Neural Induction and Regionalization

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Learning Objectives

- Learn the early stages of nervous system development, especially neural induction and early regionalization of the nervous system to the forebrain, midbrain, hindbrain, and spinal cord, from which all differentiated and functional nervous system tissues are derived.
- Identify some of the key molecules and signaling pathways essential for nervous system development, including the sonic hedgehog (SHH), transforming growth factor beta (TGFβ), canonical WNT, and Notch/Delta signaling pathways.
- Appreciate the advantages and limitations of using various invertebrate (Drosophila, *C. elegans*) and vertebrate (chick, *Xenopus laevis*, zebrafish, and mouse) model systems to understand early nervous system development in the human.
- Link some clinical disorders of early nervous system development to specific molecules and signaling pathways described in this chapter.

Highlights

- **Table** comparing two invertebrate (fruit fly and nematode) and four vertebrate (chick, frog, zebrafish, and mouse) model systems.
- Description of the genes and signaling pathways disrupted in some **disorders linked to early nervous system development**, including neural tube defects, holoprosencephaly, hydrocephalus, and neuronal migration disorders.
- Description of recent work using **induced pluripotent stem cells** (iPSCs) and **brain organoids** to improve our understanding of early nervous system development.

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Introduction to the Neural Tube and Early Regionalization of the Central Nervous System

The vertebrate central nervous system (CNS), incorporating the brain and spinal cord, begins as an epithelial sheet and through overlapping stages of neural induction, regionalization, and patterning, dorsal/ventral and anterior/posterior axes are established. Within each prospective CNS region, the prosencephalon (forebrain), mesencephalon (midbrain), metencephalon (cerebellum), rhombencephalon (hindbrain) and myelencephalon (spinal cord), neural progenitor cells (NPC)

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are generated, proliferate, undergo apoptosis, and migrate. These progenitors differentiate into neuronal and glial cell populations as well as extend axons, commence myelination, and establish synaptic connections. The prosencephalon will later be further regionalized into the telencephalon (including the neocortex and germinal matrices) and diencephalon (including the thalamus and hypothalamus). **Primary neurulation** involves fusion of the neural tube in the dorsal midline at three sites of closure in the following temporal sequence: (1) hindbrain/cervical boundary, (2) forebrain/midbrain boundary, and (3) rostral end of the neural tube [1].

The topics of CNS stem cells (Chap. 3), neurotrophins and cell death (Chap. 4), synaptogenesis (Chap. 5), axonal guidance (Chap. 6), and myelination (Chap. 7) are covered separately in subsequent chapters. This chapter will provide an overview of current concepts regarding induction, early regionalization, and patterning of the central nervous system, including discussion of the key morphogens and signaling pathways involved in these processes. In addition, congenital malformations and related disorders resulting from dysregulated neural induction, early regionalization, and patterning will be briefly reviewed.

Neural Induction

Model Systems: *Drosophila*, *C. Elegans*, *Xenopus*, Chick, and Mouse

Most of what we understand about neural induction has been learned from the use of invertebrate (*Drosophila melanogaster*, *C. elegans*) and vertebrate (*Xenopus laevis*, chick and mouse) model systems. Key facts about each model system, including advantages and disadvantages for their use in research, are presented in Table 2.1.

Early embryologic researchers proposed the "default model" of neural induction, wherein in the *absence* of specified signals favoring **bone morphogenetic protein (BMP)** signaling, the ectoderm gives rise to the neural plate [2, 3]. However, depending on the model system and experimental design used, the results obtained cannot always be explained by a simple default model of neural induction [2].

Setting Up Anterior/Posterior and Dorsal/Ventral Axes

How the differentiated CNS is generated from an unspecified sheet of epithelial cells has fascinated human embryologists, developmental biologists, and neuroscientists for decades. The developmental anatomy and ease of experimental manipulation of *Xenopus* and chick model systems permitted earlier investigators to elegantly spatiotemporally identify critical regions from which neural inducers originate by transplanting donor tissues from relevant developmental timepoints and anatomical areas.

By convention, *dorsal* is defined by the side in which the sperm fertilizes the *Xenopus* egg with *ventral* being directly opposite. Initially, the unfertilized *Xenopus* embryo has animal (anterior) and vegetal (posterior) poles, from which ectoderm and endoderm will be derived during gastrulation, respectively. From the ectoderm are derived the epidermis giving rise to skin and dermal tissues and the nervous system. Induction of the mesoderm, which gives rise to the notochord (most dorsal region), somites, and mesenchyme (eventually the skeleton, muscle, kidney, heart and blood in the mature animal), follows from the involuting marginal zone (IMZ) between the ectoderm and endoderm first specified during the blastula stage. Subsequently, signals from the dorsal lip of the *blastopore* are instructive for specifying the presumptive neurogenic region as gastrulation proceeds. In classic experiments, isolated late blastula stage *Xenopus* animal caps become epidermis, whereas gastrula-derived animal caps become neural tissue [4].

Nodes and Organizers

In *Xenopus*, the **Spemann organizer** from the dorsal lip of the blastopore *dorsalizes* adjacent mesoderm by inhibiting ventral signals from the mesoderm. The inductive properties of the organizer change during gastrulation. In the famous Spemann and Mangold experiment, when taken from the early gastrula, a graft from this organizer region translocated to the ventral side induces a second anterior/posterior (A/P) axis including a second neural tube. However, when derived from the late gastrula stage, a similar graft only induces the formation of tail structures.

Identified molecules within the Spemann organizer include those secreted from the notochord, such as chordin, noggin, and follistatin. Both chordin and noggin specifically block BMP family members, including BMP-2, BMP-4, and BMP-7. BMPs are members of the **transforming growth factor beta (TGFβ) superfamily** that are anti-neuralizing. Follistatin, also known as activin-binding protein, binds to activin, similarly interfering with TGFβ signaling. Acting downstream of BMP and TGFβ receptor signaling are the **SMADs**, vertebrate homologs of *mad* (*mothers against decapentaplegic*), the *Drosophila* homolog of TGFβ. Of the nine members of the SMAD family of transcription factors are the R-SMADS (receptor-regulated; *Smads*-1, 2, 3, 5, 8, 9), the I-SMADS (inhibitory; *Smads*-6, 7), and one co-SMAD (common partner; *Smad*4) [5](Fig. 2.1).

In the chick (*Gallus gallus*) and mouse, neural induction proceeds differently when compared to the process in *Xenopus*. **Hensen's node** arises from the most anterior end

Table 2.1 Comparison of model organisms

Organism	Fruit fly	Nematode	Mouse	Chick	Frog	Zebrafish
Latin name	*Drosophila melanogaster*	*Caenorhabditis elegans*	*Mus musculus*	*Gallus gallus*	*Xenopus laevis*	*Danio rerio*
Life cycle	Gastrula—3 h after fertilization Hatching—16-20 h 3 larval stages and pupation Metamorphosis Adult >9 days	Gastrula—5-10 h after fertilization Hatching −15-20 h 4 larval stages Adult - > 3 days	Gastrula—7 days after fertilization Birth—19 days Adult—6–8 weeks	Gastrula—16 h after fertilization Hatching—6 days after laying Adult—60 days after laying	Gastrula—15 h after fertilization Tadpole—4 days Metamorphosis Adult—60 days	Gastrula—8 h after fertilization Free swimming 2 days after fertilization Adult—90 days
Triploblast	Yes	Yes	Yes	Yes	Yes	Yes
Genome	15,431 genes	21,187 genes	33.4 K – 36.5 K genes	17,529 genes	>20 K (X. Tropicalis)	28,770 genes
Chromosomes	4 pr; polytene	6 pr	20 pr	39 pr	36 pr	25 pr
Advantages as a model system	– short life cycle – inexpensive – can be grown in large numbers – forward and reverse genetics – large scale mutagenesis – microsurgical manipulation is possible	– short life cycle – inexpensive – can be grown in large numbers – forward and reverse genetics – transparent embryos – large scale mutagenesis – invariant lineage – targeted ablation of individual cells – simple anatomy	– can model human disease – reverse genetics (gain/loss of function; conditional knockouts/ knockins) – many antibodies are available	– similar to mammals in complexity – less expensive to maintain than mice (incubator) – large eggs permit invasive procedures (transplantation, retrovirus injection, electroporation of nucleic acids) – later development is similar to mammals	– can develop in tap water – inexpensive – large, fertilized eggs easy to obtain – embryos are – hardy/ resist infection – can manipulate oocytes (mRNA injection) – fragments of early embryos can be cultured in simple media	– embryonic development is external – embryos are large and transparent – relatively inexpensive to maintain – arge number of offspring – forward genetics (mutagenesis screens)
Disadvantages as a model system	– complex anatomy – difficult to model human disease – limited antibodies	– difficult to model human disease – organs not similar to mammals – few antibodies	– expensive to maintain – small litters – forward genetics – susceptible to infections – embryonic development is internal – intrauterine manipulation is difficult – large mutagenesis screens are difficult	– forward/reverse genetics not routine – transgenic approaches are not currently an option – very early development occurs in the oviduct; difficult to study	– forward/reverse genetics not routine – limited antibodies for protein expression – later development is less similar to mammals – transgenic approaches are only a recent option and are not widely available	– reverse genetics (but can use morpholinos for knock-downs) – few antibodies – small size – may not be as useful as mouse to model human disease

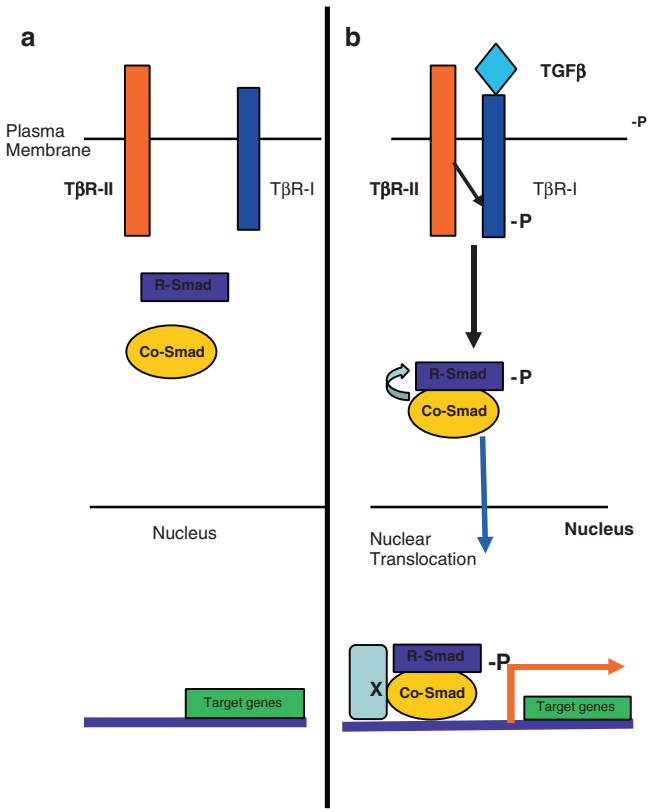

Fig. 2.1 The transforming growth factor (TGFβ) signaling pathway. (**a**) TGF-β receptor subunit type II (TβR-II) is constitutively active. (**b**) Type I TGF-β receptor subunits (TβR-I) are recruited to form a heterodimeric receptor complex upon binding of ligand to TβR-II, with transphosphorylation (-P) of the TβR-I kinase domain. R-Smads are subsequently phosphorylated by signaling from the activated receptor complex; R-Smads then bind to a co-Smad, translocate from the cytoplasm to the nucleus, and activate gene transcription with cofactor(s). [With Permission from Wigle JT and Eisenstat DD. In Moore, Persaud, and Torchia, Editors, The Developing Human, 11th Edition. Fig. 21.4, Page 466. Copyright Elsevier: Saunders [5]]

of the primitive streak (PS). The PS begins to regress after extending halfway across the blastoderm. Hensen's node subsequently moves posteriorly as the head fold and **neural plate** begin to form. As this node moves backward, the **notochord** develops anterior to it and somites begin to form on either side of the notochord. Once the notochord has formed, neurulation begins, following the progress of the notochord in an anterior to posterior direction. Posterior to Hensen's node, notochord formation, somite formation, and neurulation have not yet begun. Hensen's node can induce a new A/P axis in avian embryos. Transplants of tissue containing Hensen's node obtained from a donor quail embryo induce a second A/P axis in a chick host at the primitive streak stage. In a latter variant of the Spemann–Mangold experiment, Hensen's node explants from a chick epiblast sandwiched between *Xenopus* late blastula animal caps induce neural gene expression; however, explants derived from the posterior primitive streak or non-primitive streak epiblast cannot induce neural genes [4–7].

Inducers, Morphogens, Gradients, and Signaling Pathways

Developmental biologists have defined three criteria for an *inducer*. (1) The molecule has the correct spatial, temporal, and quantitative expression. Experimentally, this can be determined by *in situ* RNA hybridization, immunohistochemistry using specific antibodies, or more recently, by single-cell RNA sequencing. (2) Appropriate cells can respond to the factor. For example, using *Xenopus*, one can apply the candidate factor to isolated animal caps in culture or inject mRNA encoding the candidate factor into animal pole cells of the early blastula. (3) Blocking the function of the inducer factor prevents induction from taking place. This blockade can be accomplished by use of antisense oligonucleotides, RNA interference, CRISPR-*Cas9*-mediated gene editing, blocking antibodies, or dominant negative (e.g., mutant) receptors [4].

Important molecules isolated from Spemann's organizer, Hensen's node and/or the notochord include *Brachyury (a T-box gene)*, *Goosecoid* (a homeobox gene), *Hnf-3β* (an Hnf-class homeobox gene), and *Lim-1/Lhx1* (a Lim-class homeobox gene) and secreted proteins Nodal and Sonic Hedgehog (Shh).

Gradients of Nodal, a member of the TGFβ superfamily that binds to activin-type receptors, in the mesoderm (ventral, low to dorsal, high) may be specified by canonical **Wnt pathway** signaling mediated via nuclear translocation of β-catenin [5](Fig. 2.2).

Interestingly, *noggin* mRNA injected into early gastrula *Xenopus* embryos ventralized by ultraviolet (UV) treatment rescued neural induction in a manner similar to injections of polyA mRNA derived from the mesoderm of hyperdorsalized embryos resulting from treatment with lithium. Lithium inhibits glycogen synthase kinase-3 beta (GSK-3β), integral to both canonical Wnt and other signaling pathways, such as Shh. As stated earlier, intact animal caps cultured *in vitro* become epidermis, whereas dissociated cells from animal caps become neural tissue. However, adding BMP-4 to these dissociated cells blocks neural induction. In support of these experiments, expression of mRNA encoding a truncated activin receptor induces neural tissue when injected in isolated animal caps taken from *Xenopus* oocytes [2, 8].

Retinoids

Retinoids, including vitamin A (retinol) and 13-*cis*-retinoic acid, play an important role in establishing the A/P axis of the central nervous system and can serve as teratogens during early pregnancy. Retinoic acid "posteriorizes" the A/P axis, and either excessive retinoic acid or inhibition of its degradation leads to posteriorized structures. However, low levels of retinoic acid or defective endogenous retinoic acid synthesis will lead to a more "anteriorized" AP axis. Retinoic acid binds to its intracellular receptors, thereby regulating

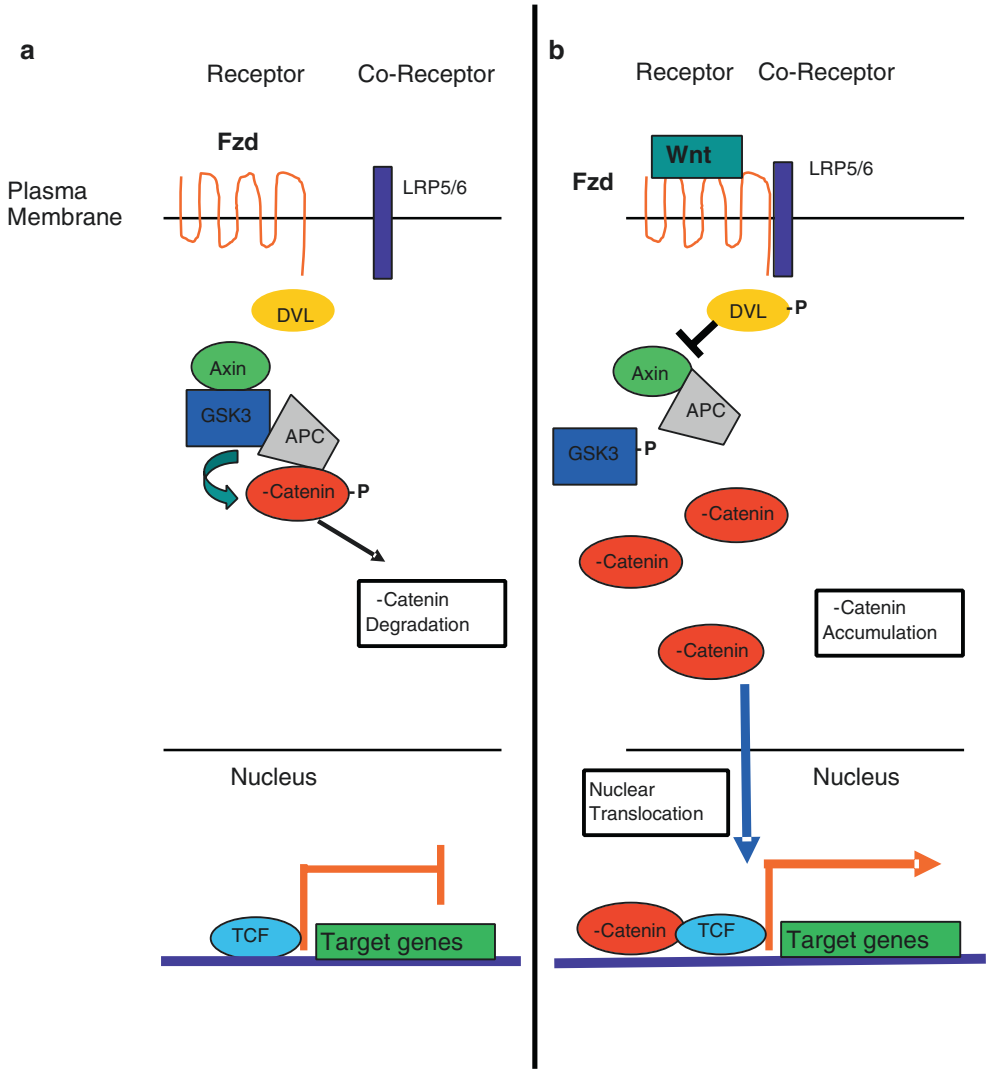

Fig. 2.2 The classic (canonical) Wnt signaling pathway. (**a**) When the Wnt ligand is not bound to the Frizzled (Fzd) receptor, β-catenin is phosphorylated (-P) by a multiprotein complex and targeted for degradation. Target gene expression is repressed by T-cell factor (TCF). (**b**) When Wnt is bound to Fzd, there is recruitment of LRP co-receptors, subsequent phosphorylation of Disheveled (DVL), and accumulation of β-catenin in the cytoplasm. β-catenin can translocate into the nucleus to activate expression of its target genes. *APC,* Adenomatous polyposis coli; *GSK-3,* glycogen synthase kinase 3; *LRP,* lipoprotein receptor–related protein. [With Permission from Wigle JT and Eisenstat DD. In Moore, Persaud, and Torchia, Editors, The Developing Human, 11th Edition. Fig. 21.6, Page 469. Copyright Elsevier: Saunders [5]]

the expression of downstream genes, including members of the *Hox* gene family of transcription factors [5].

Vertical *Versus* Planar Neural Induction

There are several postulated mechanisms of neural induction of anterior ectoderm from the underlying mesoderm and subsequent patterning of the early **neural tube**. These mechanisms may be dependent upon the experimental model systems used. In classical vertical or transverse neural induction, there is direct patterning of the overlying ectoderm by graded dorsoventral signals within the mesoderm. This patterned neuroectoderm subsequently regionalizes the neural tube along the A/P axis. In non-classical planar neural induction, these neural induction signals are derived from within the neural plate itself. These experiments were initially performed by sandwiching two explants from the dorsal blastopore lip containing IMZ cells of the early *Xenopus* gastrula (i.e., Keller Sandwiches) [4, 8].

Lateral Inhibition and Notch Signaling

Sox genes, members of the SRY high mobility group (HMG) family of transcription factors, are sufficient to induce neural differentiation through upstream activation of proneural genes such as neurogenin. In cells with activated BMP or Wnt signaling pathways, downstream expression of transcription factors such as GATA and MSX represses expression of *Sox* genes and these cells become epidermis. However, if **fibroblast growth factor (FGF)** signaling through FGF receptors is active or BMP signaling is blocked by inhibitor molecules such as noggin, chordin, or follistatin expressed from the organizer region, then *Sox* genes and subsequently downstream proneural genes are expressed [4, 8].

Furthermore, neural progenitor specification within the presumptive neuroepithelium occurs through **lateral inhibition**, a complex feedback loop process which is remarkably conserved from invertebrates to vertebrates. Conceptually,

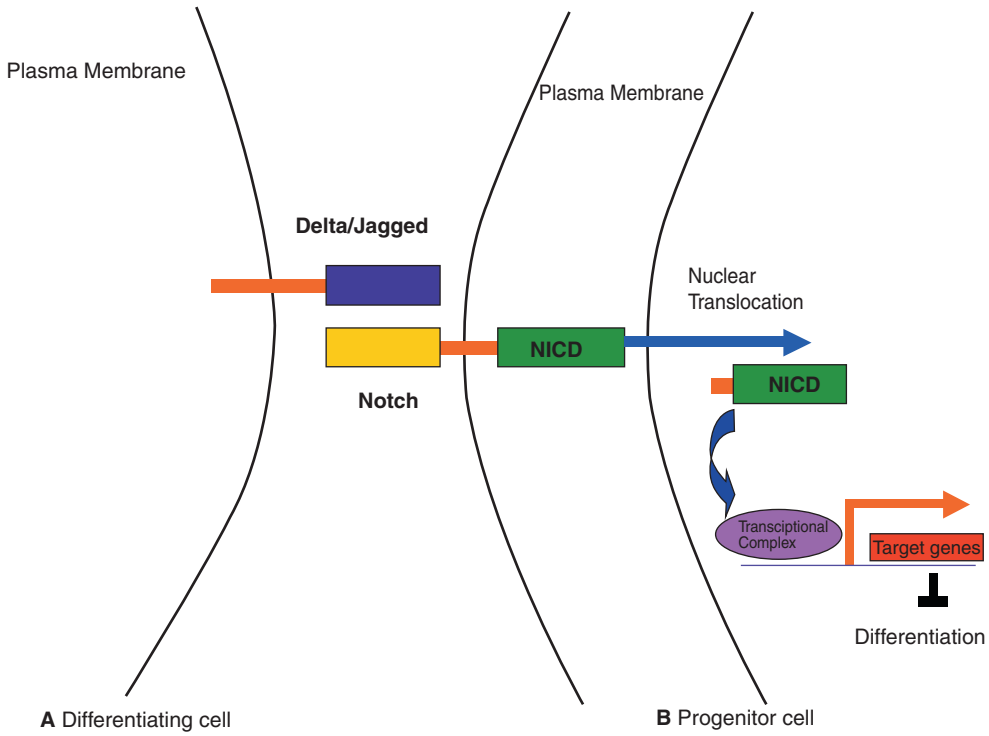

Fig. 2.3 The Notch/Delta signaling pathway. Left. Notch signaling is not active in differentiating cells. Right. In progenitor cells, Notch signaling results in cleavage of the Notch intracellular domain (NICD). Subsequently, there is translocation of the NICD to the nucleus, binding to a transcriptional complex resulting in expression of target genes, such as the bHLH gene *Hes1*, that inhibit differentiation. [With Permission from Wigle JT and Eisenstat DD. In Moore, Persaud, and Torchia, Editors, The Developing Human, 11th Edition. Fig. 21.9, Page 471. Copyright Elsevier: Saunders [5]]

one of the best described examples is in *Drosophila* sensory organ precursor specification, wherein one neuroblast is specified by cell–cell interactions within a proneural cluster and subsequently delaminates; the remainder of the cells within the cluster becomes epidermal cells, considered as a "default" cell fate. Some important *proneural* genes, such as those from the *achaete-scute* complex, are encoded by members of the basic helix–loop–helix (bHLH) family of transcription factors; these bHLH molecules dimerize and bind directly to DNA to regulate transcription of their target genes. Proneural mutants do not generate neuroblasts, only epidermal cells. Furthermore, mutations of *neurogenic* genes encoding members of the **Notch-Delta signaling pathway** result in the generation of excessive neuroblasts within a proneural cluster [4] (Fig. 2.3).

In the differentiating cell "A" destined to become a neuroblast, expression of Achaete-Scute proteins activates the Delta ligand expressed on its cell surface. Delta subsequently binds to its cognate Notch receptor expressed on the surface of the adjacent cell "B"; downstream signaling via cleavage of the Notch intracellular domain (NICD) leads to inhibition of proneural gene expression within cell "B," thereby leading to reduced activity of Delta–Notch signaling in cell "A" that will become a neuroblast. In vertebrates, the key bHLH transcription factor regulated by Delta–Notch signaling is neurogenin, which is upstream of NeuroD.

Asymmetric Versus Symmetric Cell Divisions

Another mechanism that is highly conserved from invertebrates to vertebrates is asymmetric cell division to specify a differentiated neuron from a neuroblast. There is a well-described phenomenon known as **interkinetic nuclear migration** in the developing neuroepithelium wherein early apical/basal cell polarity is established by the apical/basal migration of the nucleus within the cell during various phases of the cell cycle. M-phase (mitosis) occurs at the apical aspect directly adjacent to the ventricular surface, whereas S-phase occurs at the basal aspect. Furthermore, in the ventricular surface epithelium adjacent to the ventricles within the central nervous system, the neuroblasts that **divide symmetrically,** i.e., vertically, in the plane perpendicular to the ventricular surface, generate two equal daughter cells that have the capacity to divide further. However, the neuroblasts that **divide asymmetrically,** i.e., horizontally, in the plane

parallel to the ventricular surface, give rise to one neuroblast, capable of further cell divisions, and a more differentiated cell which can leave the cell cycle, migrate, and undergo terminal differentiation [4, 8].

Radial Versus Tangential Migration

Once a neural progenitor is generated via asymmetrical cell division, migration and terminal differentiation are frequently coupled. In general, there are two distinct modes of neuronal migration: radial migration and tangential migration. Excitatory neurons (expressing the neurotransmitter glutamate) usually migrate radially, whereas inhibitory interneurons (expressing the neurotransmitter GABA) often migrate tangentially, such as from the germinal matrix to the neocortex in humans and the ganglionic eminences to the neocortex in the mouse, where the basal forebrain is the primary source of GABAergic interneurons [9, 10].

Induced Pluripotent Stem Cells (iPSC)

Stem cells can self-renew through symmetric or asymmetric cell divisions (discussed earlier in this chapter). Several classes of stem cells have been described including **embryonic stem cells** (ESCs) and **induced pluripotent stem cells** (iPSCs). ESCs are derived from blastula's inner cell mass; they are **pluripotent** and can give rise to all differentiated cell types from the primary germ layers, the ectoderm, endoderm, and mesoderm. ESCs express several transcription factors, such as SOX2 and OCT-4, that repress differentiation. Although adult stem cells are relatively abundant in rapidly regenerating tissues, such as in the bone marrow and intestinal epithelium, there are "nests" of adult stem cells in the central nervous system and retina, in the subventricular zone and ciliary margins, respectively.

Due to ethical or practical limitations in place due to available sources of stem cells from the human embryo or adult, in the past decade, there has been significant interest in de-differentiating somatic cells such as epithelial cells and fibroblasts from adults into iPSCs. A few key master transcription factors, including OCT-3/4, SOX2, KLF4, and Nanog, have been identified that can reprogram differentiated cells into pluripotent cells and subsequently into specific neuronal populations. Furthermore, through viral and non-viral means, delivery of wild-type and edited genes through CRISPR/Cas9 technologies into iPSCs has the potential to treat many human diseases in which cell regeneration may restore structure and/or function, including neurodevelopmental disorders. Alternatively, these modified iPSCs can be screened for responses to chemical libraries toward identifying novel therapies [5, 11, 12].

Three-Dimensional (3D) Central Nervous System Organoids

More recently, there has been tremendous interest in modeling human brain development beyond the use of the commonly employed two-dimensional (2D) monolayer primary cell cultures *in vitro* or through the study of model organisms, including the zebrafish and mouse *in vivo*. Technological improvements (including spinner-flask bioreactors) and the advent of single-cell RNA sequencing have validated the diversity of cell types that can be generated from self-organizing, polarized, three-dimensional (3D) human brain organoids and their relative fidelity to the endogenous developing and adult brain with high organoid-to-organoid reproducibility. Furthermore, these models permit assessment of specific neuroanatomical regions (forebrain, midbrain, cerebellum, spinal cord, etc.), spatial organization, and cell–cell interactions including with the microenvironment. For example, using embryoid bodies, the addition of TGFβ inhibitors blocks mesendoderm lineage specification and promotes forebrain identity. BMP inhibitors block non-neural ectoderm lineage specification and promote dorsal forebrain identity. WNT inhibitors block both non-neural ectoderm and mesoderm lineages and promote forebrain identity [13].

There remain several limitations to 3D brain organoid systems, including an inability to fully replicate defined anatomical structures (such as the six-layer neocortex), missing cell types (e.g., microglia), absent vasculature, and the lack of functional neuronal networks. Recent innovations include co-culture with absent cell populations, providing an exogenous vascular supply and generating chimeric organoids from the combination of organoids from different brain regions. However, as experimental models, these 3D brain organoids provide a novel means to study normal and abnormal human brain development *in vitro*, thereby complementing studies in intact animal models and in tissues obtained from patients [13–15].

Disorders of Neural Induction, Early Regionalization, and Patterning

Holoprosencephaly

Holoprosencephaly (HPE) is a severe congenital brain malformation arising as a disorder of neural induction and regionalization with incomplete separation of the forebrain (prosencephalon). Five main types of HPE have been described (from severe to mild): (1) alobar; (2) semi-lobar; (3) lobar; (4) MIHV; and (5) microform. Its most severe phenotype includes complete lack of interhemispheric separation, a single midline forebrain ventricle, nonseparation of

deep gray nuclei and is frequently accompanied by cyclopia and severe craniofacial abnormalities. At the other end of the spectrum, there may be abnormalities of the corpus callosum and milder craniofacial anomalies observed, such as hypotelorism, coloboma, or cleft lip/palate. Neurocognitive impairment, feeding difficulties, seizures, and neuroendocrine abnormalities may be present and assessment by a multidisciplinary team as well as referral for genetic counseling is recommended.

Although holoprosencephaly can affect up to 1 in 250 conceptions, it is prevalent in only 1 on 10,000 live-born children. The etiology of HPE is very heterogeneous; HPE can occur as a single congenital disorder, as part of a syndrome (i.e., Smith–Lemli–Opitz or Kallmann syndromes) or a significant cytogenetic anomaly, including Trisomy 13. With the advent and availability of next-generation sequencing, mutations of several genes have been identified, including *SHH*, *TGIF1*, *FGFR1,* and the transcription factors *ZIC2* and *SIX3*. Other causes of HPE include submicroscopic chromosomal alterations and possibly to environmental influences, including maternal diabetes mellitus [16–19].

Anencephaly and Other Neural Tube Defects

Neural tube defects (NTD) arise due to failure of closure of the neural tube and occur in approximately 1 in 1000 live births worldwide [20]. NTD can occur anywhere along the rostral-caudal neuraxis and include disorders such as anencephaly (most anterior) to spina bifida (more posterior) and their variants. Although the majority of NTD occur as isolated congenital malformations, some are associated with syndromes and may have co-morbidities such as hydrocephalus and Chiari Malformations. The process of closure of the neural tube is discontinuous and occurs in the dorsal midline centered along three neuropores, which are open regions of neural folds: (1) hindbrain, (2) anterior (forebrain), and (3) posterior (spine). NTD can be open (anencephaly, craniorachischisis, or myelomeningocele) or closed, i.e., covered by epidermis (spinal dysraphism, spinal bifida occulta). *Primary neurulation defects* include craniorachischisis (18 days post fertilization/dpf), anencephaly (24 dpf), or open spina bifida (24 dpf). *Secondary neurulation defects* may be due to secondary neural tube tethering and can result in clinical disorders such as tethering of the spinal cord or spinal dysraphism with lipoma (35 dpf). *Postneurulation defects* include defects in skull closure, such as an occipital encephalocele with secondary herniation of the hindbrain and meninges (~ 4 months post fertilization) [1].

The causes of NTD can be genetic, environmental, or both. Closure of the neural tube has been studied in several vertebrate model systems. There is consensus that the process of **convergent extension** with convergence (mediolateral narrowing) and rostral-caudal extension is necessary. This requires the non-canonical Wnt signaling pathway via Frizzled (Fzd) membrane receptors and cytoplasmic Dishevelled (Dvl) to regulate epithelial planar cell polarity (PCP) processes. NTD can also result from dysregulation of bending of the neural folds at the median or dorsolateral hinge points of the primary neural tube. The Shh and BMP/TGFβ signaling pathways regulate these processes. Furthermore, NTD can be caused by full or partial failure of adhesion and fusion of the neural folds, experimentally supported by knockout mouse models in *ephrin-A5* or *EphA7* mutants [21]. Finally, other research has demonstrated that disordered cell proliferation and/or cell death can lead to NTD in experimental models (reviewed in [1]).

Although the majority of NTD occur sporadically, dozens of candidate genes have been implicated, often through the initial identification of NTD in single- or double-gene knockouts in the mouse model. NTDs can also be induced by **teratogens**, including the anticonvulsant medication valproic acid, which is also a histone deacetylase (HDAC) inhibitor. Various maternal risk factors include maternal fever/hyperthermia, obesity, diabetes mellitus, and nutrition during pregnancy [20]. Of significance, deficiency of the B-vitamin folic acid (folate) has been directly linked to the incidence of NTD. Clinical trials focused on primary prevention of NTD have demonstrated significant reduction in the occurrence of NTD in mothers who received folic acid supplementation. Most developed nations routinely supplement folic acid and maternal folic acid is a standard part of prenatal care. Although the mechanism linking maternal folate deficiency and NTD is not fully elucidated, it may include DNA methylation as a requirement for closure of the neural tube, as shown in *Dnmt3b* knockout mice [22].

Lissencephaly, a Neuronal Migration Disorder

Although there are many types of malformations of cortical development (MCD) with abnormal neuronal migration, this section will focus on lissencephaly (LIS). As classified [23], disorders of neuronal migration can be grouped as follows: (1) classic lissencephaly spectrum (includes smooth lissencephaly, microlissencephaly, and subcortical band heterotopia (SBH)); (2) cobblestone malformations (rough lissencephaly, polymicrogyria, leptomeningeal glioneuronal heterotopia); (3) periventricular heterotopia (nodular or linear periventricular heterotopia); or (4) dyslamination without cytologic dysplasia or growth abnormality (focal cortical dysplasia type I/FCD-I) [23]. Many patients with lissencephaly have epilepsy [24].

Classic lissencephaly (LIS) is relatively rare; morphologically there is *agyria* (absent cortical gyri) or *pachygyria* (very wide gyri) accompanied by a thickened cortical plate,

ectopic/displaced subcortical neurons and/or band/nodular heterotopias. Although LIS is usually an isolated cortical malformation, it may be part of a syndrome, such as Miller-Dieker and XLAG (X-linked LIS with ambiguous genitalia) often due to mutations of *ARX*, a transcription factor). Mutations of genes encoding cytoskeletal proteins have been implicated in classic LIS, whereas variant LIS may be linked to mutations of *REELIN* encoding a secreted protein, or other genes. *LIS1* (also known as *PAFAH1B1*, platelet-activating factor acetylhydrolase 1B) is located on chromosome 17p13.3; *LIS1* mutations are linked to classic LIS alone or as part of a chromosomal microdeletion in Miller-Dieker syndrome [25]. In part, *LIS1* encodes a cytoskeletal protein that interacts with microtubule associated proteins such as dynein required for neuronal migration. SBH is linked to mutations in *DCX* (doublecortin) located on chromosome Xq22.3-q23, encoding another microtubule associated protein [23]. Recently, several cytoskeletal disorders have been grouped together as *tubulinopathies*. Many tubulin gene disorders such as mutations of *TUBA1A*, are linked to severe malformations of cerebral cortical development, including lissencephaly and its variants.

Cobblestone LIS is due to histological defects linking radial glia (which support neuronal migration) to the basement membrane and results in dysregulated migration of neurons and glia into the subarachnoid space. Cobblestone LIS may be associated with CNS, muscular and/or ocular defects. Associated syndromes include Walker–Warburg syndrome, Muscle–Eye–Brain Disease and Fukuyama congenital muscular dystrophy (FCMD). Many of the genes associated with cobblestone LIS are part of the *α-dystroglycanopathies*, including *POMT1/POMT2*, *POMGNT1*, *FKTN*, *FKRP,* and *LARGE*. Other cases of cobblestone LIS are due to mutations of genes encoding laminins (*LAMB1/B2/C3*) [23, 26].

Hydrocephalus

Hydrocephalus is a relatively common disorder in children and sometimes occurs in adults. It can frequently accompany a closed NTD. When meningitis was a more frequently encountered disease of childhood, *communicating hydrocephalus* was a sequela of decreased reabsorption of cerebrospinal fluid (CSF). *Obstructive hydrocephalus* is often due to tumors of the CNS which frequently block CSF flow within or extrinsic to the ventricular system. In this section, the focus is on genetic disorders or syndromes for which congenital hydrocephalus is a major presenting sign. *X-linked hydrocephalus associated with stenosis of the aqueduct of Sylvius* (HSAS) is frequently due to mutations of the *L1CAM* gene encoding an adhesion molecule. Associated comorbidities may include agenesis of the corpus callosum, adducted thumbs, and X-linked spastic paraplegia. Other gene mutations resulting in congenital hydrocephalus occur in the *AP1S2* gene associated with X-linked intellectual disability and Fried syndrome with calcification of the basal ganglia, and in genes linked to α-dystroglycanopathies and cobblestone LIS briefly discussed in the preceding section [27]. Non-syndromic AR hydrocephalus is linked to mutations of the *CCD88C* and *MPDZ* genes, whereas hydrocephalus associated with the VACTERL (vertebral, anal, cardiac, tracheoesophageal, renal and limb anomalies) sequence has been linked to *PTEN* and *FANCB* (X-linked) [28].

Multiple Choice Questions

1. Which of the following overlapping stages of central nervous system (CNS) development is in the **INCORRECT** order?
   A. Induction of the neural plate
   B. Regionalization and patterning of the neural tube
   C. Migration of neurons
   D. Reflexes and behaviors
   E. Synapse formation
2. During development of the neural tube, what is the effect of **HIGHER** concentrations of retinoic acid above physiological levels?
   A. Anteriorization
   B. Dorsalization
   C. Posteriorization
   D. Ventralization
   E. Polarization
3. Which statement about cortical neurogenesis is **CORRECT**?
   A. Migrating cells result from asymmetrical cell division, perpendicular to the ventricular surface
   B. Migrating cells result from symmetrical cell division, perpendicular to the ventricular surface
   C. Migrating cells result from asymmetrical cell division, parallel to the ventricular surface
   D. Migrating cells result from symmetrical cell division, parallel to the ventricular surface
   E. None of the above
4. Which class of developing cells in the central nervous system rely on **TANGENTIAL** migration to reach their final destination in the cortex?
   A. Glutamatergic neurons
   B. GABAergic neurons
   C. Interneurons
   D. Radial glia
   E. B and C

5. Of the following genes, which one is **NOT** associated with holoprosencephaly:
   A. SHH
   B. PTEN
   C. SIX3
   D. FGFR1
   E. ZIC2

   Answers: 1D; 2C; 3C; 4E; 5B.

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