Regionalization of the nervous system

September 19, 2018
Yasushi Nakagawa
Department of Neuroscience
Complex organization of the adult brain

forebrain
midbrain
hindbrain

How are all these regions established?
Regionalization generates diversity in cell types in the nervous system

Cells in early neural tissue acquire identity that is appropriate for their location (“positional identity”). This process is called regionalization.

Positional identity contributes to the generation of different types of neurons and glial cells.

Key molecular mechanisms of regionalization in the nervous system are:
- shared throughout evolution
  - almost all molecules identified first by fly genetics
- used over and over throughout brain development as well as in the adult brain.
The neural plate is a two-dimensional structure.
The neural plate is a two-dimensional structure.
Medial-lateral axis becomes ventral-dorsal axis after neural tube closure
Fate map of the neural plate

The terminal wall of the forebrain has to be regarded as a dorsoventrally organized part of the neural wall, like the lateral walls, though it is singular in occupying the midline (Puelles, 1995, 2001; Puelles et al., 2012a,b), whereas the floor plate is a longitudinally organized brain zone.

Kingsbury (1922) was the first author who proposed that the neural floor plate does not reach the anterior neural ridge (Figure 10.2; Puelles, 1995; Shimamura et al., 1995). On the basis of the peculiar histologic appearance of the hindbrain floor, which displays a median astroglial raphe that seemed to end rostrally at the isthmic fossa, he held that the floor plate ends at the prospective isthmus (at the midbrain–hindbrain border; Figure 10.1). However, Johnston (1923) corrected this analysis, drawing attention to a less obvious but analogous floor plate glial specialization found along the ventral midline of midbrain and diencephalon, which ends roughly at the mamillary pouch (see also Kuhlenbeck, 1973; Puelles, 1995; Puelles et al., 1987a).

Johnston’s (1923) description was corroborated by observation of an early epichordal strip of midbrain and diencephalic median floor cells that differentially express acetylcholinesterase (AChE; Puelles et al., 1987a). A handful of floor plate gene markers (e.g., Shh, Ntn1, Lmx1b, Nr4a2) have become known subsequently that clearly stop rostrally at mamillary level, jointly with the primary rostral end of the notochord (Puelles et al., 2012a; see the Allen Developing Mouse Brain Atlas).

Note that a direct contact of the notochord with the neural floor is observed only at very early embryonic stages.
Where are the AP and DV axes?

Subdivisions at later stages
Outline of lectures

Regionalization 1 (9/18/15)
neural induction and early regionalization along anterior-posterior (AP) axis
AP patterning in Drosophila embryos as a model of regionalization

Regionalization 2 (9/20/15)
AP patterning by secondary organizers
dorsoventral (DV) patterning

Research talk (10/2/15)
patterning, cell division and cell fate regulation in the mouse thalamus
Key concept 1

Early regionalization is linked to neural induction

- BMP inhibition induces anterior neural tissue.

- Posterior identity is induced independently of BMP inhibition.
Neural induction and regionalization in early embryos

In Mangold/Spemann experiments, the transplanted organizer induced ectopic nervous system from cells not fated to form a neural plate.

The induced nervous system is appropriately patterned along its rostro-caudal (anterior-posterior) and dorso-ventral axes.

How are neural induction and regionalization mechanistically related?
Spemann organizer sequentially gives rise to axial mesoderm underneath the future neural tissue

-Dorsal lip cells involute and form the axial mesoderm that underlie the presumptive neural plate.
  • prechordal mesoderm: rostral, originated from early dorsal lip
  • notochordal mesoderm (notochord): caudal, originated from late dorsal lip

-Like the dorsal lip itself, axial mesoderm can also induce neural tissue in amphibians, which led to an assumption that neural induction occurs largely via vertical signaling between the mesoderm and the overlying ectoderm.

-Do rostral and caudal axial mesoderm cells have different activity of inducing rostral vs caudal neural tissue?
Temporal specificity of induction

Young (early) dorsal lip induces a secondary head.

Older (late) dorsal lip induces a secondary trunk.
Regional specificity of induction

transplantation of different rostral-caudal parts of the archiarcher roof into early gastrula

Otto Mangold (1933)

This led Mangold to propose that there are distinct organizers that induce different regions of the neural tissue separately ("head-trunk-tail organizer model")
Activation-transformation model

Grafts of early ectodermal tissues were transplanted into different parts of the future neural tissue.

The proximal part included neural tissue, whereas the distal part included non-neural tissue.

Within the induced neural tissue, the more distal part was always rostral whereas the more proximal part was always caudal.

The level of the graft in the host always determined the regional character of the most caudal neural tissue in the graft.

Niewkoop (1952)

Niewkoop and others proposed that the neural tissue is patterned by a gradient of a transformer that travels within the plane of the neural plate and induces different neural fate in a dose-dependent manner such that forebrain, midbrain, hindbrain and spinal cord form at increasing levels of this transformer (activation-transformation model).
What is the molecular basis for this classic model?
- We now know that “activation” occurs via inhibition of BMP signaling.

- Dissociated animal cap cells will generate neural cells with anterior identity (in the absence of exogenous BMP). Is this the default positional identity? If so, what molecules are responsible for “transformation”? Where are they expressed?

- Are there endogenous inhibitors of transformer activities that counteract such activities? If so, where are they expressed?

- Molecules responsible for caudalizing activity include Wnts, fibroblast growth factors (FGFs) and retinoic acid (RA).
Wnt signaling pathway

Wnts: evolutionarily conserved secreted proteins
- 19 Wnt genes in human genome
- multiple signaling pathways depending on the cellular context and receptors

“canonical” pathway

Wnt signaling OFF: • β-catenin is targeted for degradation by a destruction complex.

Wnt signaling ON: • Binding of Wnt to Frizzled and LRP6 leads to inhibition of β-catenin degradation.
• Stabilized β-catenin translocates to the nucleus and interacts with TCF/Lef1 family of HMG-box containing transcription factors to activate target gene transcription.

http://wormbook.org/chapters/www_wntsignaling.2/wntsignal.html
Tissues underlying the anterior brain express Wnt inhibitors

- Anterior endoderm (ae)
- Prechordal mesoderm (PME)
- Notochord (cm)

- Cerberus
- Dickkopf Frzb-1
- Chordin Noggin
- Follistatin

BMP inhibitor
Wnt inhibitor

Over-expression of Cerberus generates an ectopic head
Graded Wnt/β-catenin signaling acts as transforming activity

- In vivo and in vitro Wnt over-expression in Xenopus, zebrafish, chick and mouse results in caudalization of the neural tissue.

- Late Xenopus gastrula shows an AP gradient of Wnt/β-catenin signaling.

- Wnt antagonists are expressed in the anterior part of the gastrula.

Transcription factors are differentially expressed in rostral and caudal neural tissue.

Kiecker and Niehrs (2001)
Early signals come from outside of the nervous system and establish gross AP patterns

Caudalizing factors (Wnts, RA, FGFs) are generally produced by paraxial mesoderm (somites), not axial mesoderm (descendants of the organizer), and they do not induce neural tissue on their own.

Different caudalizing factors are responsible for patterning at different AP levels in the nervous system.
Mechanisms for early regionalization are similar in different vertebrate species

**Xenopus**

Cylinder-shaped mouse embryo becomes asymmetric by the formation of anterior visceral endoderm (AVE). Like the amphibian anterior endoderm, AVE produces Cerberus, a Wnt inhibitor.

Formation of AVE triggers the formation of primitive streak (ps) on the opposite (caudal) side. The primitive streak is equivalent to the amphibian blastopore, and produces Fgf, Wnt and Nodal.

Node (or Hansen’s node) is formed at the anterior end of primitive streak. Node is equivalent to the amphibian organizer, and produces chordin. Derivatives of the node (cm and PME) also produce BMP inhibitors like chordin.

AVE cannot induce neural tissue by itself, but inhibits caudalization of the neural tissue by blocking Wnt, BMP and Nodal pathways.

**Mouse**
Summary of part 1

Early regionalization is linked to neural induction

-BMP inhibition induces anterior neural tissue (default neural fate).

-Posterior identity is induced independently of BMP inhibition.
  • caudalizing (transforming) activity: Wnts, FGFs, RA
  • Inhibitors of Wnt proteins are expressed underneath the anterior neural tissue and prevent the neural tissue from becoming caudalized.

-Mechanisms of early regionalization are similar between vertebrate species.
Signaling pathways and molecules involved in regionalization of the vertebrate nervous system were initially discovered in fly genetics.

- **Signaling pathway**
  - cascade of gene regulation
  - graded expression of transcription factors forms discrete boundaries within embryos

- **Molecules**
  - *Hox* genes (encodes a family of transcription factors) are involved in identity of specific body segments.
  - secreted signaling molecules and their receptors
Embryonic AP patterning in Drosophila melanogaster

The fly consists of a head (with mouth, eyes, antennae), three thoracic segments (T1-3) and 8-9 abdominal segments (A1-9)

The segmentation starts to develop in early embryos
Genetic mutant screening identified many genes involved in AP patterning in fly embryos.

- Genetic screens pioneered by Nüsslein-Volhard and Wiechaus in the 1980s identified a hierarchy of genes that establish anterior-posterior polarity of Drosophila embryos and divide the embryo into a specific number of segments with different identities.

- Basic ideas of the identified gene regulatory cascade apply to many other aspects of animal development, including the regionalization of the vertebrate nervous system.

- Many genes identified in this screen have vertebrate homologs that are important in the patterning of the neural tissue.

Figure 22-38 Molecular Biology of the Cell 5/e (© Garland Science 2008)
Polarization starts in unfertilized oocytes

bicoid and nanos mRNAs are near the anterior and posterior end of the oocyte, respectively (egg-polarity genes)

Bicoid protein diffuses and forms a concentration gradient, regulating the graded expression of Hunchback

Hunchback, Krüppel and Giant are products of the gap genes, which mark out coarse subdivisions of the embryo
Pair-rule genes are required for alternative body segments

- Expression of *even-skipped* (eve) and *fushi tarazu* (ftz) are under the combinatorial regulation of gap genes.
- Discrete domains of gene expression are formed by a combination of upstream regulatory mechanisms.

**Figure 7-53 Molecular Biology of the Cell 5/e © Garland Science 2008**
Segment polarity genes organize the AP pattern of individual segment

Segment polarity genes stabilize boundary between segments.

Genes encoding two secreted proteins, Wingless and Hedgehog, are segment polarity genes. They promote each other’s expression as well as a transcription factor Engrailed.
Homeotic selector genes are required for the identity of each segment.

Mutations that transform parts of the body into structures appropriate for other positions are called homeotic mutations.
These proteins contain 60 amino acids of a conserved DNA-binding domain called the homeodomain.

These genes are located in two clusters (Antennapedia complex and Bithorax complex) on chromosome 3.

The order of genes on the chromosome corresponds almost entirely to the order in which they are expressed along the AP axis of the body (co-lineality).
A-P axis in vertebrates is also controlled by Hox genes

In the mouse, there are four complexes, *HoxA*, *HoxB*, *HoxC* and *HoxD* complexes, each on different chromosomes.

Each of the four complexes is the equivalent of the Drosophila set.

Members of each complex are expressed in a head-to-tail series along the AP axis, just as in Drosophila (the pattern is most clearly seen in the neural tube, from the hindbrain to the spinal cord, but is visible in other tissues such as the mesoderm).

Regulation and functions of the Hox genes in vertebrate nervous system will be discussed later.

Figure 22-46 Molecular Biology of the Cell 5/e (© Garland Science 2008)
**Hox genes ≠ Homeobox genes**

**Homeobox**: 180 nucleotide DNA sequence (encoding 60 amino acid of the conserved DNA-binding domain called the *homeodomain*).

**Homeobox genes**: genes containing a homeobox.

**Hox genes**: genes on the Hox cluster on Drosophila chromosome or the Hox A-D clusters in the vertebrates (some vertebrates have fewer than four clusters). They only comprise a small portion of homeobox genes.

In the vertebrate brain, Hox genes are not expressed rostral to the hindbrain. Many homeobox genes that are not Hox genes are expressed in the midbrain and forebrain.

"Homeotic": functional term that describes the homeotic transformation (not the same as homeobox).

---

*Figure 22-46 Molecular Biology of the Cell 5/e © Garland Science 2008*
Summary of part 2

Signaling pathways and molecules involved in regionalization of the vertebrate nervous system were initially discovered in fly genetics

- **Signaling pathway**
  - cascade of gene regulation
    - egg polarity genes, gap genes, segment polarity genes, homeotic selector genes (transcription factors, signaling pathways of secreted molecules)
  - graded expression of transcription factors forms discrete boundaries within embryos

- **Molecules**
  - *Hox* genes (encodes a family of transcription factors) are involved in identity of specific body segments.
    - *Hox* genes and other homeobox genes encode homeodomain-containing transcription factors.
    - co-lineality (*Hox* genes are not expressed in rostral neural tissue)
    - conserved in vertebrates
  - secreted signaling molecules and their receptors
    - Wingless (≃Wnt in vertebrates)
    - Hedgehog (≃Shh in vertebrates)