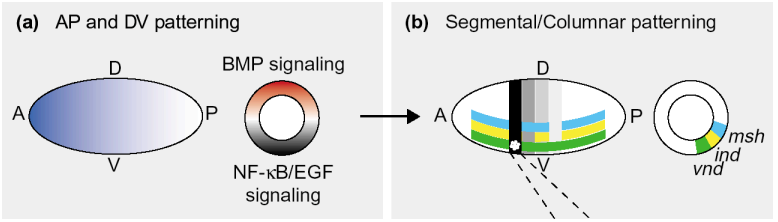


Neurectoderm patterning in insects:

- **Segmental (AP) and columnar (DV) patterning systems combine to create a Cartesian coordinate system: form checkerboard pattern of neural "equivalence groups" (cells of equal developmental potential)**
- **Gene expression profile within each group controls the identity of the neuroblasts that will form there**



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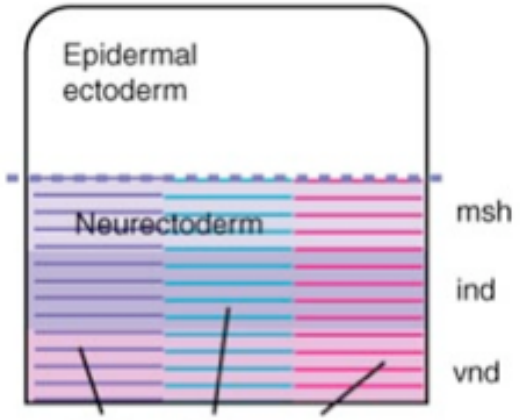
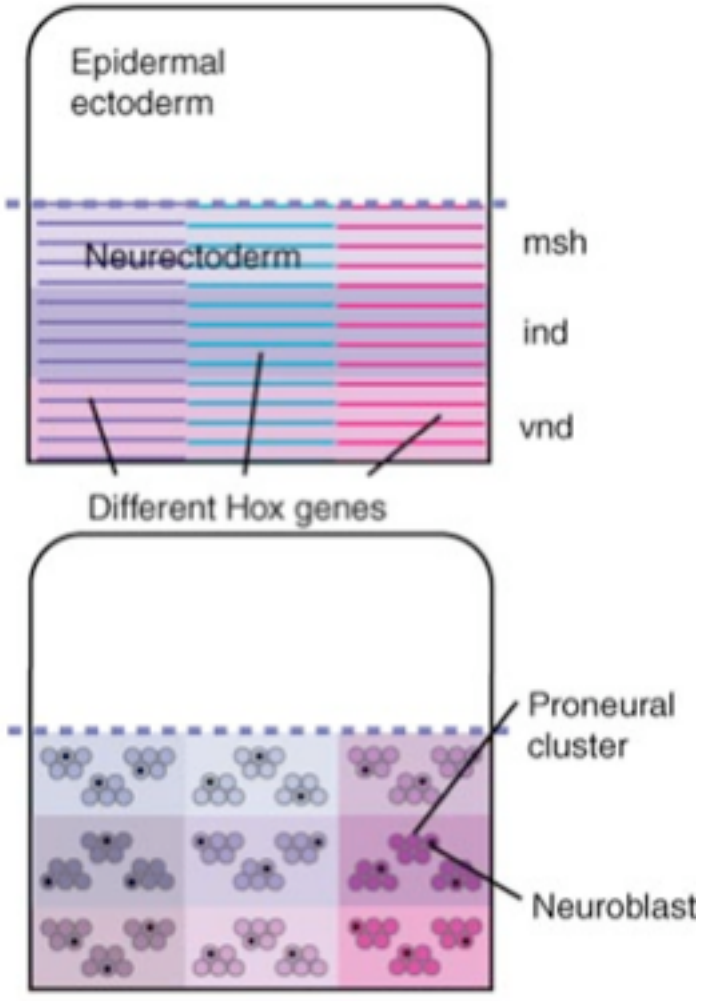


Different combo of AP pattterning genes (gap, pair-rule,segment-polarity, hox)

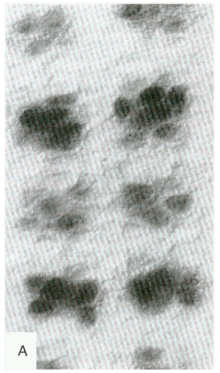
Neuroblast selection

- **Multi-step process**
  - 1) **Discrete groups of cells form proneural clusters (cells competent to form neuroblasts)**
  - 2) **Proneural cluster cells interact to determine which one will become neuroblasts (rest will become dermoblasts) : uses an** extrinsic determinant

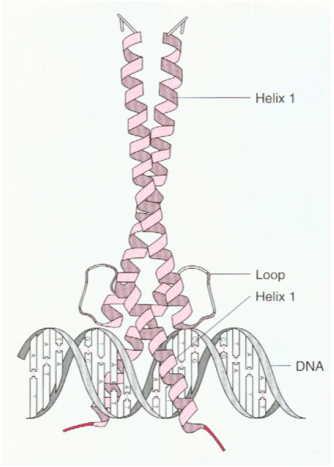


1) Formation of proneural cluster

- Combo of AP and DV patterning genes turn on expression of proneural genes in clusters of ectodermal cells (≈6 cells/cluster)
- Proneural genes: make cells competent to form neuroblasts
  - Many key proneural genes belong to a family of adjacent genes: AS-C (achaete-scute complex)
  - AS-C: encode basic-Helix Loop Helix (bHLH) transcription factors

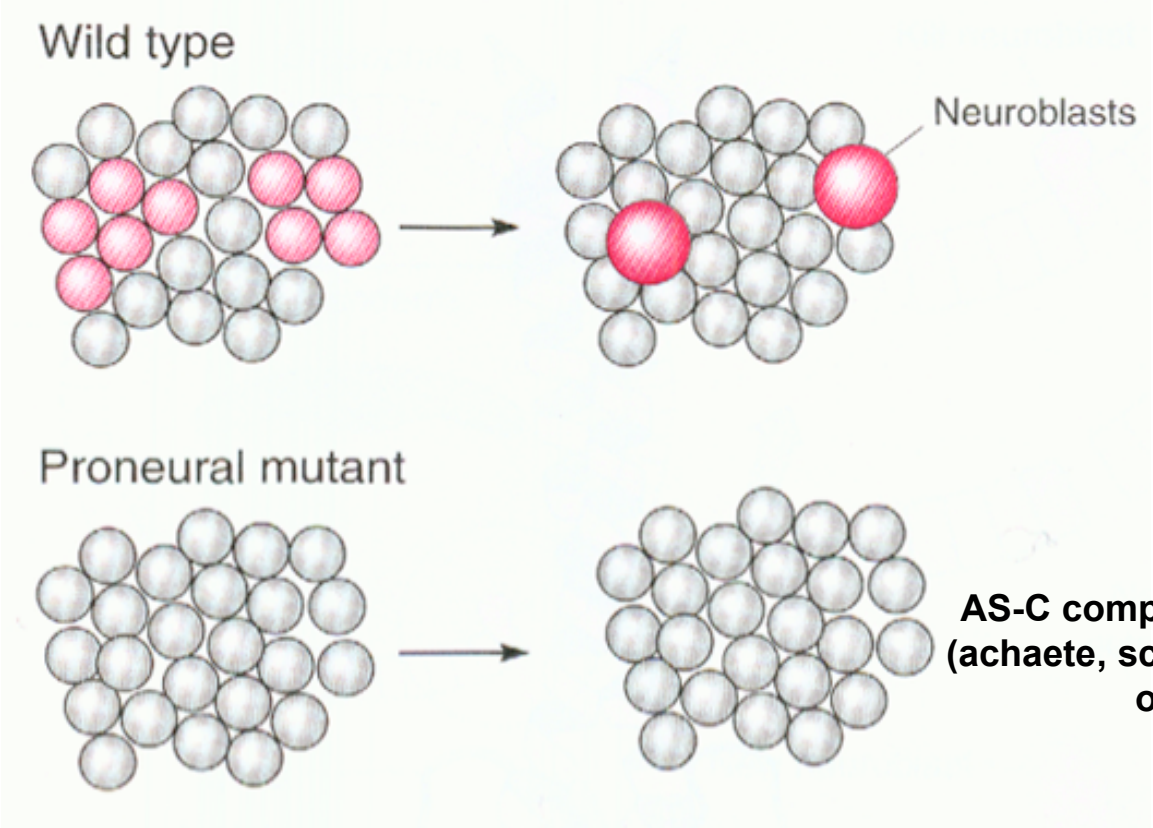


Proneural gene expression



bHLH

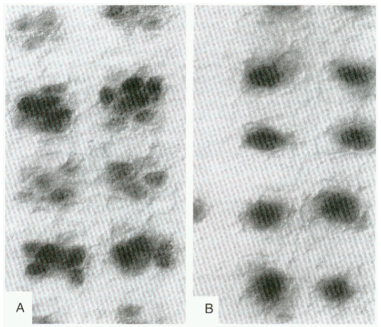
Proneural genes required for neuroblast formation



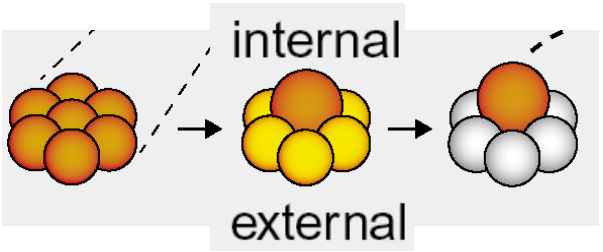
AS-C complex gene mutants (achaete, scute, asense, lethal of scute)

2) **Neuroblast specification: restriction of proneural gene expression**

- **Gradual extinction of proneural AS-C gene expression in all but one cell**



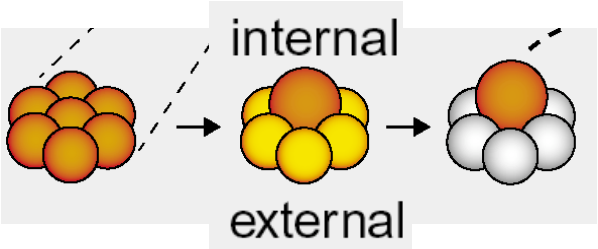
Restriction of proneural gene expression



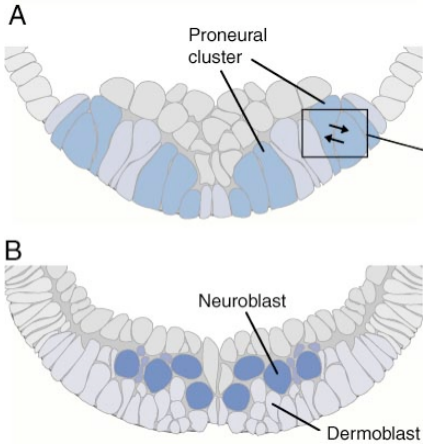
Proneural gene expression restriction

2) **Neuroblast specification: restriction of proneural gene expression**

- **Gradual extinction of proneural AS-C gene expression in all but one cell**
- **The cell expressing highest level of AS-C enlarges and eventually leaves epithelium to go inside (delamination)**
- **How is just one cell chosen to be the neuroblast?**



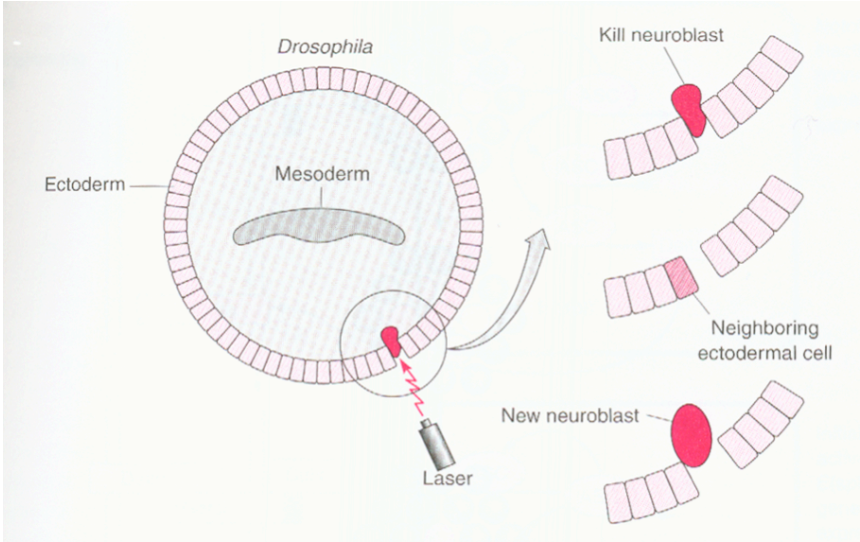
Proneural gene expression restriction to neuroblast



Neuroblast delamination

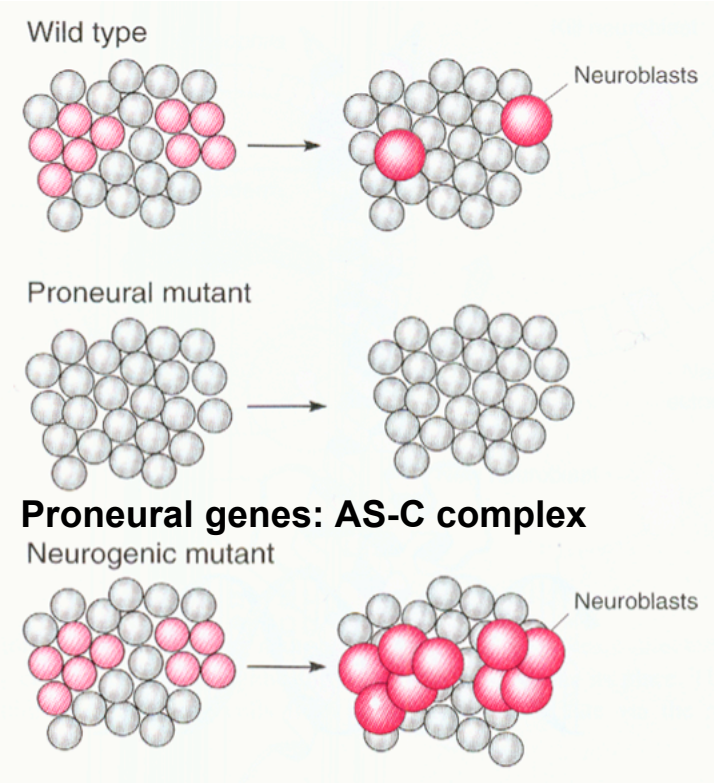
Neuroblast specification: lateral inhibition

- **Differentiating neuroblast appears to inhibit adjacent cells from becoming neuroblasts**



Molecular mechanism of lateral inhibition

- **Lateral inhibition mediated by "neurogenic genes"**
- **Neurogenic genes encode membrane of cell-cell signaling circuit:**
  - **Notch pathway**

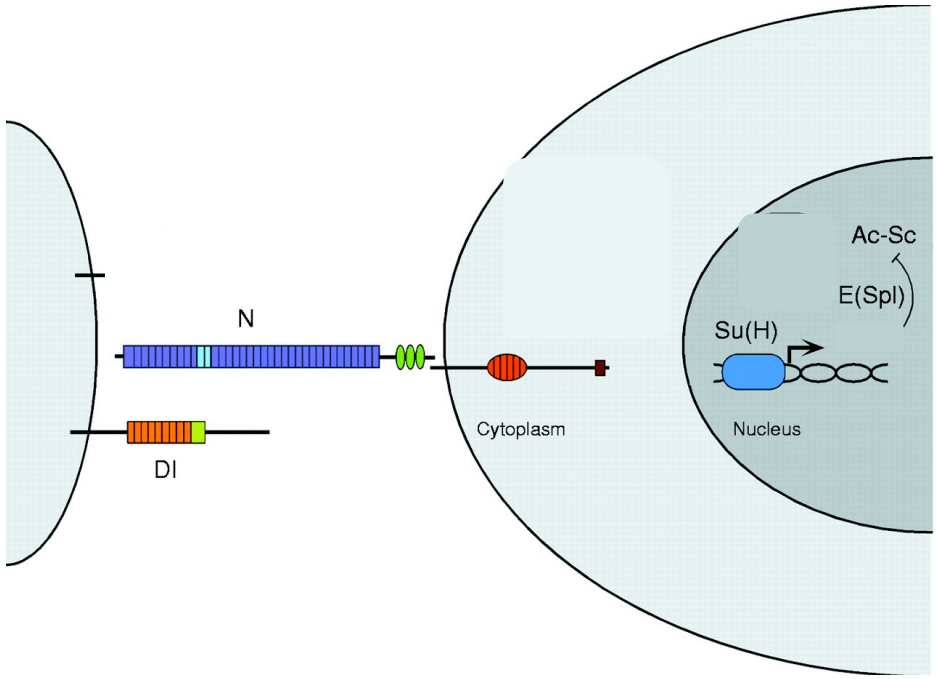


Proneural genes: AS-C complex

Neurogenic genes: Notch, Delta, etc…

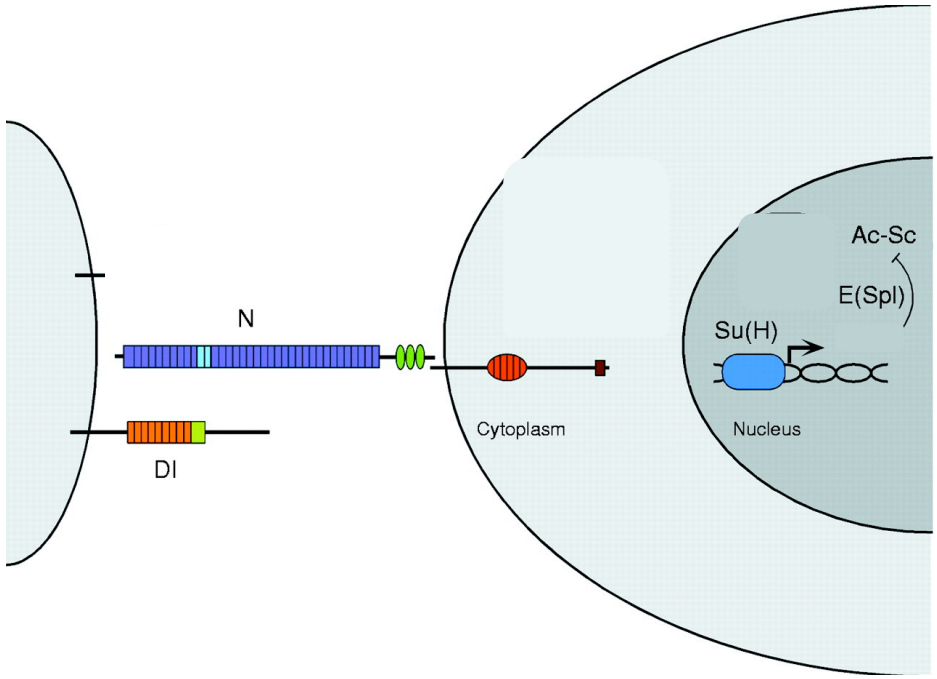
Notch pathway

- Delta - ligand
- Notch - transmembrane receptor
- Su(H) - transcription factor
- E(Spl) - transcription factor



The Notch pathway inhibits proneural gene expression

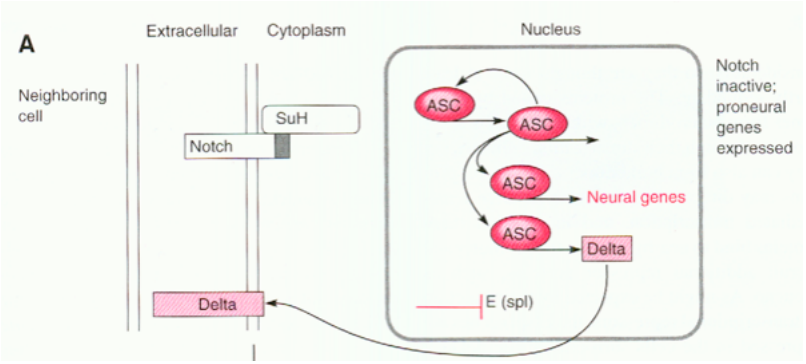
- Delta activates Notch
- Notch/Su(H) activate E(spl) transcription
- E(Spl) protein turns down AS-C transcription



Delta → Notch → Su(H) → E(spl) ⊣ AS-C

Lateral Inhibition: Step 1: Proneural clusters make Delta

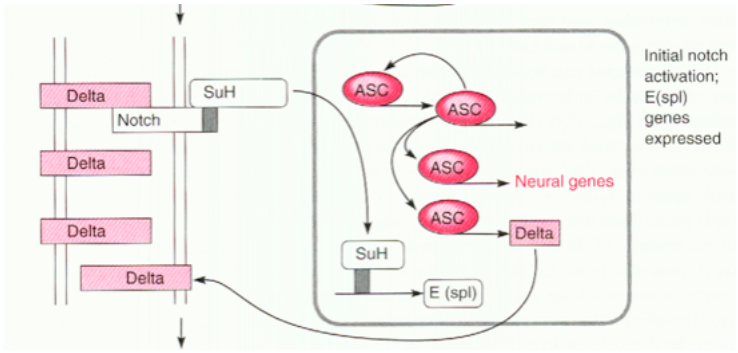
- All neurectoderm cells make Notch, Su(H)
  - Do not make Delta
- AS-C genes turn on in proneural clusters
- AS-C genes turn on neural genes + Delta



Delta → Notch → Su(H) → E(spl) ⊣ AS-C → Neural genes

Step 2: Notch pathway begins to work

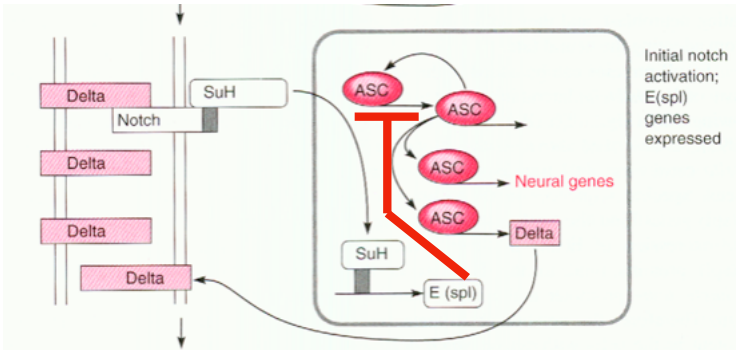
- Neighboring cells receive Delta signal
- Delta activates Notch/Su(H) which turn on E(spl)



Delta → Notch → Su(H) → E(spl) ⊣ AS-C → Neural genes

Step 3: Lateral inhibition

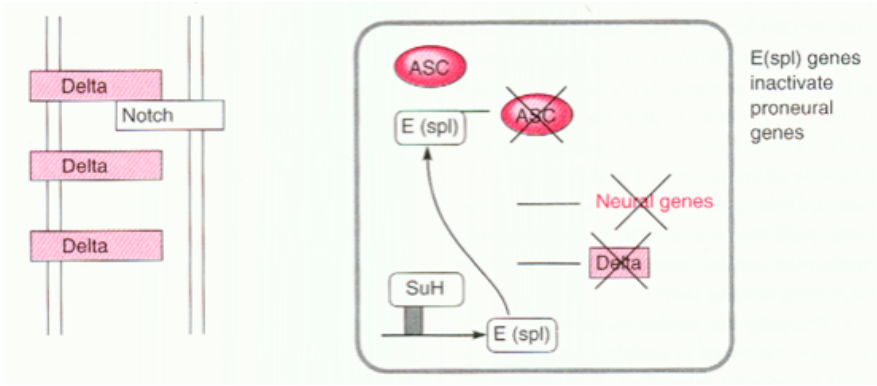
- **E(spl) turns down AS-C transcription**

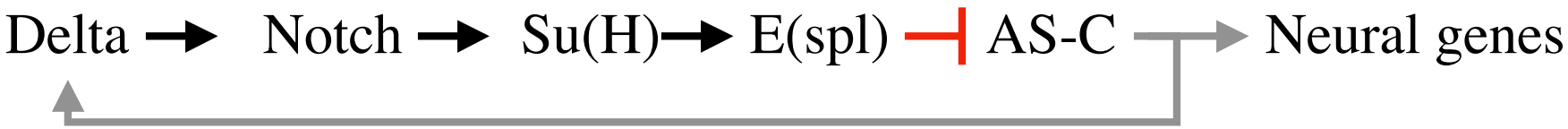


Delta → Notch → Su(H) → E(spl) ⊣ AS-C → Neural genes

Step 4: Proneural gene expression lost

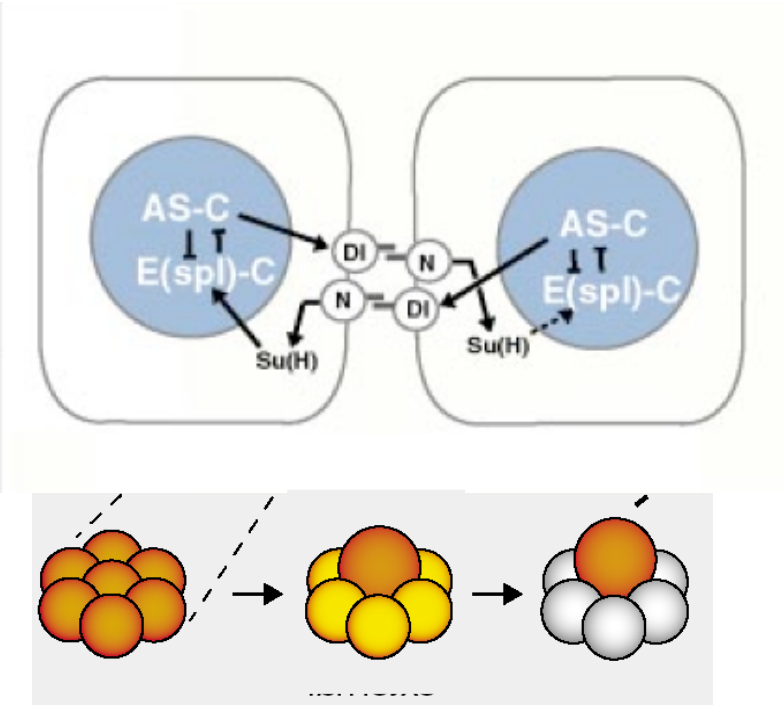
- AS-C expression lost
- Delta and Neural gene expression lost

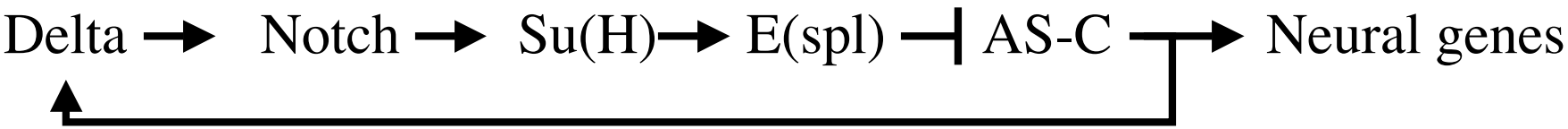




How does Notch inhibition of AS-C select a single neuroblast?

- All cells in proneural cluster make AS-C and thus Delta
- Each cell inhibits its neighbors (by activating Notch and turning down AS-C)
- Bi-stable state: cell with highest AS-C makes most Delta -- most effective at stopping neighbors from expressing AS-C and making Delta
  - "Rich get richer, poor get poorer"
- How is symmetry broken?
  - Initial underlying asymmetry?
  - Stochastic?



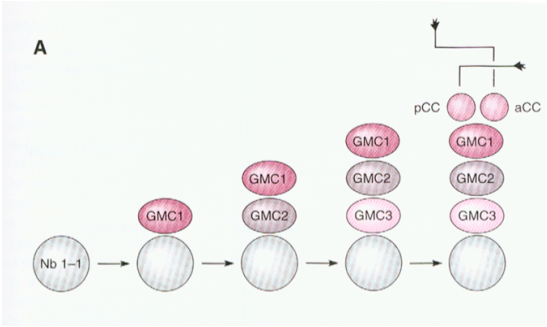


AS-C/Notch in vertebrates

- **AS-C relatives promote neural development**
  - **NeuroD mRNA injection into early blastomeres increases neuronal number in Xenopus**
- **Notch pathway members inhibit neural development**
  - **Activated Notch/Delta decreases neuron number**
  - **Dominant-negative Notch increases neuron number**

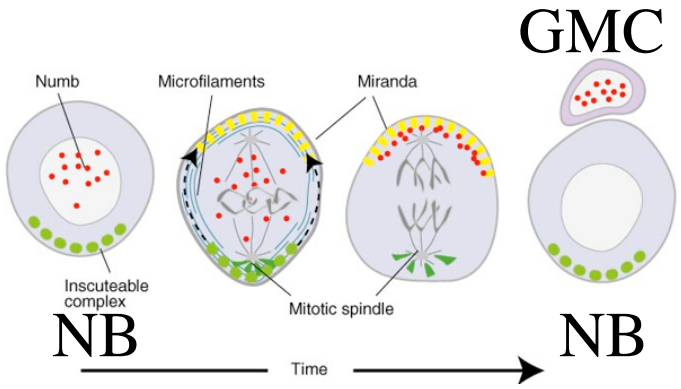
Generation of neuroblast progeny via asymmetric division

- Neuroblasts (NB) are multipotent stem cells
  - Can generate multiple cell types
  - Self-renew
- Divides asymmetrically
  - One NB/one GMC (ganglion mother cell)
- GMC divides once to generate neurons and/or glia



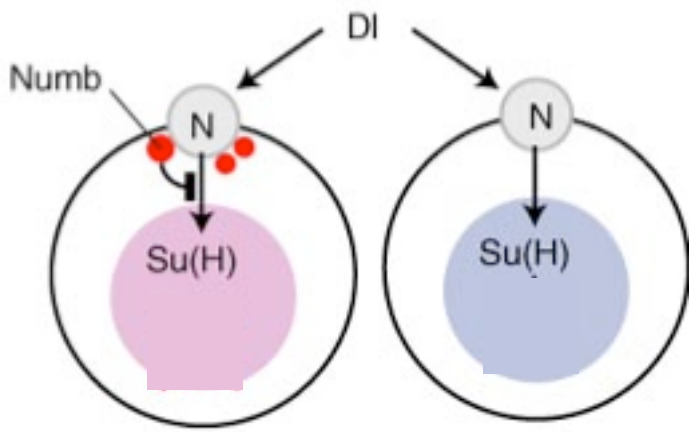
Asymmetric neuroblast division

- Inscuteable protein localizes to apical surface of NB
- Inscuteable orients mitotic spindle and localizes Miranda protein at basal surface
- Miranda traps Numb, Prospero and other intrinsic cell fate determinants
- Only GMC inherits Numb, Prospero etc…



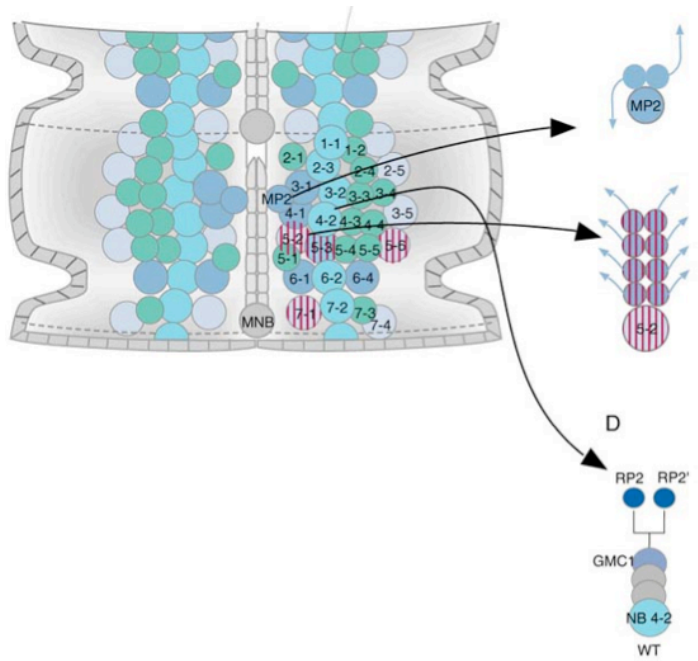
Consequences of asymmetric inheritance

- Numb inhibits Notch signaling
- Example of how intrinsic determinants can act by controlling response to extrinsic determinant
- Both types of determinants act together to generate the asymmetric outcome



Fate of Neuroblast progeny

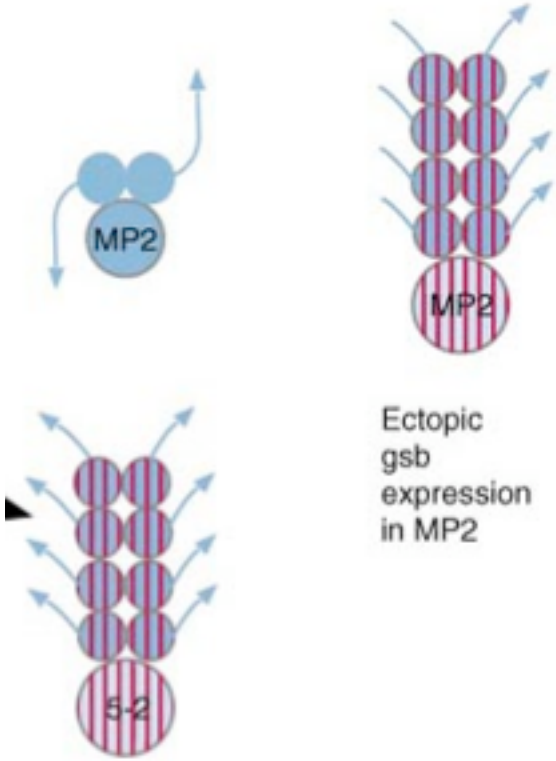
- **Each NB identifiable and gives rise to distinct and reproducible set of neurons and glia**



Partial NB map of one hemisegment
(≈30 NB/hemi generate ≈400 neurons/glia)

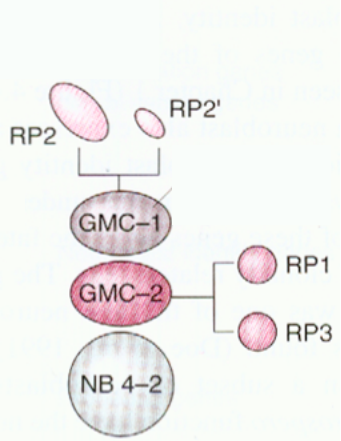
Fate of Neuroblast progeny: intrinsic determinants

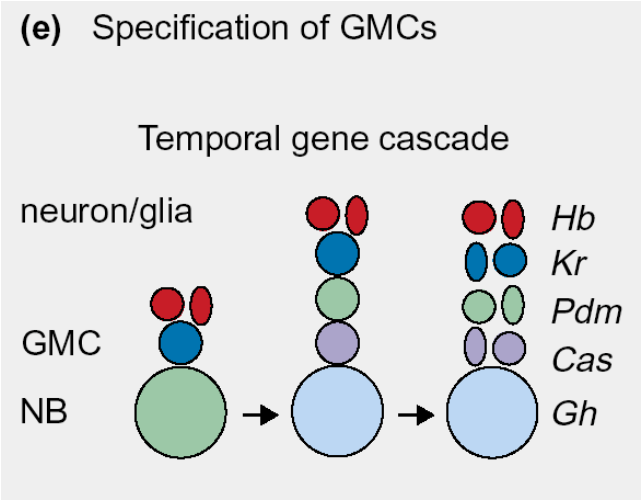
- **Intrinsic determinants control neuroblast progeny fates:**
  - **Gsb (transcription factor) usually expressed in Nb5-2 but not MP2 lineage**
  - **Express gsb in NbMP2 -- generate Nb5-2-like progeny**



A neuroblast generates a sequence of distinct GMC's

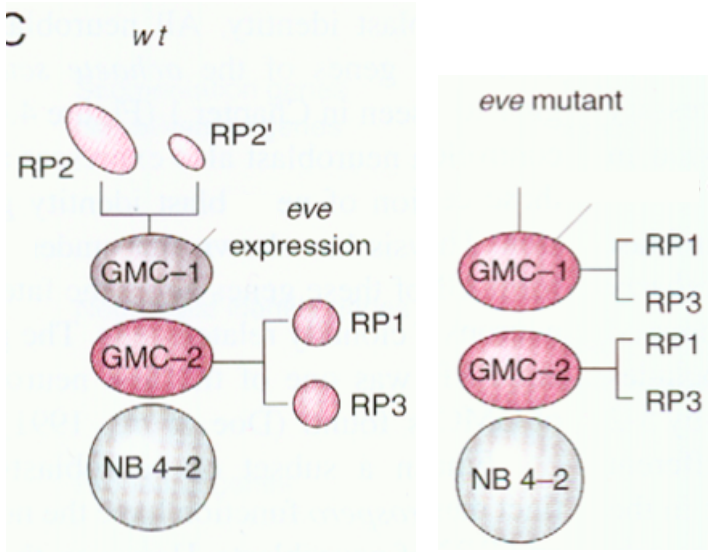
- Different GMC's from same NB produce distinct progeny
- Nb's appear to have internal clock: GMC's inherit different intrinsic factors at each division
- Most Nb's share same sequence of transcription factors: even though divide at different chronological times





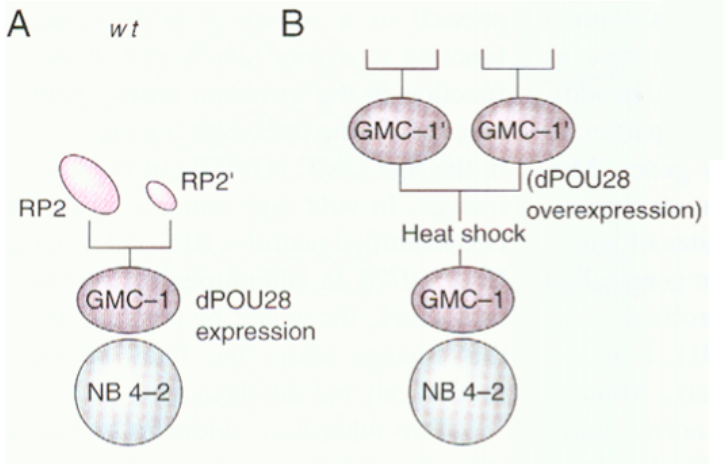
GMC fates

- **Intrinsic determinants distinguish GMCs from one another:**
  - **Eve expressed in GMC-1, not GMC-2**
    - **Eve mutant: GMC-1 transformed into GMC-2**



GMCs show dynamic regulation of intrinsic factors

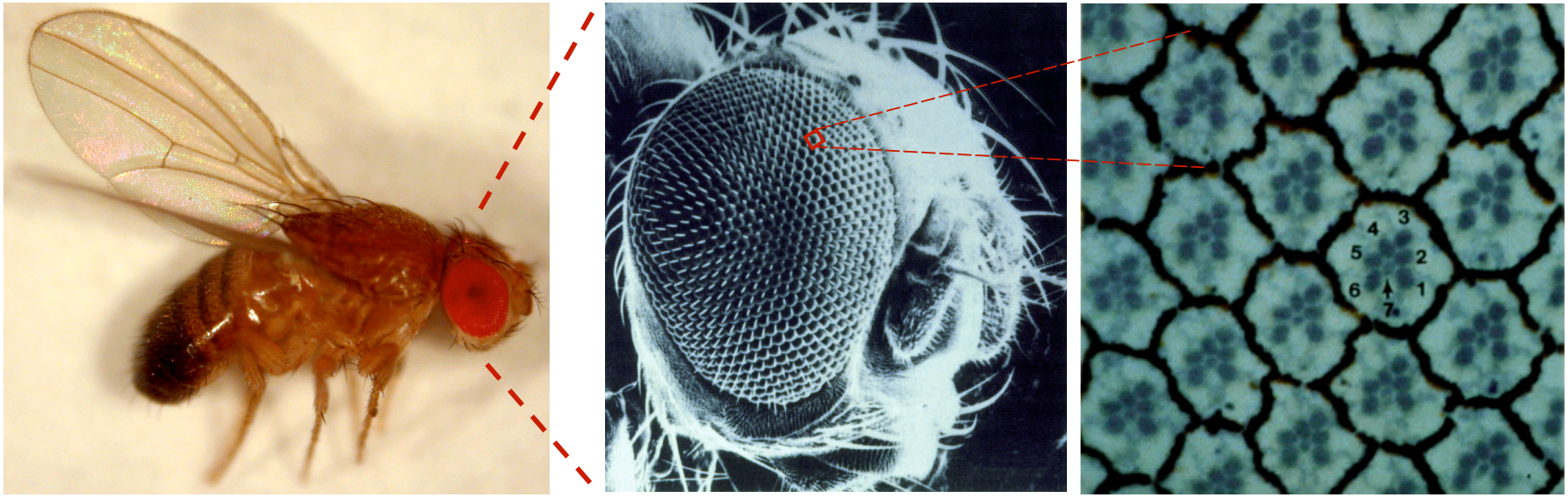
- **Distinguish GMC from progeny:**
  - **dPou28 expressed in GMC-1, not progeny**
    - **Express dPou28 in progeny: continue to behave as GMC**



Intrinsic factors in insect neurogenesis

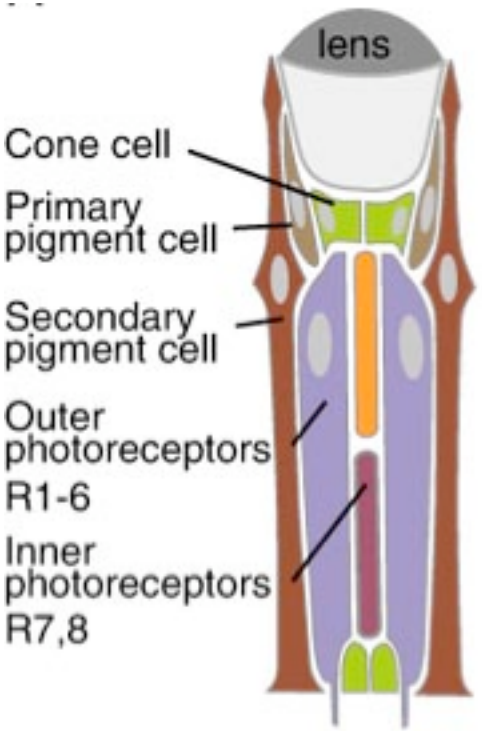
- Intrinsic determinants combine to specify behavior of a Nb and its progeny
  - Different Nb's express different transcription factors
  - Different GMC's within a single Nb's lineage express different transcription factors
  - Dynamic regulation of intrinsic factor expression helps generate diversity
- Asymmetric cell division of Nb's and GMC's generates cells containing different intrinsic determinants

Cell:cell signaling in determination of neuronal fate: *Drosophila melanogaster* retina



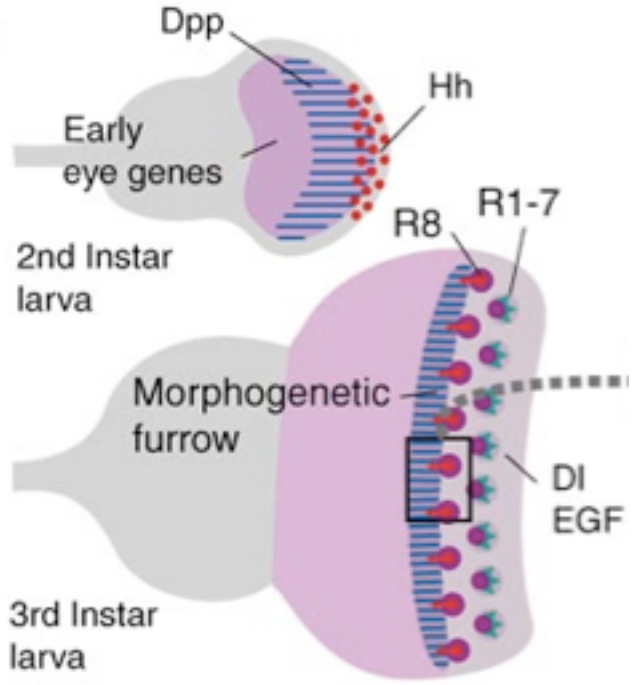
The *Drosophila* eye contains ≈750 facets

- Each facet contains 8 neurons and 12 accessory cells
- Each facet made from clonally unrelated, uncommitted precursor cells
- Cell:cell interactions between postmitotic photoreceptors and accessory cells responsible for specifying cell fates



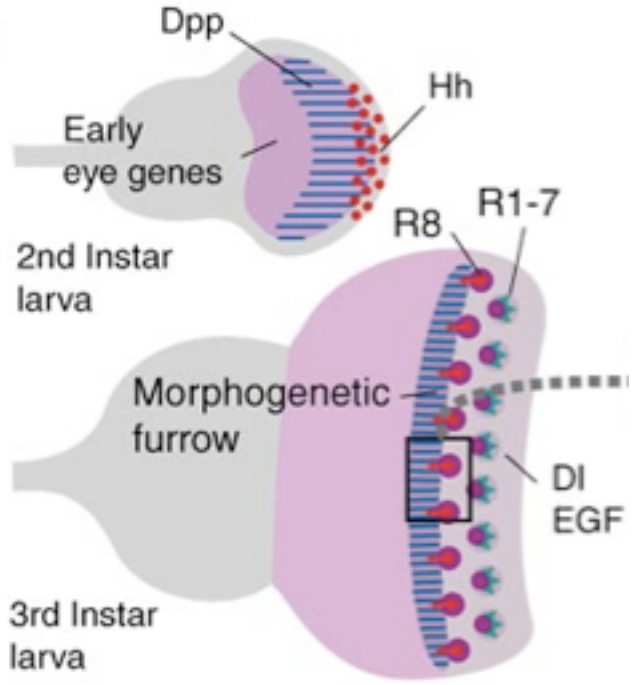
Patterning of fly retina

- Tissue made competent to form eye tissue (express eyeless etc…)
- At 3rd instar phase, signals (including Hedgehog) from posterior of eye initiate patterning
- Front of morphogenesis called morphogenetic furrow



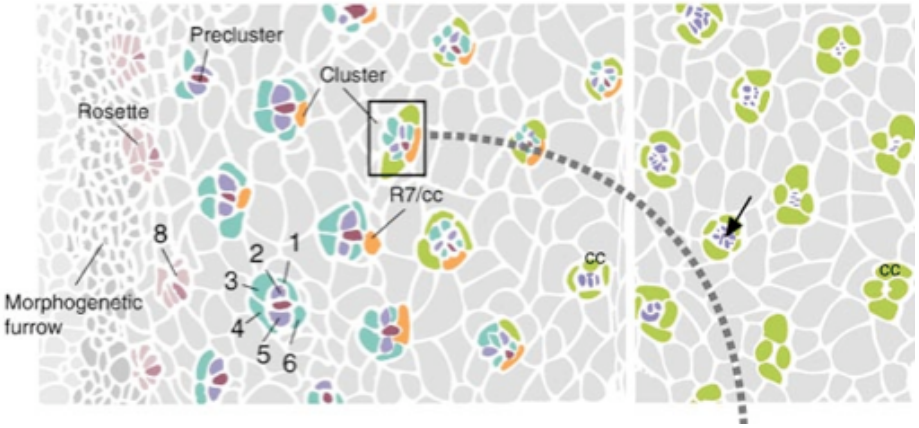
Patterning of fly retina

- At morphogenetic furrow
  - Expression of AS-C protein atonal turns on in a band
  - Notch/Delta mediated lateral inhibition then selects evenly spaced single cells (R8) to found facets

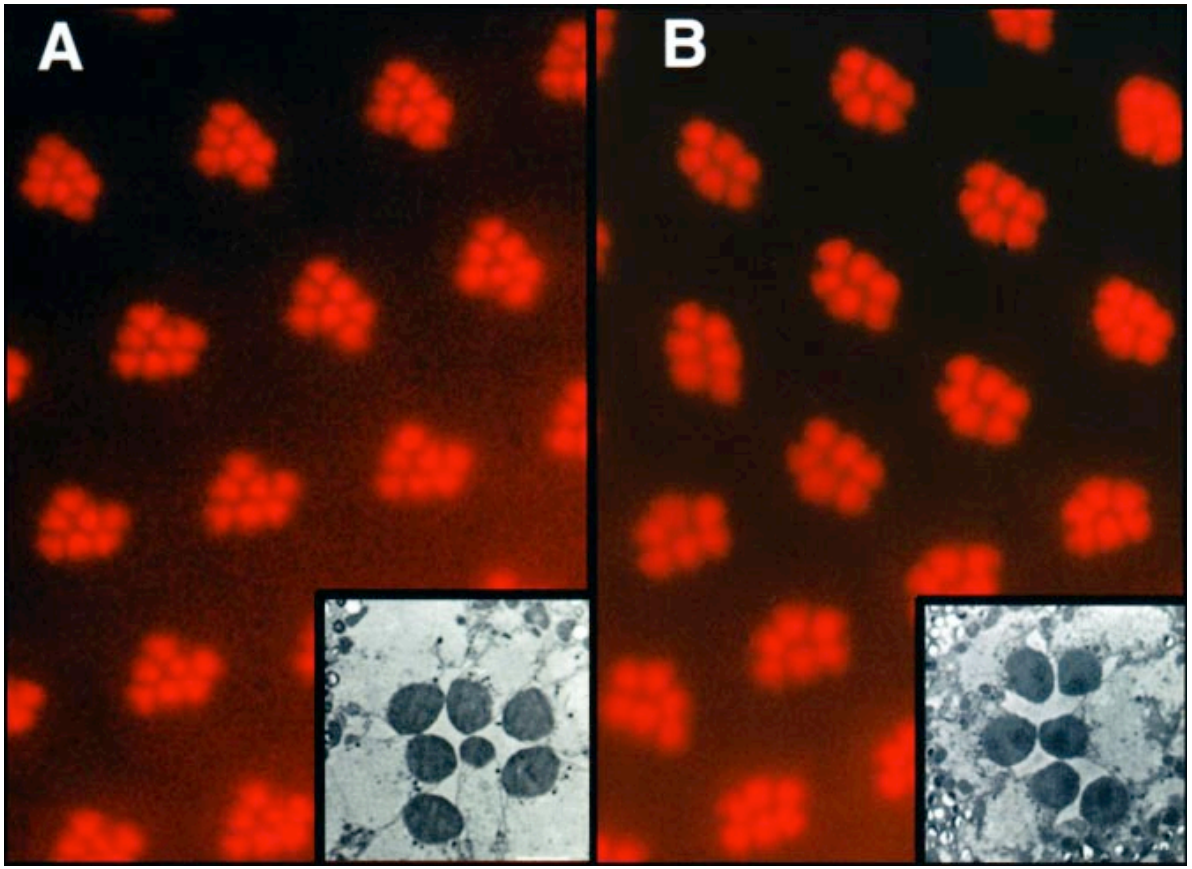


Patterning of fly retina

- R8 founder sends signals that recruit adjacent cells to become photoreceptor neurons (R1-R7)
- Induction of R7 cell differentiation by R8 classic example of extrinsic signal inducing neuronal fate



The *sevenless (sev)* mutant



wt *sev*

Genetic mosaic analysis: determine in which cell a gene's function is required

- **Two mutants with same phenotype: missing just R7 cell: may act in same pathway**
  - ***Sevenless* (*sev*)**
  - ***Bride of sevenless* (*boss*)**
- **In which cells do these gene products act?**
- **Test using genetic mosaic animals: mixture of wild type and mutant tissue**
- **Determine what cell(s) must be wild type for the R7 cell to form**

Genetic mosaic analysis

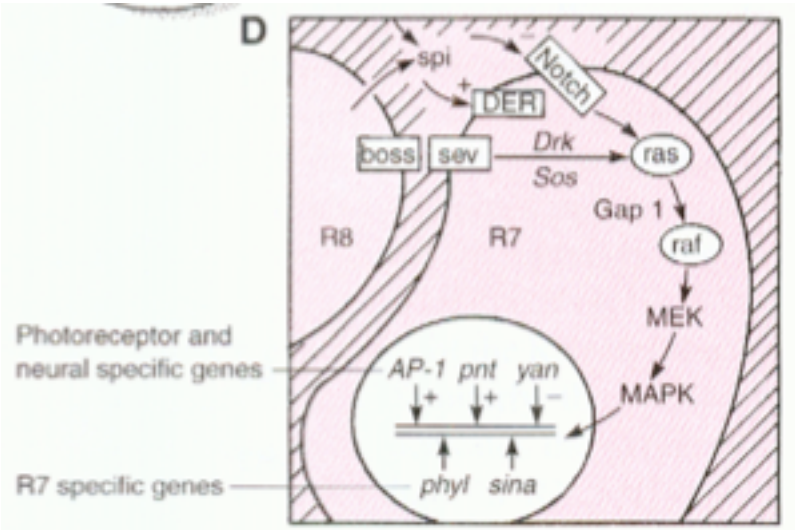
- **Generated genetic mosaic eyes with *sevenless* and *boss***
- **Result 1:**
  - ***Sev* : never see *sev* mutant R7 cell, but see R7 cells in many facets that contain other types of *sev* mutant cell**
  - ***Boss:* often see *boss* mutant R7 cell; never see R7 cell in a facet that contains a *boss* mutant R8 cell**
- **Interpretation:**
  - ***Sev* acts cell-autonomously in R7**
  - ***Boss* act cell-nonautonomously in R8 to induce R7 development**

R7 equivalence group

- **Sev: encodes receptor tyrosine kinase**
- **Sev is expressed not just in R7:**
  - **Expressed in many cells in the eye: including R7 precursor and four other cells (cone cell precursors)**
- **All five of these cells have potential to be an R7 (any can become R7 if Sev is activated in them)**
  - R7 equivalence group **(equal competence to form R7)**
- **How is one cell selected to become R7?**

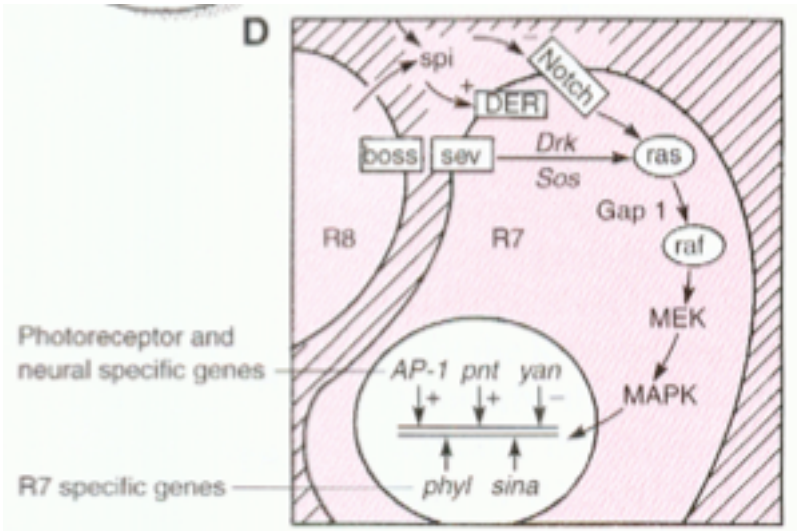
R7 induction: Boss:Sev

- R8 cell makes Boss -- transmembrane ligand of Sev
- R8 contacts only one cell in R7 equivalence group
- Sev is only activated in one cell --- becomes R7
- Use of extrinsic signal (Boss) to select number and position R7 neuron



Competence

- Sev expression not limited to the 5 cells in the R7 “equivalence group”
- A number of these other Sev-expressing cells also contact R8 and are exposed to Boss
- Why do these cells not become R7s?
- Restricted competence: only cells in R7 equivalence group competent to become R7
- Competence: reflects cell history: generates a combination of factors that determine cell’s response to a signal (what transcription factors, signaling factors etc… expressed)

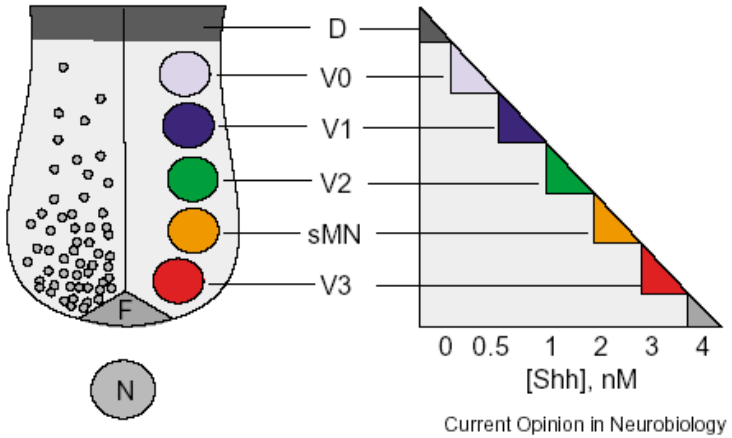


R7 determination

- Involves combination of intrinsic (competence) and extrinsic (inductive) factors
- Development: iterative process:
  - Intrinsic factors can set up expression pattern of extrinsic factors and extrinsic factors determine expression of intrinsic factors

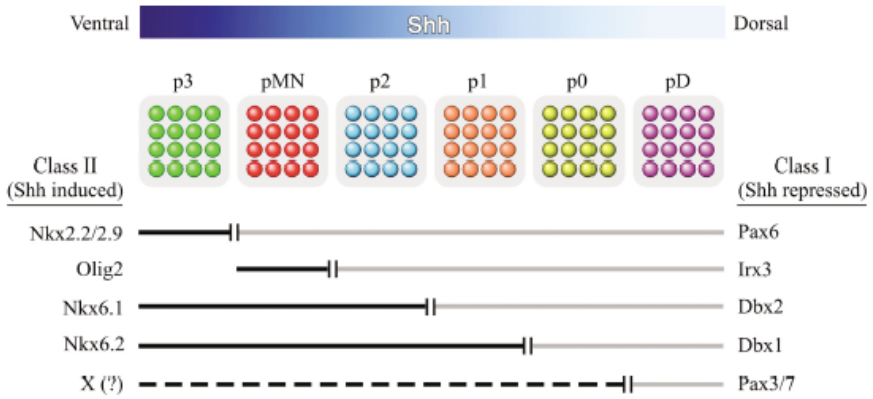
Cell fate specification in ventral spinal cord (review)

- **Gradient of Shh (extrinsic factor) patterns ventral neural tube**



Cell fate specification in ventral spinal cord

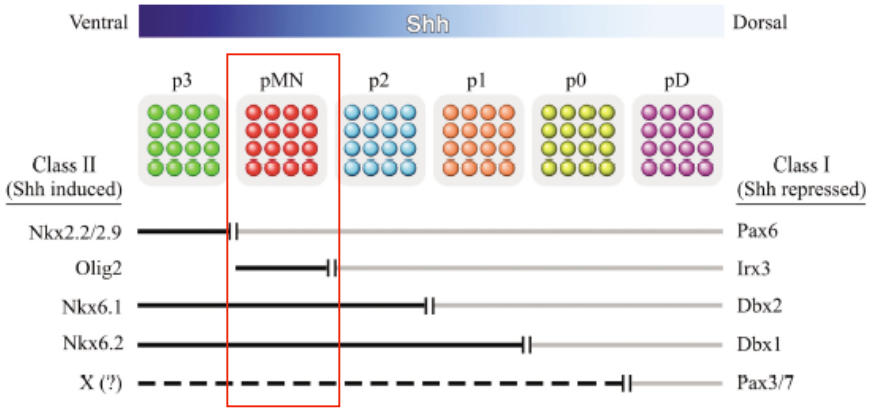
- Different levels of Shh turn on/off different transcription factors
- The transcription factors cross-repress one another to sharpen boundaries
- Combination of transcription factors induced determines progenitor identity ---the types of postmitotic neurons later produced



Beyond Shh

- **Temporal control: same progenitor domain generates different cell types at different developmental stages --- reminiscent of insect Nb's**
- **AP cues -- combine with DV cues to generate diversity along AP axis.**

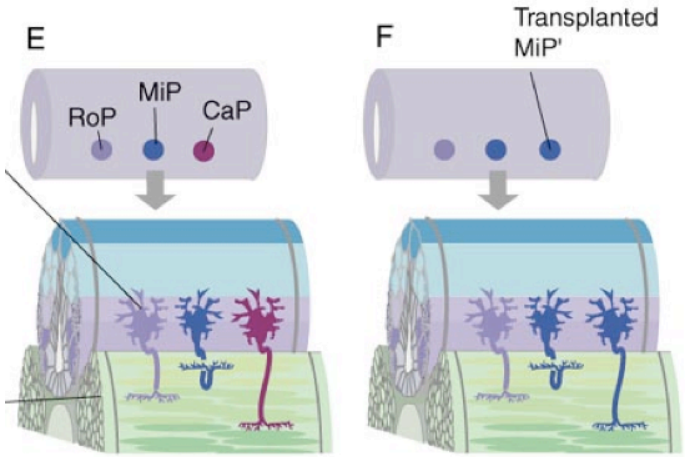
Hierarchy of motor neuron identity



- **Motoneurons can be further subdivided:**
  - **Columns:**
    - **project to different muscle groups**
    - **express different transcription factor (LIM/homeodomain) combinations**

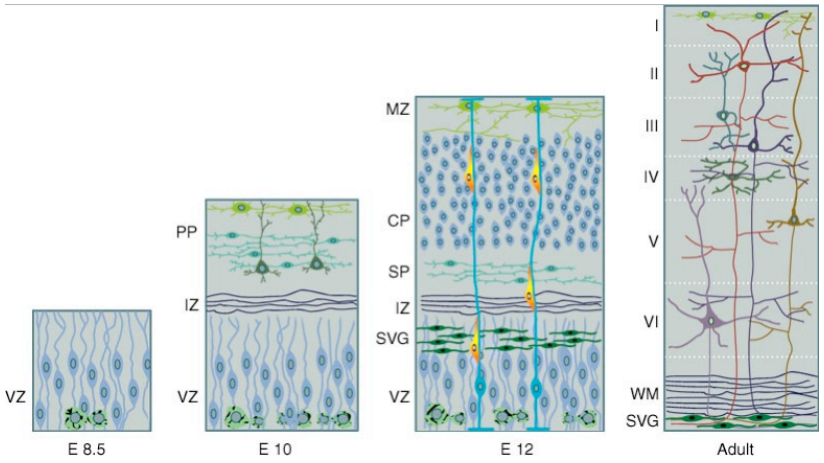
Column identity influenced by AP signals

- AP-restricted signals from notochord help determine columnar identity
- Different columns express different LIM homeodomain transcription factors
- Zebrafish:transplant individual MiP neurons: change LIM code and axon trajectory



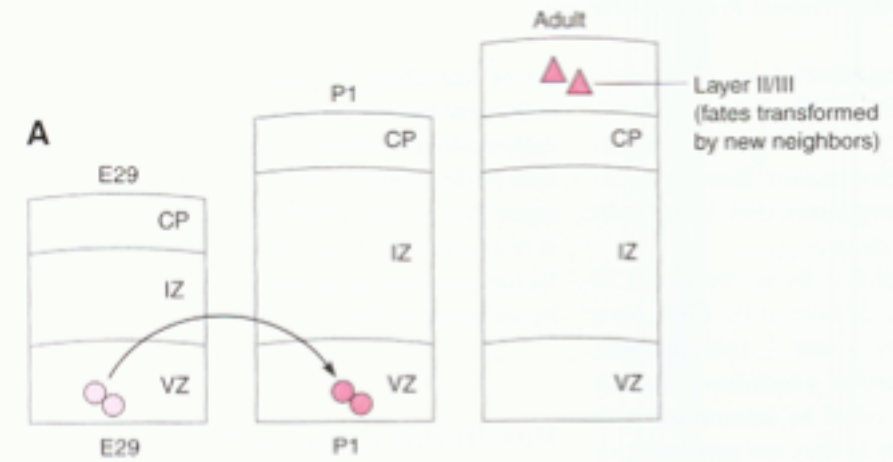
Laminar fate determination in cortex

- Cortical layers generated in inside-out temporal sequence
- When do cells receive layer-specific identity?
  - When generated?
  - When done migrating?



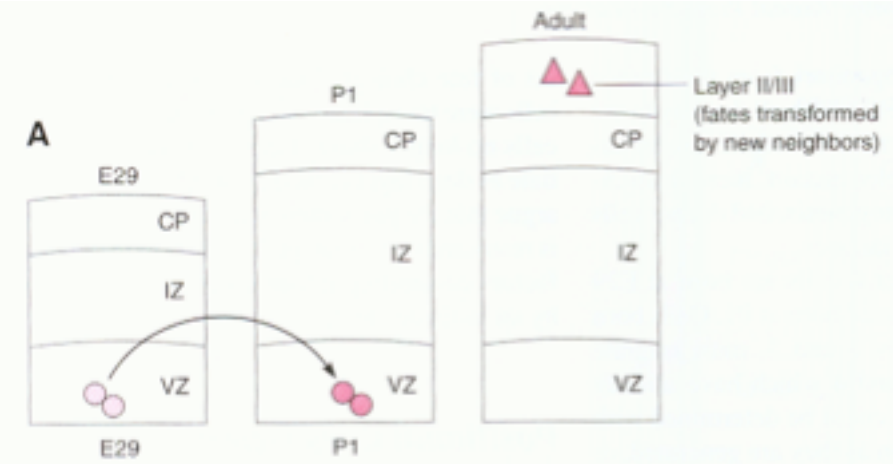
Laminar cell fate studies: Heterochronic transplantations

- Transplant VZ cells from young to old cortex
- Cells take on “old” fates
- Signals from surrounding tissue determine fate (not just age of cell)

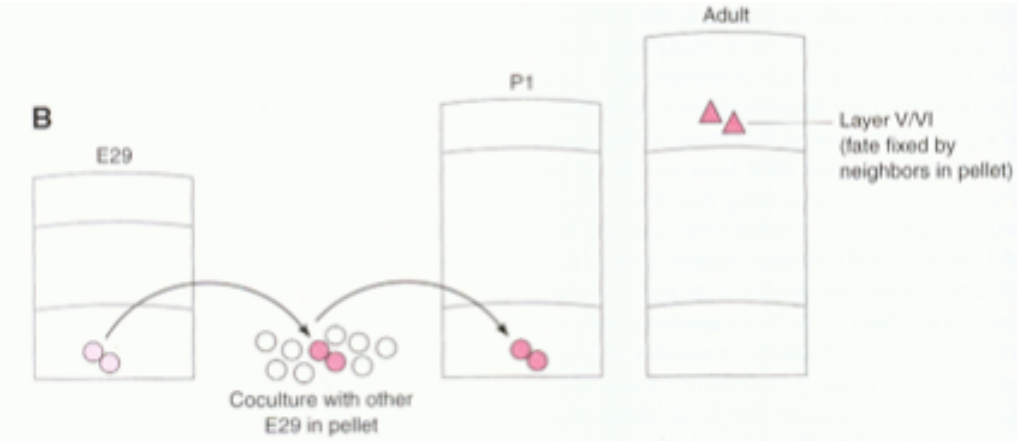


Cell:cell signaling in VZ influences cortical cell fate

- Transplant VZ cells from young to old cortex
- Cells take on “old” fates
- Signals from surrounding tissue influence fate (not just age of cell)



- If instead: First coculture with other early VZ cells for a few hours
- Cells take on “young” fates
- Signals from surrounding VZ zone cells influence fate



Neural stem cell competence changes over time

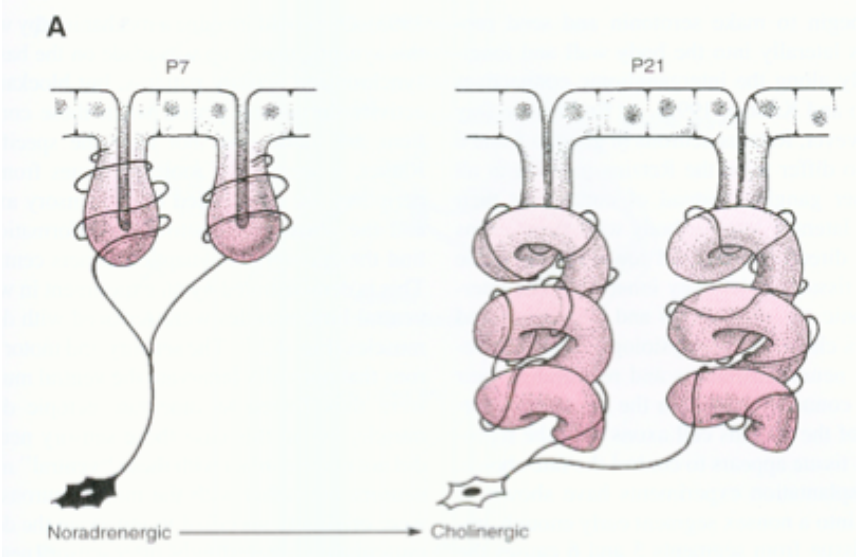
- Is fate solely determined by signals from surrounding cells?
- Transplant old VZ cells into young cortex
- Cells take on “old” fates
- Thus competence of old VZ progenitors more restricted than young VZ
- Combination of inducing factors and competence determines fate

Target tissue can also regulate cell fate

- Final cell fate determination can take place after neuron forms connections
- Target can produce key signals
  - Trophic: Survival/death
  - Neuronal phenotype: eg., neurotransmitter type

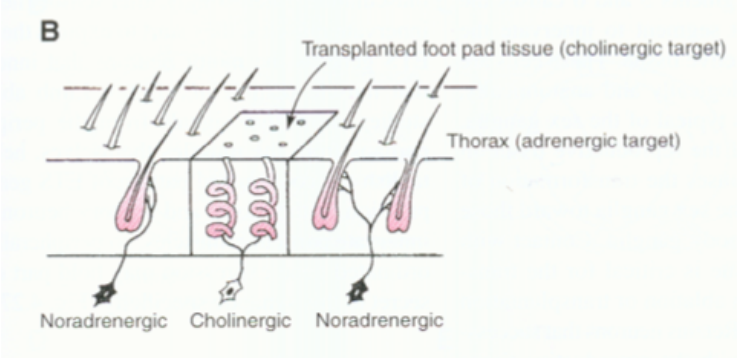
Control of transmitter phenotype by target cell

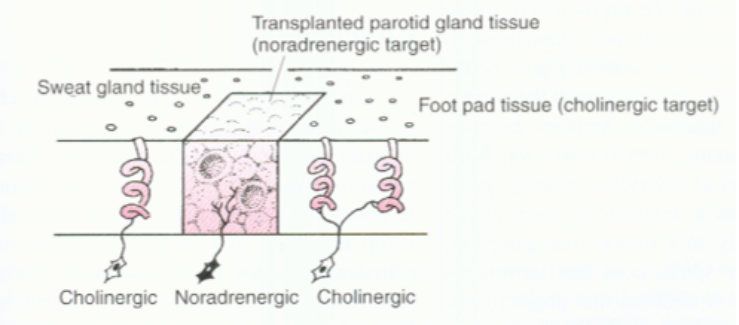
- Sympathetic neurons innervating sweat gland
- Initially adrenergic: produce enzymes & machinery for noradrenaline production and release
- As development proceeds: turn off adrenergic genes and begin to make acetylcholine



Control of transmitter phenotype by target cell

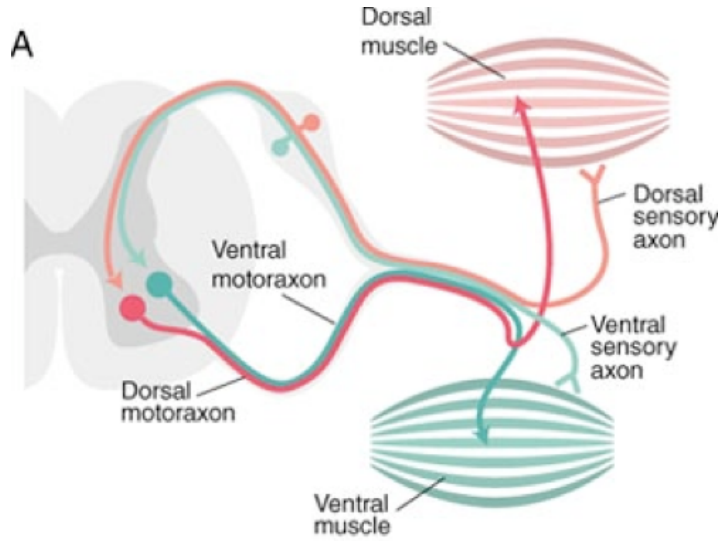
- Is sweat gland responsible for transmitter switch?
- Put sweat gland tissue into region where neurons usually remain noradrenergic
- These sympathetic neurons now become cholinergic
- Converse experiment also works: replace sweat gland tissue with other target --- switch does not occur
- Factor(s) responsible still unclear





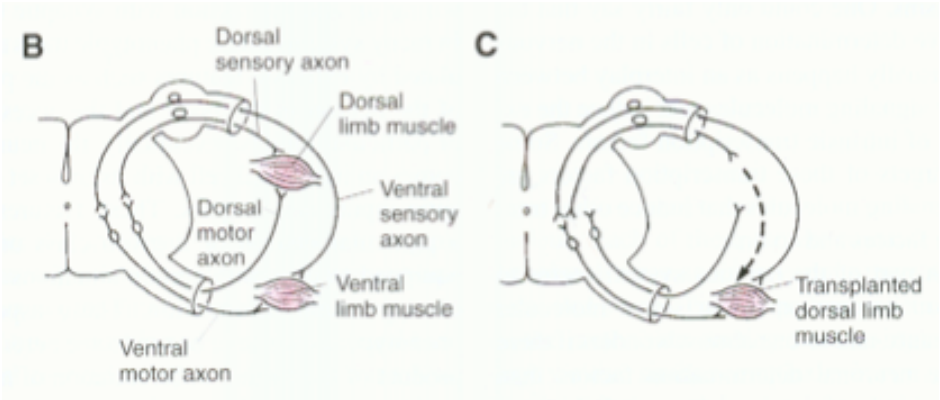
Matching fates of synaptic partners

- Stretch sensing neurons and motor neurons contacting same muscle must synapse with one another
- How matched?



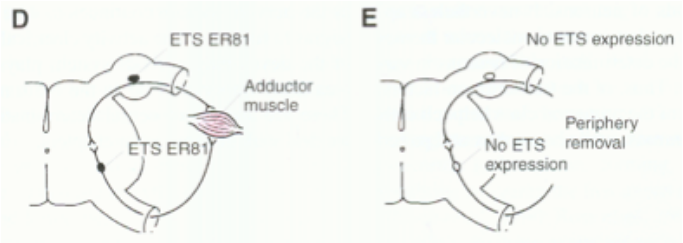
Signals from muscle target may influence fate of input

- Force sensory neurons to switch muscle target
- Synapse with different (now correct) motor neuron
- Signal from target influencing fate



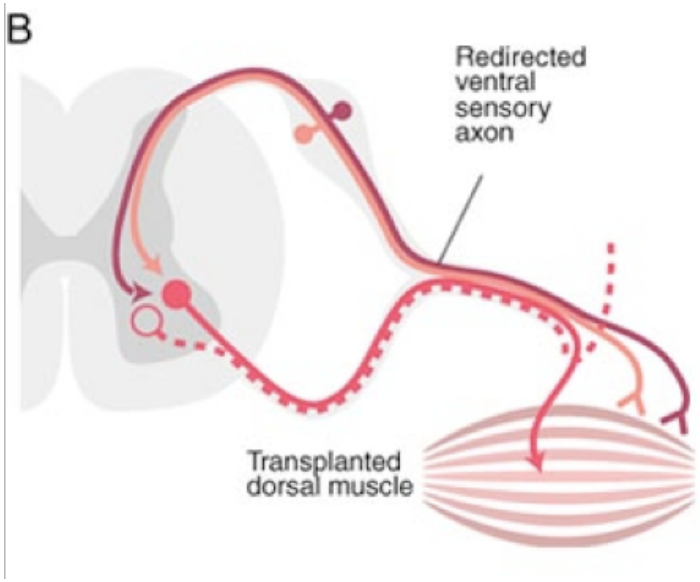
Target influences transcriptional regulator expression

- Neurons contacting same muscle co-express certain transcription factors (ETS domain)
- Remove target --- expression of such factors extinguished
- Target induces expression



Muscle target can help match fates of inputs

- Replace ventral with dorsal muscle
- All axons now contact dorsal muscle
- Redirected axons now express ETS domain protein normally found only in dorsal sensory axons
- Target can help coordinate cell fates of neurons that must connect with one another
- ETS targets include cell adhesion molecules such as cadherins



Next Class

- **Axon guidance**