Abstract

Developments in molecular biology over the past three decades have led to an increasing awareness of the importance of epigenetic phenomena in a variety of genome functions. Epigenetic aspects of complex multifactorial diseases including schizophrenia, however, have not been investigated sufficiently. Various facets of epigenetics are reevaluated through their putative relevance to four theories of schizophrenia: neurodevelopmental, dopamine dysfunction, viral, and genetic anticipation with unstable DNA. The heuristic value of the epigenetic model of schizophrenia arises from the possibility of integration of a wide variety of empirical data into a new theoretical framework. It can be hypothesized that in addition to pathological effects of DNA structural mutations and environmental factors, inherited and acquired epigenetic defects, or epimutations, may be of etiological importance in schizophrenia. In addition, the epigenetic model may lead to experiments investigating the molecular substrates of genetic-environmental interactions.

Key words: Epigenetics, neurodevelopment, dopamine hypothesis, retroviruses, genetic anticipation, unstable DNA, sporadic and familial schizophrenia, gene-environment interaction, monozygotic twins, DNA methylation, chromatin conformation.

Introduction

The 1982 book titled Schizophrenia: The Epigenetic Puzzle by Gottesman et al. perfectly represents our understanding of the etiology of schizophrenia and the zeitgeist of psychiatric research over the past three decades. It has been generally accepted that schizophrenia is a biopsychosocial disease caused by the interaction of genetic and environmental factors. Unfortunately, despite the application of sophisticated strategies, identification of such genetic and environmental factors has turned out to be a difficult task with no major breakthroughs. Several chromosomal regions of susceptibility have been identified from the genetic linkage studies (Karayiorgou and Gogos 1997); however, most of these loci could be false positives because they do not reach genomewide significance criteria (Lander and Kruglyak 1995). Absence of consistent linkage makes the prognosis for cloning the actual disease genes rather pessimistic for the near future. The search for exogenous factors that may predispose to schizophrenia is no better off. Several environmental theories have been suggested, including intrauterine insults and birth traumas (McNeil 1995; Verdoux et al. 1997), maternal nutritional deficiency during gestation (Susser et al. 1996), and viral infections (Crow 1984; Kirch 1993; Yolken and Torrey 1995). Due to the complexity of the disease on one hand and methodological limitations of these theories on the other, little insight has been gained into understanding the causes of schizophrenia.

It is interesting to note that in most studies, genetic and environmental factors have been investigated separately despite the fact that the current paradigm states epigenetic interaction between mutant genes and hazardous environments. By definition, epigenetics pertains to a set of mechanisms that lead to the phenotypic expression of an individual’s genetic information (Jablonka and Lamb 1995) and deals with potentially reversible modification of DNA mediated by DNA methylation and chromatin conformation. Over recent decades, the field of epigenetics has progressed from an abstract theory of gene-environment interaction into a separate field of molecular biology with a wide arsenal of experimental techniques.

The hypothesis developed in this paper suggests that, in addition to direct hazardous effects of DNA structural mutations and environmental factors, inherited and
acquired epigenetic defects, or epimutations (Holliday 1987), may be of etiological importance in schizophrenia. The rationale for shifting the emphasis to epigenetics came from a rapidly growing body of experimental data indicating the biological importance of epigenetic factors. A wide scope of epigenetic phenomena across various organisms has been identified, including position effect variegation, telomere position effect, repeat induced gene silencing, tissue-specific and age-dependent DNA modification, paramutation, and transvection (Bestor et al. 1994). These phenomena are achieved via a series of epigenetic mechanisms such as de novo and maintenance DNA methylation, nuclear compartmentalization, and chromatin conformation (Riggs and Porter 1996). Maynard Smith (1990) has suggested the term dual inheritance, which emphasizes the presence of epigenetic information in addition to the four nucleotide-based DNA strand.

The fundamental difference between epigenetic systems and DNA sequence-based hereditary factors lies in the ability of the former to change rapidly in comparison with the latter. Epigenetic factors may exhibit only partial stability ("metastability") when transmitted from parent to daughter cells, while DNA sequence demonstrates nearly complete interclonal fidelity. DNA methylation patterns undergo major reorganization during gametogenesis, development, and aging (Uehara et al. 1989; Monk 1990; Holliday 1996; Shemer and Razin 1996). In addition, epigenetic changes may occur under the influence of cellular-environment factors, while such factors have little influence on DNA sequence.

In human epigenetics, DNA methylation-based phenomena have been subjected to the most detailed analyses, and DNA methylation will be the focus of this article. The emphasis on DNA methylation-based processes does not exclude the importance of other epigenetic mechanisms that may exist in humans, and the pathogenic mechanisms described below can be extrapolated to epigenetic factors that are not DNA methylation-based. The haploid mammalian genome contains approximately $5 \times 10^7$ CpG dinucleotides, a portion of which are methylated at the 5' position of the cytosine residue (Leonhardt and Bestor 1993). Mammalian DNA methylation is performed by DNA (cytosine-5)-methyltransferase, which exhibits both de novo and maintenance methylation activity with the latter exceeding the former by thirty- to fortyfold (Leonhardt and Bestor 1993). Recently, genes for several new mammalian DNA methyltransferases have been cloned (Okano et al. 1998). CpG dinucleotides are the preferential target of methylation, but DNA methylation at non-CpG sites has also been reported (Woodcock et al. 1988; Kay et al. 1994; Clark et al. 1995).

Numerous cellular functions are controlled and mediated by DNA methylation: the regulation of gene activity (Yeivin and Razin 1993), genomic imprinting (Reik et al. 1990; Walter et al. 1996), and genetic recombination (Petronis 1996). Methylated cytosines are known to exhibit the highest degree of mutability in comparison with other nucleotides (Yang et al. 1996), and such mutations are frequently detected in disease genes. Similar mechanisms to DNA methylation-based repair of DNA (Modrich 1991) and mutagenesis (Klimasauskas and Roberts 1995; Yang et al. 1995) in prokaryotes may exist in higher eukaryotes. Either an abnormal position of DNA methylation or the absence of a necessary methylation signal may increase the chance of DNA replication errors not being recognized. The most impressive and straightforward argument of the biological importance of epigenetic factors, however, arises from the presence of the very wide phenotypic variability of somatic cells within an organism. While the genotypes are the same in most of the cells of the same organism (with the exception of gametes and the cells of the immune system), the cellular phenotypes and functions differ radically. The phenotypic differences are sustained and may even be initiated by epigenetic modifications of the genotype.

Despite the fact that epigenetic mechanisms play a major role in a wide variety of cellular functions, they have not been investigated intensively. Thus far, the dominating paradigm in morbid human genetics has concentrated almost exclusively on the damaging effects of DNA mutations on gene expression and protein function. In this article, the idea that DNA modification defect, or epimutation, could be an etiological factor in schizophrenia is developed. Various aspects of the putative role of epigenetic phenomena are discussed through their relevance to four theories of schizophrenia etiology: neurodevelopmental, dopamine dysfunction, viral, and genetic anticipation with unstable DNA.

**Neurodevelopmental Hypothesis**

A number of investigators have suggested that there may be abnormal development of the brain in schizophrenia (Weinberger 1996; Vicente and Kennedy 1997). A wide range of findings have been attributed to neurodevelopmental defects, including minor physical abnormalities (Green et al. 1989; Murphy and Owen 1996), premorbid neuropsychological deficits (Aylward et al. 1984; Walker and Lewine 1990), and anatomical and cytoarchitectural abnormalities in brains of individuals affected with schizophrenia (Andreassen et al. 1986; Zigun and Weinberger 1992). Several groups have detected evidence for cortical...
maldevelopment in schizophrenia: anomalous sylvian fissures (Falkai et al. 1992), and defects in planum temporale (Rossi et al. 1992) and posterior temporal cortex (Crow et al. 1989); however, other studies were not able to replicate these findings (Bartlett et al. 1993; Kulyanych et al. 1995). Cytoarchitectural abnormalities were also detected in the entorhinal cortex (Jakob and Beckmann 1986; Arnold et al. 1991) and superior frontal gyrus region of dorsolateral prefrontal cortex (Akbarian et al. 1993). Morphometric abnormalities of enlarged cerebrospinal fluid spaces and reduced cortical volumes have been also identified in schizophrenia (Zigun and Weinberger 1992). These abnormalities correlate with childhood premorbid adjustment (Breslin and Weinberger 1991) and are unrelated to gliosis (Roberts et al. 1986; Bruton et al. 1990), which would be expected if they were caused by a degenerative process. Functional neuroimaging studies have demonstrated that numerous cortical regions are hypoactive in schizophrenia (Berman and Weinberger 1991), but this hypoactivity is different from that detected in neurodegenerative disorders such as Huntington’s disease (Weinberger et al. 1988; Goldberg et al. 1990).

In addition to genetic and environmental insults that have been frequently discussed as the agents disrupting neurodevelopment, epigenetic factors are relevant to ontogenesis. The importance of epigenetics arises through its direct involvement in the mechanisms that provide temporal and spatial control of gene activity during the development of complex organisms (Holliday 1990). It is of interest that the term epigenetics was introduced to describe the mechanisms of embryonal development of complex organisms (Waddington 1956) and derives from epigenesis, a theory that states that the complexity seen in developed organisms does not exist in a smaller scale in the germ cells (Holliday 1994). Two decades ago, Holliday and Pugh (1975) and Riggs (1975) suggested a DNA modification-based mechanism of changing gene activity and maintenance of the differentiated state of cells during development. Numerous aspects of DNA modification, including the presence of methylation enzymes, de novo DNA methylation and DNA demethylation, the role of DNA methylation in mutagenesis, X chromosome inactivation, and tissue differentiation during development have been predicted in these theoretical developments. Long before these models were proposed, Morgan (1934) suggested that ‘genes ... are changing in some way as development proceeds in response to that part of the protoplasm in which they lie, and that these changes have reciprocal influence on the protoplasm.” Brink (1960) further developed the idea of DNA modification, which he named “parachromatin.” According to Brink, parachromatin represents a programmable memory of ontogeny that can be reset during each gametogenesis and that provides the developmental scenario of gene expression (Jorgensen 1994).

Numerous experiments have demonstrated that DNA methylation is involved in embryonic, fetal, and postnatal development. In mammals, DNA methylation patterns are subjected to major changes, with de novo methylation occurring during meiosis and gametogenesis, global demethylation shortly after fertilization, and then massive remethylation taking place at the time of implantation (Razin and Cedar 1993; Razin and Shemer 1995). Complex, yet incompletely understood, changes in DNA methylation in somatic cells of developing organisms lead to tissue-specific DNA methylation (Holliday 1996). The role of DNA methylation is also demonstrated by the presence of developmental defects resulting from the disruption of normal genomic imprinting (Franklin et al. 1996) and substantial changes in the pattern of gene expression and development after the application of a demethylating agent, 5-azacytidine (Jones 1985; Zagris and Podimatas 1994; Doerksen and Traslac 1996). The importance of DNA methylation is also supported by various experiments that include DNA methyltransferase gene knockout mice (Li et al. 1992). The role of DNA methylation in development has been clearly shown in plants in which DNA hypomethylation was induced either by introduction of antisense constructs to the gene of native DNA methyltransferase (Finnegan et al. 1996; Ronemus et al. 1996) or by generating mutant hypomethylated plants (Kakutani et al. 1996; Richards 1997).

All these studies have clearly shown that DNA methylation modifies DNA to specific epigenetic conditions, and this mechanism mediates developmental processes. At present, however, the causal relationship between DNA methylation and developmental differentiation has been questioned by some authors, and the definitive role of DNA methylation in the temporal, spatial, and functional organization of cells has yet to be elucidated (Bestor 1996; Holliday 1996). Although more work is required, it is clear that changes in epigenetic mechanisms such as methylation have the potential to cause subtle changes in brain development such as those observed in schizophrenia. Epigenetic defects should be considered as a group of putative etiological factors leading to neurodevelopmental abnormalities seen in schizophrenia.

**Dopamine Dysregulation Hypothesis**

During the last 30 years, a large body of pharmacological data on the dysregulation of the dopaminergic system in the brains of those affected with schizophrenia has accumulated and has led to the formulation of the dopamine...
hypothesis of schizophrenia. The hypothesis is largely based on two types of observations. First, neuroleptic drugs exhibit high affinity for dopamine receptors (Seeman et al. 1976; Van Tol et al. 1991). Antipsychotic potency of most neuroleptic medications has been shown to correlate with their affinity for the dopamine D2-like receptors (D2, D3, D4; Creese et al. 1976; Seeman 1992). The second group of findings supporting the dopamine theory of schizophrenia is related to the elevated density of D2-like receptors in postmortem schizophrenia brain (Seeman et al. 1987, 1997; reviewed in Seeman and Niznik 1990). Although it is known that chronic administration of neuroleptics results in increased dopamine D2-like receptor sites in the brain (Muller and Seeman 1977; Owen et al. 1980), neuroleptics do not seem to be the main cause of the increased density of D2-like receptors. The density of D2-like receptors was also found to be higher in a number of studies of the brains from never-medicated patients (Cross et al. 1981; Seeman et al. 1984, 1997; Wong et al. 1986). Several experimental designs have been suggested to identify the specific dopamine receptor that accounts for the increased density of D2-like receptors in schizophrenia. The most recent studies seem to favor the idea that this increase is due to dopamine D2 receptors rather than D3 or D4 (Seeman et al. 1997).

These pharmacology findings stimulated molecular genetic studies of the genes for dopamine receptors, particularly the dopamine D2 receptor gene, DRD2. Genetic linkage studies of DRD2 in schizophrenia multiplex families do not support the hypothesis that DRD2 is a major genetic factor in most familial schizophrenia (e.g., Moises et al. 1991; Coon et al. 1993; Wang et al. 1993; Kalsi et al. 1995; reviewed in Kennedy 1994). Low positive lod scores at DRD2 in a subgroup of families (Nanko et al. 1992; Mazia et al. 1995) may suggest that DRD2 can be a predisposing risk factor in some cases of familial schizophrenia. Evidence for genetic association of a variant of DRD2 (Ser311Cys) with schizophrenia in a Japanese sample has been reported (Arinami et al. 1994, 1996), but this finding has not been replicated by other groups (Asherson et al. 1994; Shaikh et al. 1994; Crawford et al. 1996; Sasaki et al. 1996; Verga et al. 1997; Spurlock et al. 1998). Direct DNA sequencing-based searches for mutations in the coding region of DRD2 were performed (Sarkar et al. 1991; Seeman et al. 1993; Gejman et al. 1994), but none of the detected rare sequence variants were associated with schizophrenia. A genetic anomaly could be located in the noncoding regions of DRD2, i.e., in the 5′-promoter region, introns, or 3′-untranslated region (Blum and Noble 1994; Gejman et al. 1994). A recent analysis of the DRD2 putative promoter region has detected a polymorphism (−141C Ins/Del) that exhibits functional differences in the luciferase test and is associated with schizophrenia in Japanese subjects (Arinami et al. 1997). The latter finding was not replicated in Europeans (Stober et al. 1998).

Epigenetic factors may produce effects on the expression of DRD2 similar to the effects of DNA structural variants in the putative DRD2 promoter region. Although the cause-effect relationship between DNA methylation and gene activity is not clear yet (see Bestor 1996; Holliday 1996), numerous studies have shown that about two-thirds of genes for which methylation pattern has been investigated exhibit a correlation between methylation status of the promoter region and transcription (Yeivin and Razin 1993). In some genes, the degree of gene expression inversely correlates with the number of methylated CpG dinucleotides in the promoter region, independent of their actual position (e.g., Murray and Grosveld 1987), while in other cases individual methylated sites exhibit differential potency to inhibit transcription (e.g., Tasseron-de Jong et al. 1989; Graessman et al. 1994). The importance of DNA methylation in promoter regions is also supported by the existence of transcription factors that have differential affinity for methylated and unmethylated CpGs within the specific binding sequence (Ehrlich and Ehrlich 1993).

It would be naive to expect that the increased D2 binding site density observed in schizophrenia is caused by a simple increase in DRD2 expression. Experimental data have shown that although the DRD2 expression may partially determine the number of binding sites, D2 binding capacity does not strongly correlate with DRD2 mRNA level at all periods of development (Srivastava et al. 1992). In addition to controlling the level of DRD2 expression, other epigenetic mechanisms may play a pathogenetic role in schizophrenia. Such epigenetic mechanisms may affect the nuclear compartmentalization of the segment of a chromosome that contains the critical gene(s) (reviewed in Riggs and Porter 1996), thereby changing local functioning of the genes. Epigenetic factors that change the location of mRNA transcripts in the nucleus may influence the efficiency of mRNA transportation into the cytoplasm and subsequent translation, as has been suggested for plants (Flavell 1994). Several cases of such "displacement" pathology have been identified in human genetic diseases. In myotonic dystrophy, transcripts of the disease gene (myotonic dystrophy protein kinase [DMPK]) containing (CTG)n expansions were detected in the nucleus of fibroblasts and muscle cells of affected individuals, while no DMPK mRNA was detected in the nuclei of cells from control individuals (Tanen et al. 1993). Aberrant subcellular localization of BRCA1 transcripts (Wilson et al. 1997) and proteins (Chen et al. 1995, but Sully et al. 1996) were also detected in breast cancer. Misplacement of DRD2 transcripts...
may lead to abnormal further processing, transportation, and function of D2 receptors.

It is interesting to note that in addition to the regulation of the level, time, and location of gene expression, recent findings have shown that an epimutation may be the cause of truncated proteins, a defect that traditionally has been attributed exclusively to nonsense mutations in the coding region of a gene. In the filamentous fungus, *Ascochloropsis immersus*, transcription of *met2* was blocked by DNA methylation, which led to the production of truncated transcripts (Barry et al. 1993). A similar finding of the blockage of transcription has been identified in *Neurospora crassa* (Rountree and Selker 1997). Cautious extrapolation of these findings to other organisms should be made because there is a high degree of DNA methylation in fungi. However, similar mechanisms, especially in combination with other epigenetic factors (e.g., DNA-protein interactions and chromatin conformation) may exist for mammals. A number of incomplete protein products may be produced by this mechanism, which may exhibit different functional characteristics compared with the normal proteins and may have a detrimental impact on cell function. The description of truncated transcripts in fungi makes the distinction between DNA structural mutation in the protein coding region and epimutations even less clear.

**Viral Hypothesis**

The viral hypothesis of schizophrenia is based on a set of epidemiological findings, including an excess of winter-spring births in affected individuals, north-south regional differences in the rate of schizophrenia, and evidence that fetal exposure to maternal influenza increases the risk for schizophrenia (reviewed in Kirch 1993; Volk and Torrey 1995; O'Reilly and Singh 1996; but Crow 1994). Several pathogenic mechanisms of viral infection have been suggested. These include the disruption of neurodevelopmental processes by viral toxic substances, virus-induced autoimmunity, and pathogenic effects arising from the integration of viral DNA into the host genome (Kirch 1993; Volk and Torrey 1995; O'Reilly and Singh 1996). The latter mechanism mostly pertains to retroviral sequences and, depending on the site of integration in the host's genome, may be pathogenic in two ways. First, the insertion of viral DNA in a functioning gene may lead to disruption of the normal protein sequence, that is, act as a traditional DNA mutation. The second mechanism is that viral DNA integrates in noncoding regions of the host's genome, and, although it doesn't disrupt a gene, it produces new viruses and toxic substances. Originally formulated for retroviruses (Crow 1984), the viral hypothesis can be extended to other types of viral and nonviral DNA sequences as well. Numerous cases of de novo transposition have been documented in human genetic diseases (Kazazian et al. 1988; Morse et al. 1988; Wallace et al. 1991). There has been some interest in the viral theory of schizophrenia, but thus far all attempts to identify the "schizovirus" have been unsuccessful (Alexander et al. 1992; Sierra-Honigmann et al. 1995; Taller et al. 1996).

Viral–host genome interactions depend to some extent on epigenetic factors. First, the integration of any exogenous genome is not random but depends on the chromatin conformation and other epigenetic conditions at the specific locus of the host genome. Only some loci are "receptive" and can incorporate a strand of foreign DNA (Sandmeyer et al. 1990; Zimonjic et al. 1994; Zou et al. 1996). Second, the activity of integrated viruses is under the control of epigenetic modification of viral DNA. Numerous experimental data have shown that DNA methylation plays a role in silencing or activating various types of viruses, including herpesvirus (Smith and Griffin 1991), lentivirus (Shaer and Mullins 1990), cytomegalovirus (Boom et al. 1987), hepatitis B virus (Bowyer et al. 1987), and human immunodeficiency virus (Bednarik et al. 1987; Spangler and Essani 1994). The findings that the majority of proviral DNA and transposable elements were methylated and transcriptionally inactive in the genomes of fungi, plants, and mammals have provided the basis for concluding that DNA methylation is part of the host defense system (Bestor 1996). In comparison with other epigenetic mechanisms, DNA methylation seems to be of key importance in genomic defense because *Drosophila*, which is known to have no DNA methylation, exhibits a significantly higher degree of insertional mutagenesis caused by exogenous sequences than other organisms that have DNA methylation (Ashburner 1992). As expected, the demethylating agent 5-azacytidine has the ability to activate dormant retroviral genes in mammalian models (Jaenisch et al. 1985).

**Genetic Anticipation and the Unstable DNA Hypothesis**

One of the most recent heuristic approaches in psychiatric genetics has arisen from the discovery of a new type of mutation, called unstable DNA. Unstable DNA mutation consists of an increase in the number of trinucleotide or other repetitive DNA sequences beyond the normal range. It has been shown that such “dynamic” mutations are the molecular cause of several neuropsychiatric disorders, including myotonic dystrophy, fragile-X mental retardation, and Huntington's disease, among others (Mandel 1997). The ability of dynamic mutations to increase in size across generations, and therefore to become more
pathogenic, was novel in human genetics. In addition, the discovery of unstable DNA was very important for the understanding of non-Mendelian aspects of clinical genetics, such as genetic anticipation. Genetic anticipation is a clinical phenomenon that exhibits increased disease severity and earlier age at onset in younger generations. It was first suggested in psychiatric disorders during the last century and it was called "degeneration" (McInnis 1996). After the detection of unstable DNA, which has served as a molecular correlate of genetic anticipation, a number of groups have performed detailed analysis of genetic anticipation in schizophrenia and other psychiatric diseases (reviewed in Petronis et al. 1995). Evidence for genetic anticipation in schizophrenia has suggested that unstable DNA may be of etiological importance in these diseases. Reevaluation of schizophrenia family and twin data from the unstable DNA perspective showed that the unstable DNA concept competes well with the traditional multifactorial polygenic theory, with many deviations from the Mendelian modes of inheritance of schizophrenia being explained by the non-Mendelian behavior of unstable DNA (Petronis and Kennedy 1995). Numerous studies have searched for both trinucleotide repeat expansions and large trinucleotide tracts in major psychoses using various experimental and methodological designs with rather controversial findings (Lindblad et al. 1995; Morris et al. 1995; O'Donovan et al. 1995, 1996; Haaf et al. 1996; Petronis et al. 1996; Vincent et al. 1996; Jones et al. 1997; Sirugiu et al. 1997; Sidransky et al. 1998). We will not review the results of these studies here, but instead will develop a hypothesis that epigenetic mechanisms may be either a molecular equivalent of or even a cause of genetic anticipation.

The putative role of epigenetic factors in genetic anticipation comes from several experimental findings of the intergenerational changes in the degree of DNA methylation that are consistent with genetic anticipation. In transgenic mice studies unrelated to unstable DNA, the transgene locus TKZ751 exhibited gradual intergenerational changes in DNA methylation in the offspring of such mice (Allen et al. 1990). The degree of DNA methylation increased or decreased in the F1, F2, and F3 offspring, depending on the genetic background of the non-transgenic parent. The transgene locus completely lost methylation in several generations when the transgenic mouse was mated to DBA/2 mice, but it became fully methylated in the BALB/c background. DNA methylation correlated with decreasing expression of the transgene across generations and was spreading by 6–10kb with each subsequent generation (Allen et al. 1990). Such intergenerational dynamics makes DNA methylation an excellent candidate for a molecular substrate of genetic anticipation. In addition, this experiment suggests that differences in the genome of the unaffected parent may determine whether an epigenetic condition progresses or regresses in the next generation, therefore contributing to phenotypic variance. Similar effect of the genetic background of the non-transgenic parent on transgene methylation was also detected by Sapienza et al. (1989). In addition, both Sapienza et al. (1989) and Allen et al. (1990) detected the effect of genomic imprinting that is observed in unstable DNA diseases exhibiting genetic anticipation (Ridley et al. 1988; Petronis 1996).

Another set of findings supporting the idea that epigenetic phenomena may be related to genetic anticipation derives from plants. A DNA hypomethylation mutant strain of Arabidopsis thaliana, addm1, exhibits a progressively more severe phenotype in subsequent generations (Kakutani et al. 1996) that could be similar to genetic anticipation in human diseases. The degree of DNA methylation was examined in several genomic loci (m105 and m118), and gradual loss of DNA methylation across generations was detected, suggesting that epimutations may be responsible for the delayed onset and progressive severity of the morphological defects (Kakutani et al. 1996).

There is a growing body of evidence suggesting the presence of epigenetic defects in unstable DNA diseases. Classic cases of fragile-X A syndrome (FRAXA) exhibit hypermethylation in the region of (CGG)\(n\)/(CGG)\(n\) expansion at the gene for FMR1, FMR1 (Hornstra et al. 1993), while full FMR1 mutations with reduced methylation do not lead to mental retardation (Rousseau et al. 1994). Similar to FAXA, incomplete penetrance among the carriers of the Huntington’s disease gene expansion Rubinsztein et al. (1996) may result from the lack of an epigenetic defect. The relatively low concordance of schizophrenia in monozygotic twins (Gottesman 1994) could be explained by similar mechanisms. Another line of evidence supporting the role of epigenetic factors in unstable DNA diseases has arisen from the detailed analysis of homologous recombination in the disease meioses. In myotonic dystrophy and Huntington’s disease, evidence for aberration of meiotic recombination has been identified from linkage studies. Such deviation from the reference genetic map may be caused by an epigenetic defect at the disease locus (Petronis 1996). Although the intergenerational dynamics of the epigenetic findings discussed above have not been investigated yet, epimutations may play a role in determining the age at disease onset. This hypothesis challenges the traditional trinucleotide repeat expansion-based mechanism of genetic anticipation. The degree of trinucleotide repeat expansion inversely correlates with the age at onset in unstable DNA diseases, but this does not necessarily mean that dynamic trinucleotide mutations are the sole determinants triggering disease at a specific occasion.
age. Dynamic epimutations can be alternative or additional explanations of genetic anticipation.

There are several more reasons to consider DNA methylation as one of the molecular mechanisms for determination of disease age at onset. DNA methylation status is an age-dependent characteristic. A strong body of experimental data has consistently shown the age-dependent changes in the level of DNA methylation in various tissues (Singhal et al. 1987; Drinkwater et al. 1989; Mays-Hoopes 1989; Golbus et al. 1990; Tawa et al. 1990). At birth, an epigenetic defect around or within a gene may be below the critical threshold level, and several decades may be required for such a defect of DNA methylation to cause disease. Regarding the mechanisms of changes in DNA methylation across generations, methylation spreading, a propensity of DNA-methyltransferase to de novo methylate CpG dinucleotides near pre-existing methylated CpG, may be involved (Tollefsbol and Hutchinson 1997).

Over the last several years, a large number of complex genetic diseases, including cancers of various kinds, and developmental, degenerative, and autoimmune disorders, have been subjected to the analysis of intergeneration dynamics of the age at onset. In the overwhelming majority of diseases, evidence for genetic anticipation was detected (reviewed in Paterson et al. 1998). Most of these studies examining anticipation can be criticized for various methodological reasons (Vieland and Huang 1998), and more thorough analysis is required. If genetic anticipation is proven in the complex genetic diseases with already known nontrinucleotide repeat mutations such as breast cancer or Alzheimer’s disease, this would favor the epigenetic origin of genetic anticipation instead of one based on trinucleotide repeat instability (Petronis et al. 1997).

Epigenetic Model: An Integrative Theory of Schizophrenia

All the theories discussed above have attempted to explain the origins of schizophrenia in different ways, and they use different scientific terminology and alternative experimental techniques. The diversity of approaches in schizophrenia research is not surprising considering the rich phenomenology of schizophrenia and the fact that understanding normal brain function is still a major challenge. Such a variety of theories is useful for the successful development of the field, because only competing theories can stimulate the growth of scientific knowledge. On the other hand, the danger is that “compartmentalization” of the empirical data and theoretical schemes, as well as orientation of various traditions toward isolated and sometimes nonessential aspects of the disease, may lead to seeing the trees but not the forest. In this situation, breaking down the “walls” between the theories may provide the basis for new heuristic developments. Some signs of theoretical and experimental integration of research programs can already be seen in several recent schizophrenia studies: molecular genetics techniques are now used for detection of the exogenous putative etiological factor, viral DNA. Another example is the neurodevelopmental theory, which, in addition to looking for pathogenic insults in the intrauterine environment, searches for gene variants causing or predisposing to abnormalities of brain development.

The epigenetic model could further facilitate the convergence of the etiological theories of schizophrenia. The possibility of integration of these theories comes from the multifaceted roles of epigenetic phenomena and allows for rearrangement of seemingly unrelated empirical findings into a new theoretical framework. Numerous scenarios for epigenetic combination of different theories and putative etiological factors in schizophrenia can be suggested. For example, viral DNA becomes incorporated into the genomic DNA, and the site of integration is close to the gene for a neurotrophic factor. The host’s DNA methyltransferase recognizes the foreign DNA and inactivates it by hypermethylation of the viral sequence. DNA hypermethylation locally changes chromatin conformation and slightly reduces the expression of the neurotrophic factor gene during embryogenesis. Shortage of the neurotrophic factor leads to a minor defect in the formation of synaptic connections between the neurons, which then predisposes to schizophrenia. Another putative scenario is that an epigenetic defect around a dopamine receptor gene causes the disruption of the normal functioning of the dopaminergic system. The epimutation may become more severe across generations, leading to more severe malfunctioning of the dopamine system and producing psychotic symptoms at an earlier age in younger generations.

In addition to integration of the existing models, the epigenetic theory brings two unclear issues of schizophrenia into a new light. One of them is that most schizophrenia cases are sporadic. Sporadic cases, as opposed to familial ones, represent single affected individuals within families with no other affected first- or second-degree relatives. Traditionally, sporadic and familial cases have been treated as two etiologically distinct groups, although no clinical differences have been observed between these groups. Sporadic cases have been explained as resulting from a complex interaction of multiple additive genes and hazardous environmental factors—a model of rather limited power to identify these genes and environmental factors. The epigenetic explanation of sporadic cases arises from metastability of the epigenetic code, which means
that epigenotypes can be reprogrammed during the gametogenesis. An epimutation may be corrected during the "recharging" of the germ line cells during meiosis, and the disease haplotype will not cause schizophrenia in the offspring of an affected proband.

The second issue for schizophrenia arises from a new experimental opportunity to investigate the gene-environment interaction and possibly find the molecular substrate of such interaction. As stated earlier, although the current paradigm states that both genetic and environmental factors are important in the genesis of schizophrenia, genetic and environmental factors have rarely been investigated together, and in fact there has been some degree of antagonism between the two sides (e.g., Torrey 1992 vs. McGuffin et al. 1994). New potential arises from the fact that the epigenetic systems have the ability to react to intracellular and extracellular factors, which leads to changes of the epigenotype (Jablonka and Lamb 1995). Psychological stress may cause similar changes in the epigenotype of critical genes compared with those changes evoked, for example, by a local stochastic error (Woolf 1997) in DNA methylation during brain development or by a stable epimutation transmitted through the germ line. These three types of epimutations of different origin may have the same molecular substrate and the same target locus in the genome, and therefore can be investigated using a single set of experimental approaches.

Epigenetic factors can be investigated with a range of experimental techniques; here we will briefly describe the methods for DNA methylation studies. Although a wide spectrum of experimental ideas have been suggested for DNA methylation analysis (Saluz and Jost 1993), two laboratory techniques are now used most frequently and should be performed in the affected tissue expressing the gene of interest. The first of them is based on Southern blot-hybridization using methylation-sensitive restriction enzymes such as HpaII, Hhal, and others. For each methylation-sensitive enzyme, an insensitive isoschizomer is selected (e.g.,MspI and HpalII for identification of C\textsuperscript{N6}CGG). The patterns of DNA hybridization for the digests of each pair of enzymes are compared, and the difference in the size of DNA restriction fragments as well as differences in the intensity of hybridization signals are the signs of DNA methylation in the restriction site(s). Although this approach is relatively simple to perform, it provides limited information as only the specific restriction sites can be tested.

The disadvantage of the Southern blot-based approach in DNA methylation analysis is overcome by the method of direct sequencing of sodium bisulfite-treated DNA (Frommer et al. 1992). This method allows for mapping of all 5-methylcytosines in the DNA strand. The technique is based on the reaction of genomic DNA with sodium bisulfite under conditions such that unmethylated cytosine is deaminated to uracil, but 5-methylcytosine remains unchanged. In the DNA sequencing reaction, all uracil and thymine residues are detected as thymine, and only 5-methylcytosine residues remain as cytosine. Methylation has to be analyzed specifically on both DNA strands by using polymerase chain reaction (PCR) primers specific for sites where cytosine deamination occurs, because hemimethylation of cytosines may occur. By cloning and sequencing multiple PCR products after treatment with bisulphite from each individual DNA sample, the degree of intraindividual mosaicism of methylation can be evaluated.

The preferable targets of DNA methylation analyses have been the genes' regulatory regions that play a key role in the control of gene activity. The recent analyses of the promoter regions of the genes for retinoblastoma and breast cancer have shown that DNA methylation defects are quite common in these diseases (Dobrovic and Simpfendorfer 1997; Lohmann et al. 1997). Similarly, epigenetic analysis of regulatory regions of candidate genes in schizophrenia may lead to interesting discoveries. Although large-scale DNA methylation studies are not feasible at present because DNA sequencing techniques are still relatively inefficient, new developments such as DNA chip-based DNA sequencing (Chee et al. 1996) may significantly expedite screening for epigenetic defects.

When the target genomic locus is unknown a priori, a set of indirect methods for DNA methylation analysis can be applied. The restriction landmark genomic scanning (RLGS) method uses two-dimensional gel-electrophoresis and enables simultaneous visualization of a large number of genomic loci (Hayashizaki et al. 1993). If methylation-sensitive restriction enzymes are applied, the loci that are differentially methylated across the organisms or various tissue of the same organism can be identified (Kawai et al. 1993; Watanabe et al. 1995). This approach has recently been used to demonstrate differences in DNA methylation patterns in peripheral lymphocytes between monozygotic twins discordant for schizophrenia (Tsujita et al. 1998). Another experimental strategy, called representational difference analysis, which uses subtractive hybridization and was originally designed for identification of DNA sequence differences, now has been modified for DNA methylation analysis (Ushijima et al. 1997). Following the preliminary finding of aberrant meiotic recombination in Huntington's disease and myotonic dystrophy (Petronis 1996), a comparative analysis of meiotic recombination may lay the basis of a new strategy for detection of epigenetic defects. Genetic loci exhibiting a different pattern of meiotic recombination in individuals affected with schizophrenia in comparison with controls would suggest the presence of an epigenetic defect.
In summary, the epigenetic model of schizophrenia may become an important development in the understanding of the etiology and pathogenesis of this devastating disease. An epigenetic research program can substantially enrich schizophrenia studies with a number of theoretical and experimental developments. On the other hand, since schizophrenia demonstrates a wide variety of molecular, cellular, and clinical abnormalities, it is an excellent target for the application and further development of epigenetic strategies. The so-called multifactorial origin of schizophrenia may become unified under the epigenetic umbrella. In all likelihood, both molecular psychiatry and epigenetics will significantly benefit from the alliance.

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Huntington's diseases. Neuropsychopharmacology, receptors in schizophrenia, Alzheimer's, Parkinson's, and

Dopamine D2-like sites in schizophrenia, but not in


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The Authors

Arturas Petronis, M.D., Ph.D., is an Assistant Professor; Andrew D. Paterson, M.B., Ch.B., B.Sc., is a Post-doctoral Fellow; and James L. Kennedy, M.D., F.R.C.P.(C), is L’Anson Associate Professor, Department of Psychiatry, University of Toronto, Clarke Division, Centre of Addiction and Mental Health, Toronto, Ontario, Canada.
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