Part I

Basic aspects

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Genes and brain development

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The mechanisms underlying development of the mammalian central nervous system (CNS) are of fundamental importance for research into psychiatric disorders. The processes of neurulation, patterning, neuronal specification, and synaptogenesis, as well as the functional dynamics of neurotransmission, are governed by the coordinated actions of products from a wide array of genes. Our knowledge of the expression and complex interactions between the products controlling these processes has been broadened by developmental studies using animal models (primarily fruit fly and nematode but increasingly in the mouse); biochemical, histochemical, and imaging studies; and analysis using high-throughput, non-selective techniques such as microarray hybridization. Undoubtedly, the identification of novel brain-expressed transcripts through the Human Genome Project has also provided a solid framework for investigation of CNS function.

A neurodevelopmental etiology of schizophrenia is suggested by neuroimaging and postmortem studies revealing significant and replicated lateral ventricular enlargement, hippocampal and gray matter deficits, and cellular disarray, independent of duration of illness and antipsychotic treatment. As these features remain some of the best non-behavioral correlates of schizophrenia, the genetic investigation of neurodevelopmental candidate genes, especially those with well-characterized neural function and within chromosomal regions demonstrating prior linkage or association with schizophrenia, is an important research focus.

This chapter will provide an overview of the major mechanisms involved in the development of the mammalian CNS, with specific reference to the identity and patterning of genes that are known to regulate these developmental phases (Table 1.1). This pattern of gene expression will be related to the etiology of schizophrenia through evidence provided by genetic, postmortem, imaging, electrophysiological, and behavioral investigations of the disorder. In this fashion, a set of possible determinants of both aberrant neurodevelopment and psychosis will be suggested.

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Table 1.1. Neurodevelopmental genes and reports of expression-based and genetic analysis in schizophrenia

Gene	Function	Locus of interest	Genetic and expression-based analysis
Mash1	Neurulation -		
Notch		NOTCH4 (6p21.3)	(Wei & Hemmings, 2000 and see text)
Delta		DLL1 (6q27)	
Neurogenin		NGN1 (5q23-q31)	
NeuroD		NEUROD (2q32)	
Sonic Hedgehog		SHH (7q36)	
Wnt		WNT1 (12q12-q13)	(Cotter *et al.*, 1998; Miyaoka *et al.*, 1999)
Krox20	Patterning	EGR2 (10q21.1-q22.1)	
Hox		HOXB (17q21.3)	(Kennedy and Kidd, unpublished)
Dlx		DLX1 (2q32)	
Emx		EMX2 (10q26.1)	
Gbx		GBX2 (2q36-q37)	
Nkx		TITF1 (14q13)	
Otx		OTX2 (14q21-q22)	
Pax		PAX6 (11p13)	(Stober *et al.*, 1999)
POU		POU3F3 (3p14.2)	
NCAM	Cell migration/ neurite extension	NCAM1 (11q23.1)	(Vicente *et al.*, 1997; Doherty *et al.*, 1990)
L1CAM			
N-Cadherin		NCAD (18q11.2)	
Reelin		RELN (7q22)	(Fatemi *et al.*, 2000; Guidotti *et al.*, 2000)
NGF	Neuronal	NGFB (1p13.1)	
BDNF	Survival	BDNF (11p13)	(Muglia *et al.*, 2003)
NT-3		NTF3 (12p13)	(Nanko *et al.*, 1994 and see text)
NT-4/5			NTF5 (19q13.3)
GDNF		GDNF (5p13.1-p12)	(Lee *et al.*, 2001)
CNTF		CNTF (11q12.2)	(Thome *et al.*, 1996 and see text)
SNAP25	Presynaptic/ exocytosis	SNAP25 (20p11.2-p12)	(Tachikawa *et al.*, 2001; Wong *et al.*, 2003)
Syntaxin		STX1A (7q11.23)	(A. Wong *et al.*, unpublished data)
Synaptobrevin		VAMP1 (12p)	
Synapsin		SYN3 (22q12.3)	(Ohmori *et al.*, 2000) and see text
Complexin		CPLX2 (5q35.3)	(Harrison and Eastwood, 1998 and see text)
Synaptophysin		SYP (Xp11.23-p11.22)	
Synaptotagmin		SYT1 (12cen-q21)	

Neurulation

The coordinated development of the human brain, from a single cell to some 10^{12} neurons with a possible 1000 connections between each, is a feat of unimaginable intricacy. The function of individual groups of neurons within this framework in the control of essential processes such as learning and memory, perception, mood, and motor activity adds a further level of complexity. While the differentiative pathways followed by neurons are topologically similar to those in other cell types, one unique aspect of nervous system morphogenesis is the essential connectivity of its units through axonal and dendritic outgrowth, allowing for interactions in a highly plastic system. Despite the apparent difficulties of dissecting such a detailed system, the changes involved in neuronal specification and overall CNS development are becoming increasingly well understood. Much of our present state of awareness in the field has been gathered through research using simple organisms such as sea slug, squid and nematode, while recombinant DNA technology has allowed generalization to vertebrates through investigations in mice. The cascade of events proceeding from early gastrulation of the embryo to maturation can now be adequately described.

The events leading to formation of the neural tube are known collectively as neurulation. Two distinct developmental programs may be used: primary neurulation, during which the chordamesoderm instructs the overlying ectoderm to divide, invaginate, and separate from the surface to form the neural tube, and secondary neurulation, during which a cylindrical zone of cells descends into the embryo and hollows to form the tube. In mammals, this appears to occur in regionally differentiated processes, with primary neurulation in the anterior region and secondary neurulation posterior to somite 35.

The first signals responsible for the determination of anterior/posterior identity in the ectoderm during gastrulation emanate from Hensen's node, a small group of cells at the anterior end of the primitive streak. The neural ectoderm is induced through vertical signals from the mesodermal tissues beneath and adjacent to it. The neural ectoderm gives rise to the neural plate, which is polarized through the dorsal–ventral and anterior–posterior axes. The edges of the plate fold upwards to join at the dorsal midline, producing the neural tube. Cells overlying the dorsal midline of the neural tube form neural crest cells and migrate to form the brain and spinal cord from the anterior and posterior portions, respectively. A commitment to cell fates is revealed with the closure of the tube, with ventricular CNS generated from the interior of the tube and epithelial cells at the interior periphery eventually forming neurons and glia.

The brain becomes further divided through a series of constrictions into the forebrain (prosencephalon), midbrain (mesencephalon), and hindbrain

(rhombencephalon). Further compartmentalization results in regions within these vesicles known as neuromeres, where mixing of cells becomes restricted to others within the same region (as within rhombomeres of the hindbrain), laying the ground plan for specification of neurogenesis. The forebrain gives rise to the telencephalon (forming the olfactory lobes, hippocampus, basal ganglia, and cerebral cortex in the adult brain following proliferation and folding) and the diencephalon (forming the thalamus, epithalamus, hypothalamus, and the retina). The midbrain forms the mesencephalon (including regions of connectivity between rostral and caudal brain, the optic lobes, and tectum), while the hindbrain develops into the metencephalon (producing the cerebellum and pons) and myelencephalon (forming the medulla).

The localization and development of these highly specialized regions of the brain is regulated principally through the actions of transcription factors: DNA-binding proteins that direct the expression of specific genes. The phenotype of all neurons is dictated by the precise set of genes expressed within the cell, allowing for functional specialization. While the initial effect of transcription factors on the cell may be brief, the influence of individual factors may be carried through numerous cell divisions and persist beyond migration and diversification of a specific cell lineage. The coordinated boundaries of expression of these factors serve to delineate clearly morphological boundaries and to impart organization to the adult CNS. Much of the information that we now possess regarding the determination of neural cell fate and patterning in the early embryo has been made possible through research conducted in *Drosophila*, *Xenopus*, and mice, where factors such as single neuroblast ablation, ease of manipulation, and the ability to knock out specific genes are advantageous. The extrapolation of findings from these organisms to humans is facilitated by the remarkable degree of conservation of the factors controlling neural fate across species.

The selection of distinct areas within the ectoderm to form neural precursor cells is regulated through the expression of proneural genes, encoding transcription factors of the basic helix-loop-helix (bHLH) class. The expression of these genes provides the potential for the cell to become neural. Proneural genes are typified by *Mash1*, encoding a factor in the central and peripheral nervous systems that selects for neural fate and acts in subsequent differentiation. Knockout analysis of *Mash1* reveals its role in neurogenesis within the ventral telencephalon (Casarosa *et al.*, 1999). Overexpression of the *Xash3*, the homologue of *Mash1* in *Xenopus*, leads to selection of neural over epidermal cell fate and ectopic neuronal differentiation (Ferreiro *et al.*, 1994). Lateral specification within the cluster of proneural cells then allows specific cells within the equivalent group to become neural. The transmembrane proteins encoded by the neurogenic genes *Notch* and *Delta* permit the division between neuronal and epidermal cell fates to become

established in a mechanism incorporating cell-to-cell signaling and an amplified feedback loop. Neural precursors express the Delta ligand, which, on binding to the Notch receptor, represses further proneural gene expression and downregulates the expression of Delta. When *Notch* transcription is absent from the embryo, all ectodermal cells develop into neural precursors in *Drosophila* (Artavanis-Tsakonis *et al.*, 1983), while markers of neurogenesis are markedly increased in the absence of *Notch1* transcription in mouse (de la Pompa *et al.*, 1997).

Neurogenins are proneural bHLH proteins that function as transcriptional activators of neuronal differentiation genes. The neurogenin Ngn1, for instance, promotes neurogenesis and inhibits differentiation of neural stem cells into astrocytes (Sun *et al.*, 2001). Another neurogenin, Ngn2, restricts cell migration from the cortex to the striatum (Chapouton *et al.*, 2001) and is expressed in dorsal telencephalic cells, defining a boundary through repression by Mash1, which defines the ventral telencephalon (Parras *et al.*, 2002). A regulator of differentiation known as NeuroD is directly regulated by the neurogenins in many regions of the brain and maintains expression in fully differentiated neurons (Lee, 1997). The overexpression of NeuroD in *Xenopus* will direct the development of non-neural ectoderm into neurons and accelerate the differentiation of neural precursors (Lee *et al.*, 1995). A host of additional factors act to convey positional information to groups of cells, instruct the development of specific phenotypes through cascades of gene expression and repression, and preserve the differentiated states of various cell lineages in the CNS.

Sonic hedgehog (*Shh*), a member of a vertebrate gene family corresponding to the *Drosophila* gene *hedgehog*, is a secreted factor that acts in a concentration-dependent fashion to induce cells of the floor plate and neural tube (Ericson *et al.*, 1995), in addition to its role in axial specification over various regions of the body. Regionalization within the developing telencephalon is controlled by the ventralizing properties of *Shh* expression (Kohtz *et al.*, 1998) and distinct dopaminergic and serotonergic neuronal subpopulations are induced along the anterior–posterior axis at different times by Shh signaling (Hynes and Rosenthal, 1999). Signaling mediated by the Wnt family of glycoproteins is tightly connected to those controlled through the *hedgehog* family. The expression of *wnt1* (and the gene for fibroblast growth factor 8) is coordinated with establishment of the mesencephalon–metencephalon boundary; and mice lacking Wnt1 fail to develop midbrain structures and cerebellum (McMahon and Bradley, 1990).

The recent description of a strong association between a promoter base-pair substitution and the exon 1 $(CTG)_n$ repeat of *NOTCH4* with schizophrenia (Wei and Hemmings, 2000) in a region (6p21.3) previously associated with the disease (Schwab *et al.*, 1995; Straub *et al.*, 1995) has ignited interest in this candidate gene. Of six *NOTCH* homologues identified in vertebrates, *NOTCH4* is the most divergent phylogenetically (Kortschak *et al.*, 2001) and shows a pattern of expression that

is primarily endothelial and myocardial (Li *et al.*, 1998) with minimal expression in brain. A number of follow-up reports on *NOTCH4* have failed to replicate the original finding for both individual markers and haplotypes using case–control and family-based association approaches (Fan *et al.*, 2002; Imai *et al.*, 2001b; Klempan *et al.*, 2001; McGinnis *et al.*, 2001; Sklar *et al.*, 2001; Swift-Scanlan *et al.*, 2002; Ujike *et al.*, 2001). The extreme biases in transmission of *NOTCH4* alleles witnessed in the first study now appear more likely to be a false-positive association, although an unusual population specific effect cannot be excluded since differences between the African-American and European populations have been noted at the *NOTCH4* locus (Luo *et al.*, 2004).

Abnormalities of the Wnt signaling pathway have been suggested by several recent studies which have described reductions of β-catenin and γ-catenin staining in the CA3 and CA4 hippocampal subregions and increases in Wnt1 staining in these regions of schizophrenic brains relative to controls (Cotter *et al.*, 1998; Miyaoka *et al.*, 1999). Furthermore, levels of glycogen synthase kinase-3β (GSK-3β) are significantly reduced in the prefrontal cortex of schizophrenia patients and Wnt is known to act as a repressor of GSK-3β (Beasley *et al.*, 2001). GSK-3β also participates in apoptosis, a form of programmed cell death, and, therefore, aberrant GSK-3β expression may provide a rationale for observations of irregular neuronal distributions found in schizophrenia (Kozlovsky *et al.*, 2002). Many of the signaling components of the Wnt pathway have been localized and some of these map within susceptibility regions for psychosis (Rhoads *et al.*, 1999).

Segmentation

Patterning of the specialized cell groups that will eventually specify distinct regions of the CNS is carried out by other groups of transcription factors, whose expression is confined to discrete segments of the developing embryo. The compartmentalization granted by division of regions of the brain into segments (prosomeres in the forebrain and rhombomeres in the hindbrain) prevents the mixing of various lineages of cells and the activity of expressed genes and restricts the targets and navigational properties of axons within these segments.

One gene, for example, that is known to be critical in the establishment of neural tube boundaries is the zinc-finger transcription factor Krox20. *Krox20* is expressed in the neural plate in alternating segments (rhombomeres r3 and r5) prior to distinct rhombomere formation, as revealed by lineage tracing studies in the chick. Mice that are null for *Krox20* display a disruption of segmental identity with a fused r2/r4/r6 region (Schneider-Manoury *et al.*, 1997). The function of *Krox20* is considered comparable to that of the *Drosophila* pair-rule genes, which translate information

from previously expressed genes into periodic stripes of further expression. At the early stages of segmental specification, however, there is very little conservation of specific genes between flies and vertebrates.

One family of genes that is critical in the determination of regional identity along the anterior–posterior axis is known as the homeobox (*Hox*) family. Based upon homeotic genes in *Drosophila*, these master regulatory factors are strongly conserved from fly to mammals. Homeobox proteins are characterized by a 60 amino acid residue motif (the homeodomain), which binds to specific sequences of DNA. The *Hox* genes show colinearity with positions of genes within clusters along the chromosome corresponding to their domains of expression along the embryo. Those genes located further 3′ within the cluster are expressed both earlier during development and in a more anterior location. The limits of rostral expression of the *Hox* genes are strongly coordinated with the divisions between rhombomeres, suggesting that they may be involved in the specification and/or maintenance of segment identity (Krumlauf, 1994). The influence of specific *Hox* members on actual segment phenotype is illustrated by *Hoxb1*. The expression of *Hoxb1* is particularly strong in rhombomere r4 and loss of Hoxb1 in mutant mice transforms r4 to an r2 phenotype (Studer *et al.*, 1996), while ectopic *Hoxb1* expression in chick produces the opposite result (Bell *et al.*, 1999).

In addition to the *Hox* family of transcription factors, a number of other homeodomain-containing proteins impart positional information to the developing brain. Mice lacking both *Dlx1* and *Dlx2*, for instance, do not demonstrate proper migration of cortical cells from subcortical regions and show disrupted cell migration within the striatum (Anderson *et al.*, 1997). In the absence of functional Emx1 protein, the corpus callosum fails to develop, while *Emx2* mutants lack hippocampal dentate gyrus and Cajal–Retzius cells of the neocortex. These *Emx2* mutants are also similar to the reeler mouse, with disturbances of lamination and neuronal migration (Mallamaci *et al.*, 2000). The expression of *Gbx2*, required in rostral hindbrain differentiation (Wassarman *et al.*, 1997), acts in concert with Otx2 to establish the isthmic signaling region (Broccoli *et al.*, 1999). The function of Otx2 is perhaps even more critical in neural induction, as targeted mutations of *Otx2* result in absence of rostral brain areas.

Another family of transcriptional regulatory factors expressed in the mammalian forebrain with persistence into adulthood is known as the POU domain family. Members of this group contain a POU homeodomain and a POU specific domain and are generally expressed in restricted regions late in forebrain development. The severe defects produced by mutations in many early patterning genes (such as *Hox*) make these unlikely candidates for the subtle structural alterations witnessed in schizophrenia; however, many members of the POU class of factors

are expressed specifically in frontal cortical and hippocampal areas implicated in disease processes (Turner *et al.*, 1997). The POU-III subclass of factors includes Brn-1, Brn-2, and SCIP (among others), which have been extensively studied in null mutant mice. *Brn-1* null mice show cellular disorganization of the hippocampus and transitional cortex and disorganized cortical lamination, while *Brn-2* mutants are defective in hypothalamic development (Schonemann *et al.*, 1995). Brn-1 and Brn-2, coexpressed in layer II–V cortical neurons, have roles in the initiation of radial migration (McEvilly *et al.*, 2002). Reelin, also involved in radial migration of cortical neurons, is reduced in a subpopulation of cortical plate neurons normally colocalizing with Brn-1 expression in *Brn-1* mutants, suggesting cross-regulation between these pathways.

Transcriptional regulatory molecules can act in a hierarchical fashion to enhance or inhibit the expression of downstream targets and sometimes may compensate for others in their absence. The roles played by these factors can be diverse, acting in neuroepithelial patterning, differentiation, and survival through cues given at different times throughout embryogenesis. Numerous genes have been identified as targets for these molecules, as seen in the enhancement of neuronal cell adhesion molecule (NCAM) expression by Otx2 (Nguyen Ba-Charvet *et al.*, 1999) and the regulation of the *SNAP25* promoter by Brn-3 POU transcription factors (Morris *et al.*, 1997). The cascade of gene expression induced by these factors would classify them as interesting targets for investigation in schizophrenia, yet few studies have been undertaken, perhaps because it is felt that the consequences of their misregulation would be dire. One report has shown a mild association between a high-activity variant of a *Pax6* (paired-box family transcription factor) polymorphism, and paranoid schizophrenia (Stober *et al.*, 1999), while others have suggested that Pax6 may contribute to schizophrenia through disrupted retinoic acid signaling (LaMantia, 1999).

Cell adhesion

In the developing brain, once a neuron has become committed to its phenotype it must migrate to its proper layer of the maturing brain (Ruiz i Altaba, 1994). Cell adhesion molecules (CAMs) are cell membrane proteins that mediate adhesion between neural cells, exerting a key role in the morphogenesis, differentiation, and migration of neurons as well as in the guidance of outgrowing axons in the developing brain (see Fig. 1.1). CAMs can be classified functionally into calcium-dependent and calcium-independent groups. The calcium-dependent category contains at least 80 proteins belonging to the cadherin superfamily, while the Ca^{2+}-independent CAMs comprise the immunoglobulin (Ig) superfamily (IgSF). Most of the cadherin

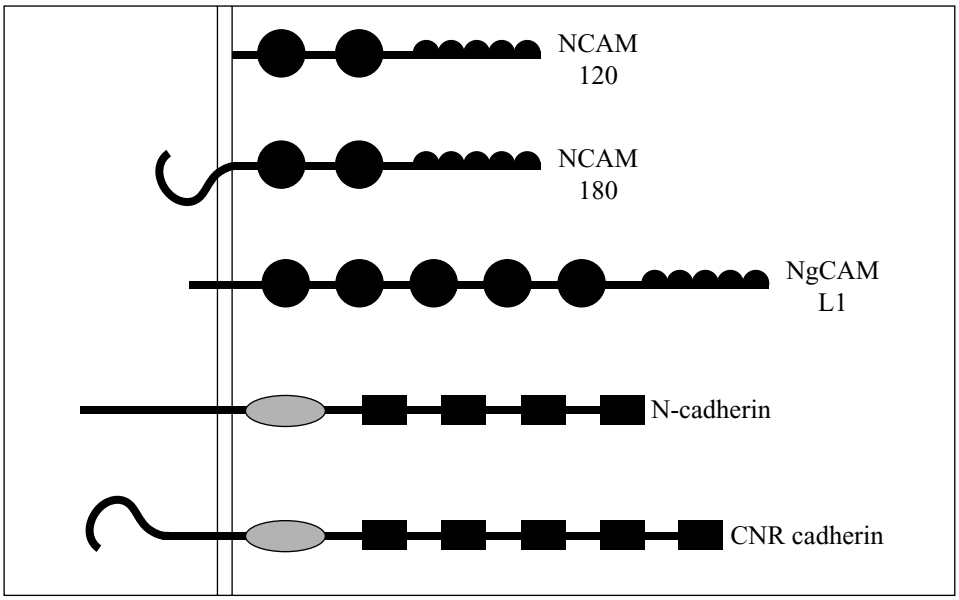

Fig. 1.1. Members of the cadherin and immunoglobulin superfamilies of cell adhesion molecules. Several prominent members of these transmembrane protein families are depicted, with known roles in neurite outgrowth and morphogenesis. CNR, cadherin neuronal-related receptor; NCAM, neuronal cell adhesion molecule; Ng CAM, neurogenin cell adhesion molecule.

superfamily genes are expressed in the brain, and the protein structure is characterized by a unique domain named the cadherin motif, which is involved in calcium binding (Takeichi, 1990). The cadherin motif (also called the EF motif) is repeated a variable number of times in the different members of the superfamily (Yagi and Takeichi, 2000). In addition to the classic cadherins, other members of the protocadherins superfamily are cadherin neuronal-related receptors (CNRs) and the so-called seven-pass transmembrane cadherins. The seven-pass transmembrane cadherins have a transmembrane structure similar to G-protein-coupled receptors and appear to be involved in polarity orientation of the developing neuron, as shown in *Drosophila* (Usui *et al.*, 1999). The CNRs are coded by a cluster of 13 genes that map to 5q31.1 in humans and play a role in both the formation of neuronal circuits at the synaptic level and the strengthening of the synapse during long-term potentiation (LTP) (Yagi and Takeichi, 2000). It is interesting to note that a significant linkage region for schizophrenia, derived from meta-analysis of data from 20 genome scans, is located in a 30 cM stretch across 5q23–34 (Lewis *et al.*, 2003), which includes the CNR gene cluster. CNRs are distinguished from the other