Genomic Disorder and Gene Expression in the Developing CNS

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The central nervous system (CNS) is composed of billions of neurons with trillions of synaptic connections specifically designed for large amounts of information processing through parallel representational neural networks. The level of diversity required for higher order human cognition is, in large part, genetically regulated and, for this reason, highly susceptible to genomic instability resulting in neurological dysfunction. Cellular diversity is regulated by molecular mechanisms at multiple levels: DNA, RNA, and protein. This article focuses on the level of DNA – specifically, how genomic changes influence gene expression in the normal brain and in human genomic disorders.

Genomic Regulation of CNS Development

Development of the CNS requires an array of cellular processes to produce a functioning brain, including proliferation, programmed cell death, differentiation, migration, axonal/dendritic outgrowth, and synaptogenesis. A large number of genes are involved in the transition from neural stem cell to progenitor cell to postmitotic neuron, and in the age of microarrays and genome sequencing, new genes are constantly being discovered. Genomic and environmental regulation of gene expression determines the ultimate fate of individual neurons and their integration into functioning networks. Within the highly interconnected brain, each neuron has the potential to exert far-reaching effects on numerous other cells. Therefore, altered gene expression within even a single brain cell can impact nervous system development and function.

Neural stem cells lining the surface of the embryonic neural tube proliferate and give rise to lineage-committed neural progenitor cells, specifying neural patterning (see **Figure 1**). A large number of proneural transcription factors are involved in directing the fate of neural progenitor cells. In addition, epigenetic mechanisms (discussed below) are important in selectively silencing genes, committing cells to neuronal or nonneuronal lineages. As stem and progenitor cells divide, genomic instability can produce daughter cells with distinct genotypes.

Upon exiting the cell cycle, neurons and glia migrate away from their proliferative zone, guided by soluble and cell surface molecular cues. A host of factors can influence the subsequent maturation of neural populations, including those intrinsically encoded within the genome as well as an orchestrated milieu of activity-dependent intra- and intercellular signaling pathways.

Cell death is a prominent aspect of brain development, with substantial overproduction of neurons that are subsequently culled. Two waves of cell death occur over the course of nervous system maturation, the first during neurogenesis and the second during synaptogenesis, which together shape the final organization of the mature brain. Neuroproliferative cell death is likely a means of eliminating new cells with undesirable genotypes, resulting from a host of potential variables. Later in development, postmitotic cell death occurs as neurons are forging synaptic contacts. Competition, largely for limited neurotrophic factors, permits the survival of only a subset of neurons. The high rate of cell death in the developing CNS suggests that there is a selection process whereby neurons with more advantageous phenotypes (based on a combination of physiological environment and genotype) survive to populate the mature brain.

Genomic Changes That Alter Gene Expression

Multiple changes at the level of the genome have been shown to alter gene expression in neural cells. Genomic changes that occur during neurogenesis give rise to populations of cells with distinct genotypes. Altered genomes are often associated with brain malfunction; however, accumulating evidence points to genomic mosaicism as a feature of normal vertebrate brains.

The most extreme form of genomic change is aneuploidy – the loss and/or gain of whole chromosomes. Aneuploidy can occur as a result of missegregation during mitosis, through events such as lagging chromosomes, supernumary centrosomes, and nondisjunction (**Figure 2(a)**). With several hundred genes on each chromosome, loss or gain of a single chromosome leads to widespread changes in cellular gene expression. Changes in gene expression that result from aneuploidy include gene dosage effects and allelic variation. Increases or decreases in gene dosage for genes expressed on the aberrant chromosome (*cis* effects) and on other chromosomes (*trans* effects) have been reported in aneuploid neurons. Allelic variation results in the gain or loss of one parental allele, altering the balance of allele-specific gene expression. Allelic variation is due to genetic imprinting, expression of recessive mutated alleles (as seen in loss of tumor

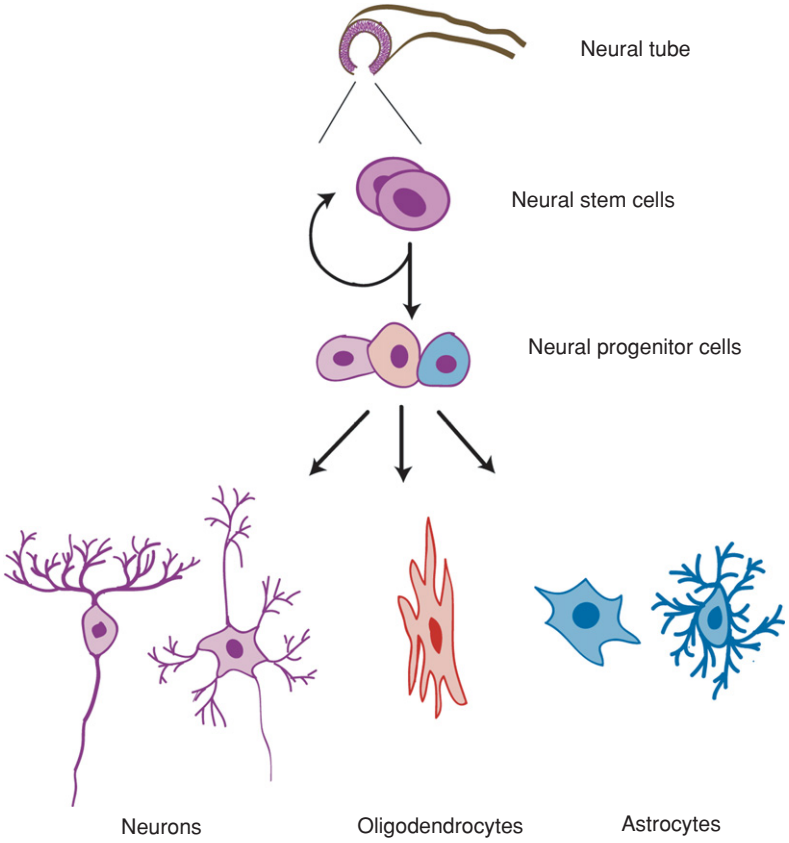

Figure 1 Process of neurogenesis. Neural stem cells dividing within the neuroepithelium of the neural tube give rise to lineage-committed neural progenitor cells. Neural progenitors differentiate into multiple diverse cell types that constitute the mature nervous system.

suppressor genes in aneuploid cancer cells), and polymorphisms. This degree of genetic diversity will affect cellular function through changes in any number of signaling pathways and, in turn, influence nervous system development. Given the range of possible functional changes produced by aneuploidy, the implications of this recent finding extend to normal neuronal diversity, behavioral diversity (as seen in monozygotic twins with exactly the same genome yet individual personalities), and neuropsychiatric diseases.

Retrotransposons, primarily those within the long interspersed nuclear element 1, or LINE1 (L1), can cause genomic mutations by disrupting gene coding regions or noncoding promoters/regulatory elements (Figure 3). L1 protein binds to untranslated L1 mRNA and escorts it back to the nucleus where the L1 endonuclease nicks a single strand of DNA and re-inserts itself through reverse transcription. Both germ line and somatic transposition events have been noted. L1 retrotransposons have been recently reported as a mechanism for genomic alteration in neurons.

Genetic imprinting is a mechanism that can regulate gene expression without altering the DNA sequence, yet the specific modifications are heritable. A classic example that affects CNS function is Prader-Willi syndrome, in which partial deletion of chromosome 15 results in mono-allelic expression of a subset of genes, causing mental retardation. Specific genomic imprinting modifications include methylation of CpG sites within a gene or promoter region and histone modifications which are encoded during DNA replication and thus passed on in proliferating cells. Genetic imprinting likely contributes to other brain functions, as there are estimates of 100–200 known imprinted genes in humans. This mechanism plays an important role in determining maternal or paternal monoallelic expression of many genes involved in brain development.

Genomic Disorders Affecting CNS Development

Down Syndrome

Down syndrome (DS), or trisomy 21, is perhaps the best-known genomic disorder affecting the CNS. It is characterized by stereotypical craniofacial abnormalities, moderate to severe mental retardation, and an

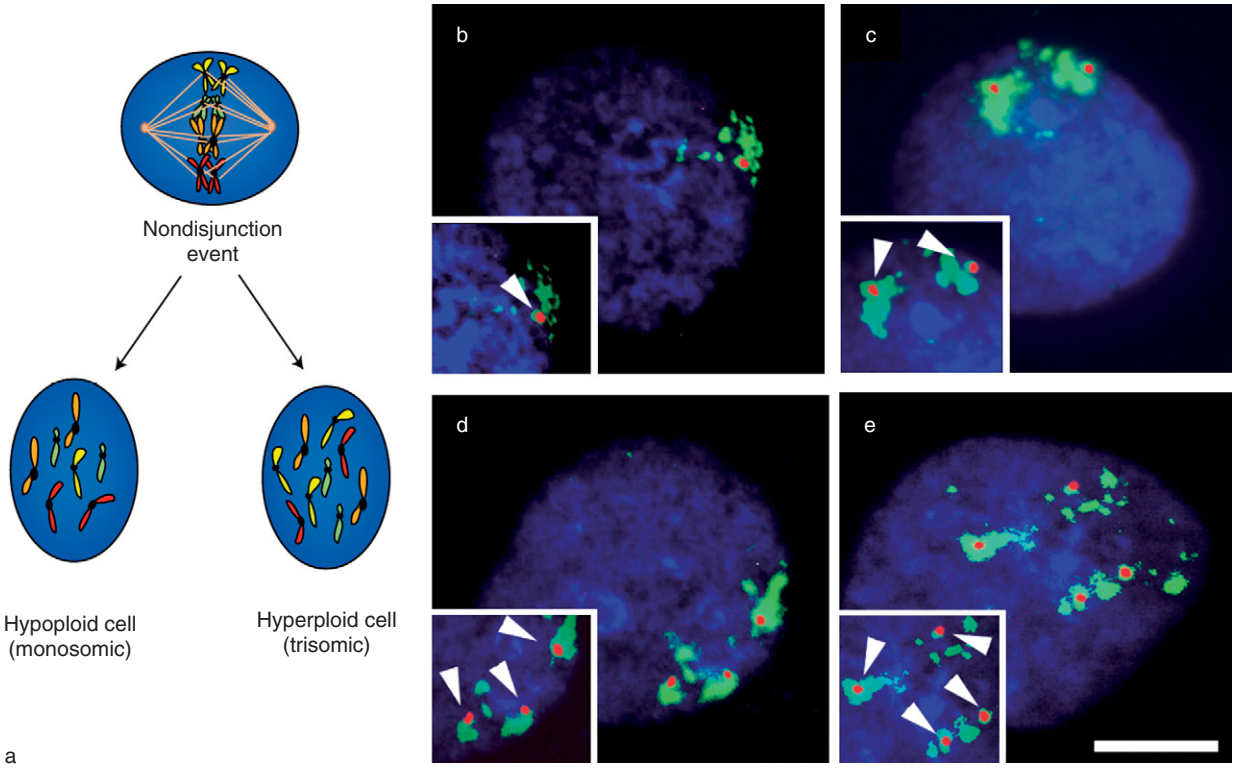

Figure 2 Aneuploidy is one mechanism for altering the genome of individual neurons. (a) Missegregation events, such as nondisjunction, can result in daughter cells that have lost (monosomy) or gained (trisomy) chromosomes (yellow chromosome). Here, genetically distinct mature brain cells from an adult human cortex are shown to have different copy numbers of chromosome 21 (b–e). Nuclei are visualized with DAPI and chromosome 21 is detected with a whole chromosome-specific paint (green) and a locus-specific point probe. Arrowheads denote distinct chromosomes. Error bar = 10 μm.

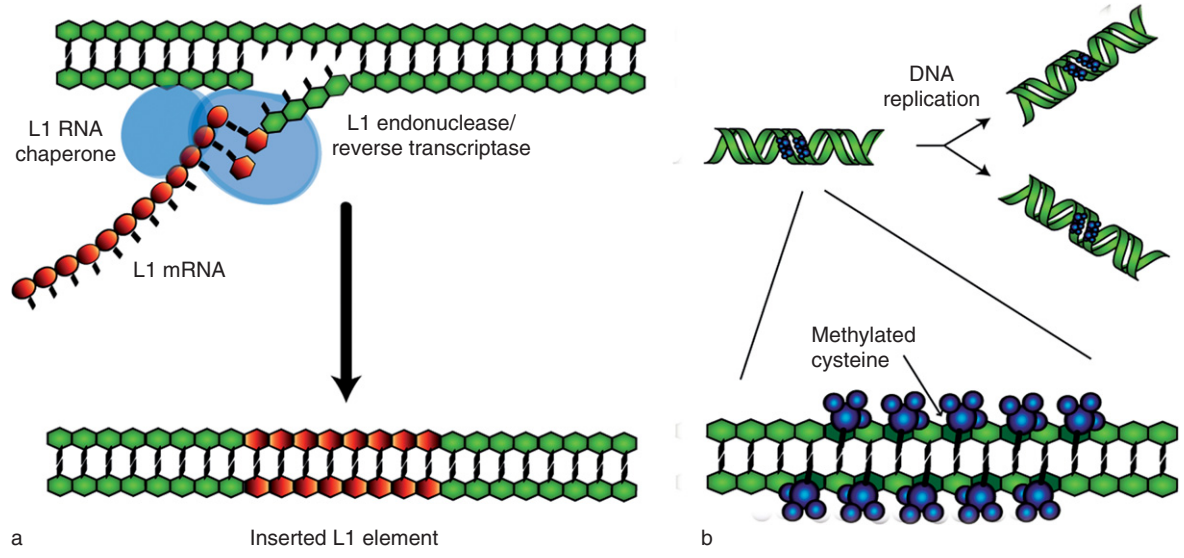

Figure 3 Intrachromosomal mechanisms can also alter the genome of individual neurons. (a) LINE1 (L1) retrotransposons randomly insert their sequence into the genome. L1 proteins escort L1 mRNA into the nucleus, nick a single strand of DNA, and reverse transcribe the L1 sequence. The cell's replication and repair machinery incorporate the new sequence into the genome. (b) Methylation of cysteine nucleotides, particularly in CG repeat sequences, silences gene transcription. This epigenetic event is reproduced during DNA replication; thus, it is permanently imprinted on the genome.

increased risk for leukemia and cardiovascular disease. Patients with DS often live into the forth or fifth decade of life, during which they prematurely develop Alzheimer's disease (AD)-like amyloid plaques and neurofibrillary tangles. Edwards (trisomy 18) and Patau (trisomy 13) syndromes are other major trisomic disorders compatible with postnatal life, and they similarly show pronounced mental retardation and physical dysmorphism.

Much research has focused on defining the genotype-phenotype relationship in DS. Chromosome 21 contains roughly 360 known genes, and the main effects of trisomy are thought to be related to the 1.5-fold increase in gene dosage for genes on that chromosome. Gene expression studies on trisomic cells from DS individuals find that most genes on chromosome 21 are overexpressed, although the pattern of altered gene expression depends on tissue type and developmental stage. It has been noted that none of the features of DS are unique to DS, suggesting a shared etiology in disorders with overlapping symptoms. An example is the possible relationship that has been reported between DS and AD in which elevated gene dosage for the *APP* (amyloid precursor protein) gene on chromosome 21 has been implicated in amyloid plaque formation, although data linking trisomy with AD is lacking. Most phenotypic features do not appear to follow this single-gene/single-phenotype pattern, suggesting that *trans*-regulation of genes on other chromosomes could result in the disruption of many common signaling pathways. Such loss of genetic homeostasis, produced by aneuploidy in brain cells, could produce general neuronal dysfunction and result in mental retardation, common to all genomic brain disorders.

Chromosomal Deletion Disorders

A long list of chromosomal disorders in which partial deletions of various chromosomes exist: Miller-Dieker syndrome (del 17p13), Angelman and Prader-Willi syndromes (del chr 15q11–12; see previous discussion), DiGeorge syndrome (del 22q11), Cri-du-chat syndrome (partial del 5p-), and Wolf-Hirshhorn syndrome (partial del 4p-), among others. Invariably, these chromosome deletion syndromes are associated with brain malformation and/or mental retardation, supporting the idea that systemic genomic abnormalities disrupt general nerve cell function and brain development.

Turner syndrome (X0) is an intriguing disorder that results in females with normal verbal intelligence but impaired numerical and spatial cognition. In addition, social skills and emotion recognition are poorly developed in these patients, possibly related to structural abnormalities of the amygdalae. Since normal XX cells are known to inactivate one X chromosome early in development, much research has focused on gene expression in Turner syndrome. The phenotype is attributed to haploinsufficiency of genes with biallelic expression on chromosome X, representing up to 20% of the genes on that chromosome. It is not known how these biallelic genes are regulated in normal females and males, and to what degree biallelic expression is required in various cell types.

Fragile X Syndrome

Fragile sites are unstable chromosomal regions that are prone to double-strand breaks and rearrangements. The most common genomic disorder associated with a fragile site is the inherited disorder fragile X syndrome, in which a CGG trinucleotide repeat causes methylation-mediated silencing of the *frm1* gene on chromosome X. The gene product, FMRP, is a mRNA-binding protein that appears to traffic specialized mRNAs to the dendrites of neurons necessary for synaptic plasticity. CGG repeat length expands during germ line transmission and the full phenotype of fragile X syndrome does not manifest until there are more than 200 repeats. Mental retardation (IQs ranging from 20 to 60) is the main clinical phenotype in fragile X syndrome, although other symptoms can include autistic behaviors, anxiety disorder, attention deficit-hyperactivity disorder, and seizures. In addition to the predominant germ line mutations, repeat length instability has been reported in somatic tissues, including the developing brain, indicating that the phenotype may partially depend on neural genotypes distinct from the inherited genotype.

Ataxia-Telangiectasia

Ataxia-telangiectasia (A-T) is the best-known disorder resulting from disruption in a DNA repair pathway. The disorder is characterized by cancer predisposition and radiation sensitivity as well as progressive neurodegeneration. A mutation in the *atm* gene (A-T mutated) has been linked to the disorder. ATM phosphorylates multiple substrates involved in cell cycle regulation and genomic stability, playing an important role in genome surveillance. Loss of ATM function has been proposed to result in an accumulation of neurons with genomic abnormalities, leading to the progressive loss of neurons. Interestingly, mice lacking *atm* have an increase in aneuploid brain cells, suggesting that aneuploidy is one form of genomic instability associated with loss of genome surveillance mechanisms. It is notable that both defects in DNA repair and aneuploidy are also associated with medulloblastoma formation. Other

DNA repair disorders with neurological phenotypes include Nijmegen-breakage syndrome, Cockayne's syndrome, and xeroderma pigmentosa.

Mosaic Variegated Aneuploidy

Mosaic variegated aneuploidy (MVA) is a rare disorder characterized by mosaic aneuploidy in multiple somatic tissues, with the requirement of at least three distinct aneuploidies for diagnosis. Up to 65% of cases of MVA show premature centromere division, leading to a high frequency of different trisomies. In other cases, chromosome missegregation and mitotic checkpoint failure are suggested as mechanisms of aneuploidy. The gene *BUB1B,* which encodes the spindle checkpoint protein BUBR1, has been implicated in some forms of this disease. Rates of aneuploidy in blood lymphocytes and fibroblasts have been reported ranging from less than 10% to more than 80%, but no information is known about the effect of this high rate of aneuploidy on gene expression or cell function. Neurologically, the disorder is associated with microcephaly, mental retardation, and myoclonic seizures, presumably related to high levels of aneuploid mosaicism in the brain, similar to other chromosomal disorders.

Neuropsychiatric Disorders

It has been hypothesized that sporadic neuropsychiatric disorders may be the result of as-yet-undetected genomic changes. Evidence for this includes Tourette's syndrome, which has been linked to mutations in microRNAs that regulate gene expression; epilepsy, which has an increased risk associated with most chromosomal disorders, including DS; idiopathic mental retardation, in which *de novo* microdeletions have been identified in several cases; and neurodegenerative diseases, in which single gene mutations account for only a small minority of cases. Since many neuropsychiatric disorders present with clinical symptoms that overlap with known genomic disorders, it is not unreasonable to postulate that undetected genomic abnormalities may be present in these patients.

Recently, fragile sites have been implicated in some neuropsychiatric disorders. FRA13A is a fragile site that maps to chromosome 13q12–14, a region flanked by tumor suppressor genes and containing the neurobeachin gene *NBEA*. In humans, haploinsufficiency for NBEA has been linked to sporadic autism, suggesting that instability of FRA13A may be a contributing factor in some cases of autism. Interestingly, the unstable nature of the FRA13A site may be related to a high number of LINE1 elements within the region. Another known fragile site, FRA6E, maps to chromosome 6q26, which includes the *PARK2* locus. Mutations in *PARK2* have been associated with autosomal recessive early parkinsonism, and it is compelling to speculate that genomic instability of FRA6E could be a factor in sporadic forms of Parkinson's disease.

Genotype-Phenotype Variability within Genomic Disorders

The clinical phenotype of most genomic disorders is highly variable, especially regarding cognitive ability; however, the basis for this clinical presentation is unclear. Genomic mosaicism in the CNS is a compelling mechanism for explaining at least some degree of the phenotypic variability seen in neuropsychiatric disorders. Genomic mosaicism produced by multiple mechanisms (e.g., aneuploidy, genetic imprinting, and retrotransposons) exists within all human brains. The precise function of this mosaicism in normal and diseased brain is an area of active research, and will likely have repercussions for our understanding of many of the genomic diseases discussed in this section. Future research in this area will provide further insight into the link between genomic mosaicism and neurological phenotype.

Acknowledgment

The authors would like to acknowledge NIH grant number MH076145.

See also: Alternative Splicing in the Nervous System; Gene Expression Regulation: Activity-Dependent; Gene Expression Regulation: Chromatin Modification in the CNS; Gene Expression Dysregulation in CNS Pathophysiology; Genetic Influence on CNS Gene Expression: Impact on Behavior; Plasticity, and Activity-Dependent Regulation of Gene Expression.

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