

MOL 33266

**DNA (cytosine-5) Methyltransferase Inhibitors:  
A Potential Therapeutic for Schizophrenia**

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MOL 33266

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References: 10

Nonstandard abbreviations: CpG, cytosine-guanine dinucleotide; DNMT, DNA (cytosine-5) methyltransferase; GAD67, glutamic acid decarboxylase 67;

MOL 33266

## **Abstract**

In this issue of Molecular Pharmacology, Kundakovic et al. present compelling evidence suggesting that the promoters for reelin and GAD67 are coordinately regulated. The regulation occurs at the level of DNA (cytosine-5) methylation. Moreover, the authors present evidence that suggests pharmacologic inhibition of DNA methyltransferase results in reversal of methylation, loss of methyl-DNA binding proteins and relief of repression. Repression of both reelin and GAD67 have been implicated in the pathogenesis of Schizophrenia. Therefore, these results suggest that the reelin and GAD67 promoters are subject to continuous repression by DNA methyltransferase, and that inhibitors of DNA methyltransferase represent a potential treatment for Schizophrenia.

MOL 33266

Schizophrenia is a debilitating neurological disease that afflicts about 1 percent of Americans. The symptoms of Schizophrenia are severe and include hearing voices, generalized paranoia and severe cognitive dysfunction. As a result of these phenomena, Schizophrenics are unable to function in normal social situations and tend to be fearful, withdrawn and exhibit disorganized thought and speech. Onset of Schizophrenia occurs late in adolescence or early adulthood and is oftentimes associated with a precipitating, stressful life event. Contemporary treatments relieve some symptoms, however most individuals with Schizophrenia continue to experience symptoms throughout their life.

The nature of Schizophrenia suggests that the initial, triggering event results in a robust set of neuroadaptations that significantly derange normal brain function. How then, could one event result in widespread, maladaptive changes in neural function that last a lifetime? Given that the entire composition of the human brain turns-over every 2 months, the triggering event must impinge upon a process that is stable or is perpetuated throughout the lifetime of an individual. DNA is perhaps the only molecular component of neurons that is not continuously degraded and resynthesized, and represents a unique and powerful substrate for storage of cellular information.

DNA exists in the nucleus as a highly compressed protein-DNA complex known as chromatin. In addition to compressing DNA into the nucleus, chromatin acts as a molecular platform for signal integration and long-term information storage (Levenson and Sweatt, 2005). For example, every cell in a metazoan must “remember” its phenotype. This information is stored in the cell in the form of stable marks applied

MOL 33266

directly to chromatin which result in permanent changes to chromatin structure, gene expression and cellular physiology.

The process of marking DNA and its associated proteins is commonly referred to as epigenetics. Epigenetics has various meanings depending on the context that it is used. The broadest definition of epigenetics refers to processes of transmission and perpetuation of information through mitosis or meiosis that do not rely on DNA sequence. In this context, epigenetics encompasses DNA, RNA and protein-based mechanisms. As applied to the adult nervous system, epigenetics refers to a mechanism for stable maintenance of gene expression whereby the DNA or its associated proteins are physically marked. These marks come in a variety of forms (for review, see Levenson and Sweatt, 2005). Methylation of cytosine residues is an epigenetic mark applied directly to DNA. Methylation of a cytosine can occur only when it exists as a cytosine-guanine dinucleotide (CpG). Methylated CpG dinucleotides are bound by methyl-DNA binding proteins, which recruit chromatin remodeling enzymes such as histone deacetylases and function to actively repress gene expression. DNA methylation is an enzymatic process governed by DNA methyltransferase and an as-yet unidentified DNA methylase.

There is an increasing body of literature that indicates the adult nervous system utilizes epigenetic marks to chromatin for integration and storage of information. Moreover, there are several examples whereby a single, precipitating event in the lifetime of an organism results in an epigenetic mark to chromatin, lasting changes in expression of one or more genes and lifetime changes in behavior (Champagne et al., 2006; Kumar et al., 2005; Weaver et al., 2004). Given the sudden onset and lifetime

MOL 33266

persistence of symptoms, it is reasonable to hypothesize that the pathogenesis of Schizophrenia is due, at least in part, to aberrant epigenetic marking of one or more genes, resulting in lifetime changes in their expression.

Post-mortem studies of gene expression in the brains from individuals diagnosed with Schizophrenia have revealed that expression of glutamic acid decarboxylase 67 (GAD67), an enzyme critical for synthesis of the inhibitory neurotransmitter GABA, is significantly reduced in interneurons. Moreover, expression of reelin, an extracellular matrix-associated protein important for development and cognitive function (Qiu et al., 2006; Weeber et al., 2002), is also significantly reduced in these same neurons. Animal models of reelin haploinsufficiency exhibit reduced GABAergic inhibitory tone and a deficit in sensorimotor gating, hallmarks of Schizophrenia (Qiu et al., 2006; Tueting et al., 1999). Collectively, these results suggest that an aberrant decrease in reelin and GAD67 gene expression could contribute to the pathogenesis of Schizophrenia.

In this issue of Molecular Pharmacology, Kundakovic et al. explore the hypothesis that methylation of the proximal promoters of reelin and GAD67 are co-regulated, and if so, could this methylation status be manipulated using DNA (cytosine-5) methyltransferase (DNMT) inhibitors. Previous studies have demonstrated that a CpG island exists in the proximal promoter of reelin (Chen et al., 2002). CpG islands are regions in the genome where CpG dinucleotides occur at a frequency greater than would normally be expected. Interestingly, while CpG islands in general are usually hypomethylated, post-mortem studies suggest that the reelin promoter is actually hypermethylated in individuals with Schizophrenia (Abdolmaleky et al., 2005; Chen et al., 2002). This hypermethylation could explain the reduction in reelin expression associated with

MOL 33266

Schizophrenia. Kundakovic et al. postulated that this hypermethylation could also serve as a basis for therapeutic intervention.

Kundakovic et al. exploited NT-2 neuronal progenitor cells to study the regulation of reelin expression. NT-2 cells are ideal for these studies as they exhibit low levels of reelin expression and hypermethylation of the reelin promoter (Chen et al., 2002), modeling some of the molecular derangements associated with Schizophrenia. Chronic treatment of NT-2 cells with the DNMT inhibitor doxorubicin significantly increased expression of reelin and GAD67. Surprisingly, the increase in reelin and GAD67 was preceded by a significant decrease in expression of DNMT1 protein. Further studies revealed that doxorubicin decreased levels of DNMT1 and the methyl-DNA binding protein MeCP2, and increased levels of histone acetylation at the reelin promoter. These results suggest that, at least in NT-2 cells, the reelin promoter is actively repressed through the action of DNMT and this repression is mediated via MeCP2-dependent chromatin remodeling (Fig. 1A). Furthermore, these results support other findings that suggest DNMT inhibitors may block DNMT activity by promoting degradation of DNMT (Fig. 1B), perhaps through formation of nonfunctional complexes (Yokochi and Robertson, 2004).

The findings of Kundakovic et al. are striking when one considers the ramifications for intervention in Schizophrenia. Hypermethylation of the reelin and GAD67 promoters, whether due to a triggering event during development or adolescence, represents an epigenetic mark that persists for the lifetime of an individual. However, the use of DNMT inhibitors to treat Schizophrenia or any disease is years away at best, as drugs of this nature will likely result in upregulation of numerous genes that are actively repressed via

MOL 33266

DNMT. Despite the potential drawbacks, the ability to pharmacologically erase an epigenetic mark through the use of DNMT inhibitors and reverse some or all of the derangements in brain function associated with Schizophrenia is revolutionary.



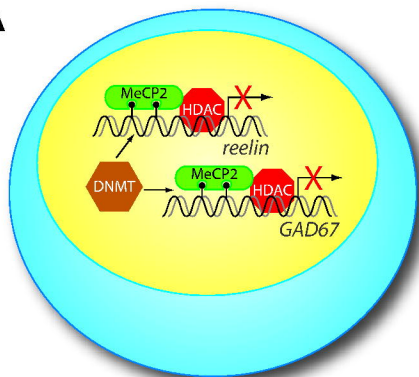
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MOL 33266

## Figure Legends

**Figure 1.** *Active repression of reelin and GAD67 by DNA methyltransferase.* **A)** Under normal conditions, the expression of reelin and GAD67 is repressed through active methylation of their promoters by DNMT. Methyl CpGs are bound by the methyl-DNA binding protein MeCP2, which recruits chromatin-remodeling enzymes including histone deacetylase (HDAC). This results in a decrease in histone acetylation and repression of gene expression. **B)** Application of a DNMT inhibitor, such as doxorubicin (DOXO) results in formation of DNMT-DOXO complexes and degradation of DNMT. In the absence of DNMT, active methylation and repression of the reelin and GAD67 promoters is relieved, and expression of reelin and GAD67 can occur.

**A****B**