Neuronal Determination and Differentiation

Paul Garrity
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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
Extrinsic determinants

- Extrinsic determinants: external signals
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

*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

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

**Amphibians:**
All dorsal neurectoderm cell appear to become neural stem cells

**Insects:**
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


Lecture 4-13
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

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


Different combo of AP patterning 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 genes required for neuroblast formation

Wild type

Proneural mutant

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
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?
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

Wild type

Proneural mutant

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

\[
\text{Delta} \rightarrow \text{Notch} \rightarrow \text{Su(H)} \rightarrow \text{E(spl)} \rightarrow \text{AS-C} \rightarrow \text{Neural genes}
\]
Step 2: Notch pathway begins to work

- Neighboring cells receive Delta signal
- Delta activates Notch/Su(H) which turn on E(spl)
Step 3: Lateral inhibition

- $E(spl)$ turns down AS-C transcription
Step 4: Proneural gene expression lost

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

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

\[
\begin{align*}
\text{Delta} & \rightarrow \text{Notch} \rightarrow \text{Su(H)} \rightarrow \text{E(spl)} \rightarrow \text{AS-C} \rightarrow \text{Neural genes}
\end{align*}
\]
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

- Insccuteable protein localizes to apical surface of NB
- Insccuteable 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
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.
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