

Fooling Mother Nature: Epigenetics and Novel Treatments for Psychiatric Disorders

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NEW TREND IN PSYCHOPHARMACOLOGY

Modern formulations of psychiatric disorders hypothesize that mother nature goes awry, causing both genetic and epigenetic disease actions. Genetic disease actions are the consequences of naturally inherited risk genes that have an altered sequence of DNA. This altered DNA sequence theoretically leads to the production of altered gene products in neurons, causing inefficient information processing in various brain circuits, and biasing those circuits towards developing symptoms of a mental illness. Epigenetic disease actions are theorized either to activate risk genes to make an altered gene product or to activate normal genes to make normal gene products but at the wrong time. Epigenetic disease mechanisms theoretically turn normal genes into risk genes by causing normal genes to be expressed in neurons when these genes should be silenced or by causing normal genes to be silenced when they should be expressed. Such epigenetic disease actions are thought not only to be regulated by inherited mechanisms and to be triggered by stress and

the environment; they are also hypothesized to contribute to inefficient information processing in brain circuits, increasing the chances of developing symptoms of a psychiatric disorder. Since manipulating genes themselves in psychiatric disorders may not be feasible, ethical, or safe (it is not nice to try to fool mother nature this way), targeting epigenetic mechanisms is increasingly being seen as a possible therapeutic approach for developing novel treatments of psychiatric illnesses (perhaps the nice way to fool mother nature?). Three specific epigenetic mechanisms currently under investigation include attempts to silence risk genes by enhancing the methylation of gene promoters or their downstream products; attempts to activate helpful genes by inhibiting an enzyme called histone deacetylase; and preventing the formation of defective or unwanted proteins in a neuron by interfering with RNA. Here we review the progress in applying such epigenetic therapeutics in psychiatry and determine whether such a strategy has shown any success in fooling mother nature's role in psychiatric disorders, emphasizing findings in schizophrenia.

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If you would like to comment on this column or submit a suggestion to Dr. Stahl for future columns, please e-mail lla@mblcommunications.com.

INTRODUCTION

Genetics is the sequence of DNA that is inherited and epigenetics is a parallel process determining whether a given gene (ie, a sequence of DNA coding for transcription) is expressed into its RNA or is silenced.¹⁻⁴ Epigenetic actions hypothetically can serve as mechanisms of disease action in some psychiatric illnesses¹⁻⁵ and the evidence for this is extensively reviewed in a companion article.⁵ The hope now is that epigenetics can also serve as a target of potential new therapeutics for psychiatric illnesses. Various contemporary approaches to therapeutic epigenetics in psychiatry are reviewed here.

EPIGENETICS AS A THERAPEUTIC TARGET FOR PSYCHIATRIC ILLNESSES

If psychiatric illnesses are caused by risk genes, then why do we not just fix the risk genes? Simple question, complex answer. Although modifying human genes could be done in principle, in practice it is complex, ethically troublesome, and potentially dangerous if the wrong targets are modified. Trying to change your genes may be no more feasible than trying to change your mother after you are born. As the saying goes, it is not nice to fool mother nature, at least this way.

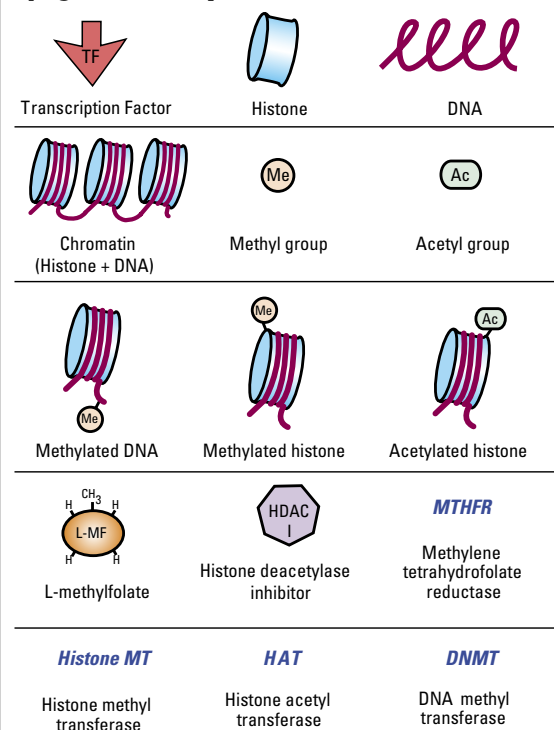
The strategy of targeting epigenetic mechanisms as a route to new therapeutics in psychiatry may be more feasible. That is, good or bad, the sequence of your genes is faithful throughout your lifetime. However, the developmentally programmed silencing and activation of some of your genes may be open to change if enticed by seductive epigenetic molecular mechanisms. This approach is based upon the idea that if mental illnesses are caused by the expression of risk genes that make altered proteins or by the expression of normal genes that turn them into risk genes by making normal proteins but at the wrong time, then maybe silencing risk genes or activating compensatory genes would treat or even prevent mental illnesses.^{2,4}

In order to turn your genes on or off, epigenetic mechanisms must be employed. Epigenetic molecular switches turn genes on and off by modifying the structure of chromatin in the cell nucleus.²⁻⁵ Chromatin is an octet of proteins called histones around which your DNA is wrapped (Figure 1).²⁻⁵ DNA contains genes and also promoters that tell genes when to make

RNA, which can then go on to make proteins. To silence genes, histones can be methylated or gene promoter DNA sequences can be methylated (Figures 1 and 2A). Methylation is often followed by another chemical process called de-acetylation which occurs at histones and which also inactivates nearby genes (Figures 1 and 3A). To activate genes, the reverse is done: histones and genes are demethylated (Figure 2A) and histones are acetylated (Figure 3A). All of these processes are regulated by numerous enzymes and methylation is regulated by the availability of methyl donors as well.²⁻⁵

Therapeutic epigenetics seeks to silence undesirable genes without silencing desirable genes and to activate desirable genes without activating undesirable ones. This is a complex process because there are over 20,000 genes and it is not yet proven which genes conspire to cause which mental disorders, let alone how to target the right genes selectively without also going off target and silencing or activating the wrong genes as well. Furthermore, psychiatric illnesses are likely to be caused by a conspiracy of many genes and

FIGURE 1.
Epigenetic key

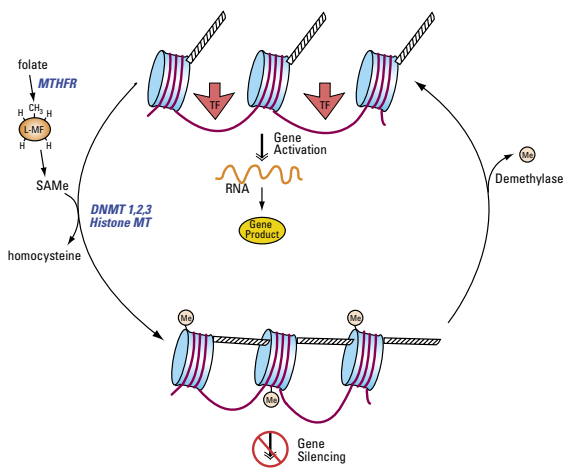


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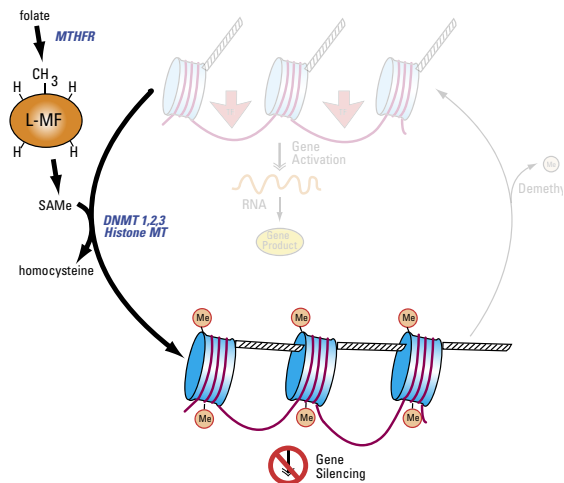
many epigenetic mechanisms that act simultaneously, so therapeutics may ultimately require multiple simultaneous genes to be silenced while others are activated.⁵ This can appear to be a formidable task, but some progress is already being made in epigenetic therapeutics in psychiatry by taking three main approaches to this: silencing genes by promoting methylation, activating genes by blocking de-acetylation, and stopping genes from being translated into proteins by interfering with their RNA.

FIGURE 2.
Methods of gene silencing

A. Regulation of Gene Silencing by Methylation



B. Enhancing Gene Silencing by Promoting Methylation



TF=transcription factor; MTHFR=methylene tetrahydrofolate reductase; L-MF=L-methylfolate; Me=methyl group; DNMT=DNA methyl transferase; Histone MT=histone methyl transferase.

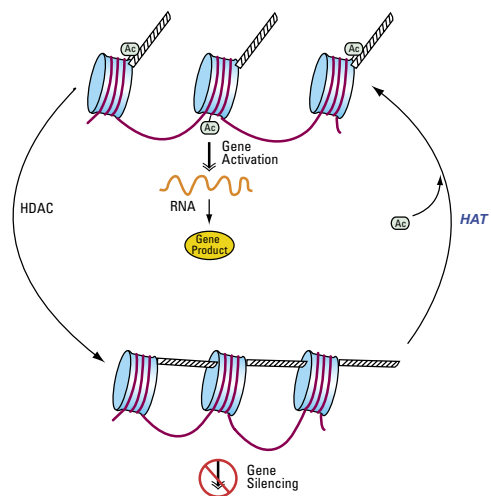
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THERAPEUTIC METHYLATION: HOW, WHERE AND IN WHOM?

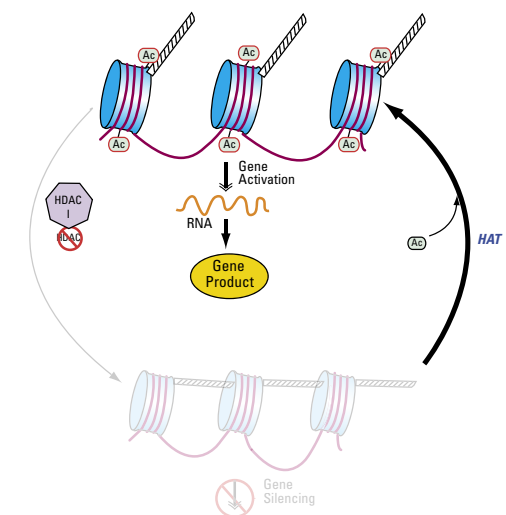
You may not be able to change your mother, but maybe you can silence her (or your father) by methylating your chromatin. Image that! Suppose your parents have installed risk genes in your brain. Sometimes we can literally see our parents in our brains when they do this.

FIGURE 3.
Methods of gene activation

A. Regulation of Gene Activation by Acetylation



B. Enhancing Gene Activation by HDAC Inhibition: Blocking De-Acetylation Enhances Acetylation and Gene Activation



Ac=acetyl group; HDAC=histone deacetylase; HAT=histone acetyl transferase.

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For example, if you inherited from your father the val form of the enzyme catechol-O-methyl transferase (COMT), which regulates dopamine (DA) levels in prefrontal cortex, your information processing is less efficient when you do cognitive tasks and you will light up your brain more during functional imaging than someone who inherited the met form of COMT.⁵⁻⁸ Like father, like daughter.

Secondly, if you also inherited from either of your parents epigenetic mechanisms telling your brain not to methylate the promoter for the gene that makes COMT, your DA signaling may be further compromised.^{5,9,10} Thirdly, to add insult to injury, if you inherited as well the T form of the enzyme methylene-tetrahydro-folate reductase (MTHFR), which regulates methyl donor availability, these three inherited events may have dealt your information processing a triple whammy.⁵ That is, this triple combination will potentially compromise your DA signaling in your prefrontal cortex and your cognitive functioning by the interaction of these three molecular mechanisms.^{5-7,9-16} The precise molecular basis for this is explained in more detail in the accompanying article.⁵ If you have inherited this triple whammy, you will literally be able see this in your functional brain images⁶ and you will probably also have an increased chance of getting schizophrenia or bipolar disorder.^{6,7,13} Thanks, Mom and Dad.

In theory, this situation could be fixed if you could silence your mother (or father) by increasing the methylation of your COMT promoter for your undesirable form of COMT and also by increasing the availability of methyl donors for your DNA, histone, and downstream metabolic methylation reactions by your compromised form of MTHFR. Investigators are pondering how to find drugs that can promote methylation in patients with psychiatric illnesses, how to identify the targets to methylate, and how to identify the targets to avoid. Furthermore, investigators are trying to determine which patients to treat with this approach, since not all patients are turning out to have the same genetic and epigenetic profiles, even if they have the same diagnosis.

Hints about how to identify the targets for therapeutic methylation come from understanding the enzymes that mediate normal gene methylation and thus normal gene silencing. Some enzymes try to maintain the status quo of a cell, enzymes such as DNA methyltransferase

1 (DNMT1, also called maintenance DNMTs), which maintain the methylation of specific areas of DNA and keep various genes quiet for a lifetime, a process called maintenance methylation.^{2,4,5,17} For example, this process keeps a liver cell always a liver cell, including when that cell divides into another one. This process also keeps a neuron a neuron, by always silencing a different set of genes. Presumably methylation is maintained at promoters for genes that one cell does not need, even though another cell type might. If DNA that should be silent is suddenly expressed, this could hypothetically wreak havoc in the brain.

Other enzymes such as DNMT2 and 3, also called do novo DNMTs, can methylate new DNA promoters in mature cells to alter their function and silence their genes in reaction to events in one's life, drugs, stress, and the environment.^{2,4,5,17} This process of de novo DNA methylation as well as de novo histone methylation may mediate everything from normal learning, to therapeutic response to psychotropic drugs, to fear conditioning, chronic pain states, addiction, and the unfolding of schizophrenia over time.⁵ For example, mutations within genes encoding for various histone methyltransferases are linked to mental retardation and autism.^{4,5} Mutations in the gene for a methylated DNA binding protein (MeCP2) that normally silences genes are linked to the behavioral abnormalities of Rett syndrome.^{4,18}

THERAPEUTIC METHYLATION IN SCHIZOPHRENIA

In schizophrenia, the situation is complex and not yet unraveled. However, hope springs from the fact that you might not have schizophrenia even if your identical twin does. Only half of co-twins of a schizophrenic patient also have schizophrenia. It is now assumed that epigenetic silencing in the unaffected twin prevents the schizophrenia that is occurring in their co-twin. If those same epigenetic mechanisms could be harnessed, maybe the schizophrenia in your affected co-twin could be reversed or prevented. Maybe these same therapeutic mechanisms would work in schizophrenics or individuals at risk for schizophrenia who do not have a twin.

For example, certain specific histone methyltransferases are essential for hippocampal synaptic plasticity and might be involved in cortical dysfunction of some cases of schizophrenia.^{4,5,19}

Upstream epigenetic alterations in DNA methylation of some genes or gene promoters such as those for COMT,^{9,10} various types of glutamate receptors,^{5,8} glutamic acid decarboxylase, the enzyme that synthesizes γ -aminobutyric acid (GABA),¹⁹⁻²³ and the critical synaptic structural protein reelin have been described in some²¹⁻²⁷ but not all²⁸⁻³⁰ studies of cerebral cortex of patients with psychosis. Prominent theories suggest that some forms of schizophrenia are caused specifically by inherited and environment- or experience-triggered disease related changes in gene expression for glutamate and GABA systems, potentially explained by alterations of histone trimethylation or other histone modifications.¹⁹ A deficit in histone methyl marks at the promoter of the gene for GABA synthesis has been described in postmortem schizophrenic brain. Altered methylation of DNA of a more global nature has also been described in schizophrenia.^{31,32} Most theories of schizophrenia that involve glutamate and GABA, however, suggest that multiple simultaneous co-conspirators, acting either epigenetically or genetically, must plot together in the conspiracy in order for schizophrenia to occur.^{5,8} Whether the abnormalities in methyl marks on DNA that are postulated to be present in psychiatric disorders such as schizophrenia are due to inherited factors, acquired factors, or both, is not yet known, but both are suspected. If deficient methyl marks could be therapeutically replenished, the hope is that this would have a therapeutic effect.

DNA and histone methylation are suspected to be compromised when the neuron is starved of methyl donors for methylation reactions, such as occurs with various inborn errors of metabolism, or when folate is deficient from the diet or when the methyl donors folate, L-methylfolate and S-Adenosylmethionine (SAME) are deficient in the body and brain from other factors such as pregnancy, gastrointestinal disease, smoking, alcohol addiction, or taking various drugs.^{5,33} Inborn errors of metabolism such as severe MTHFR deficiency, although rare, can deprive neurons of the methyl donor L-methylfolate to such an extent as to cause not only elevated homocysteine levels, but also psychosis and developmental delay.^{34,35} The more common inborn error of metabolism is a form of MTHFR deficiency, known as the T form, that also causes elevations in homocysteine levels and a less profound reduction in enzyme activity.^{5,36,37} This T form of MTHFR is associated

with marginal functional availability of methyl donor groups and although not sufficient in itself to cause schizophrenia, does appear to increase the risk for schizophrenia or for cognitive dysfunction in schizophrenia.^{5,36-40}

Two other inborn errors of metabolism of folate are associated with schizophrenia but do not appear to be sufficient in themselves to cause schizophrenia.^{5,41} That includes low enzyme activity variants of methylene tetrahydrofolate dehydrogenase (MTHFD1) and methionine synthetase (MTR). Variants of both of these enzymes are associated with increased risk of schizophrenia.^{5,41}

Interestingly, homocysteine levels have been reported to be high and folate levels low in patients with schizophrenia whose MTHFR, MTHFD, and MTR enzyme activities and genotypes are unknown.^{5,38-40} Also, folate levels correlate inversely with the severity of negative symptoms in schizophrenia.³⁰⁻³⁸ These findings highlight the importance of assessing serum homocysteine levels or obtaining genotypes of folate related enzymes to detect inborn errors of methylation in patients with schizophrenia. Such patients may require treatment with folate or L-methylfolate as well as antipsychotics.

One simple approach is already in hand to treat such methyl donor deficiency states: namely, to boost the availability of methyl donors in well selected patients by administering folate or L-methylfolate (Figure 2B). That is, administration of folate or L-methylfolate raises L-methylfolate levels and reduces homocysteine levels.^{5,36-40} In schizophrenic patients, both folate and L-methylfolate have been reported to improve positive, negative and cognitive symptoms.³⁸⁻⁴⁰ Folate itself is not active in brain since it needs to be converted into L-methylfolate by MTHFD and then by MTHFR,³³ enzymes that can be inherited in an inefficient form.⁵ L-methylfolate, the product of MTHFD and MTHFR, gets into brain and bypasses these enzymes and thus any inborn errors of metabolism caused by deficiencies of these enzymes. L-methylfolate is then converted into methionine and finally into SAME, which is often^{5,33} but not always⁴²⁻⁴⁴ the direct methyl donor for methylation reactions.⁵ Administering methionine and SAME, however, can cause build up of the unwanted metabolite homocysteine that can interfere with epigenetic mechanisms, and can also eventually deplete methyl precursors for SAME itself.⁴² Also, methi-

onine can exacerbate schizophrenia^{5,45} and the effects of SAME in schizophrenia are variable.^{5,46}

In practice, therefore, L-methylfolate may be the most efficient form in which to administer methyl donors and the most consistently effective for schizophrenia. Whether the administration of L-methylfolate works through epigenetic mechanisms that enhance methylation and thus silencing of desired chromatin targets is not yet known (Figure 2B). It is certainly too soon to know for sure whether L-methylfolate will turn out to be an "epigenetic neuroleptic," but the evidence so far is promising. Epigenetic balance in uremic patients with hyperhomocysteinemia and DNA hypomethylation has been reported to be re-stored by methylfolate.⁴⁷ It would indeed be exciting if such an epigenetic restoration by L-methylfolate could be demonstrated in schizophrenia, especially in patients with inborn errors of methylation. Current research is attempting to determine how to identify the best patients for this approach (Figure 2B), including genotyping for the triple whammy and beyond, measuring red blood cell folate levels, blood homocysteine levels, or identifying risk factors for functional deficiencies in L-methylfolate availability such as pregnancy, various concomitant drugs, and various concomitant illnesses.

Another potential use of L-methylfolate for schizophrenia may be in prenatal treatment. We should also not forget that broadly defined, your "environment" at least for a while, before you are born, includes your mother's uterus. Thus, methyl donor deficiencies in your mother could affect your own embryonic development, methylation, and epigenetics, and theoretically could enhance your risk of schizophrenia. It could be that the MTHFR genotype of your mother is just as important as that of your own in determining your risk of schizophrenia. But this is rarely determined in practice and treating the epigenetic in utero environment of individuals at risk for schizophrenia (ie, maybe your mother) is often not considered and is certainly not well studied. Interestingly, L-methylfolate is one of the few agents not only proven to be safe during pregnancy,⁴⁸⁻⁵⁰ but also to be recommended during pregnancy to prevent just such methyl donor deficiencies and related complications, such as spina bifida. Perhaps there should be more aggressive treatment of pregnant women with the centrally active L-methylfolate if that mother has schizophrenia, risk for schizophrenia, or risk for methyl donor deficiency from various genetic and epigenetic causes.

HDAC INHIBITORS

Histones are reversibly acetylated by enzymes known as histone acetyl transferases, which open chromatin gates and facilitate gene expression (Figure 3A).²⁻⁵ Acetyl groups are removed by enzymes known as HDACs, which thus close the chromatin gates and silence those same genes (Figure 3A). Inhibitors of HDACs are currently the major pharmacological mechanism for experimentally manipulating epigenomic mechanisms (Figure 3B).⁴ Stopping the removal of acetyl groups would serve to keep genes activated, so HDAC inhibitors are essentially gene activators (Figure 3B). Novel and selective inhibitors for the numerous forms of HDACs are in development. However, it is not well characterized which selective HDAC inhibitors activate which genes and until this is clarified, clinical testing of such agents will not begin.

Also, some drugs are "accidentally" HDAC inhibitors, such as valproate.⁵¹ Topiramate may also be an HDAC inhibitor.⁵² These drugs were developed because they have other pharmacological mechanisms, but once on the market were discovered to have HDAC inhibitory properties as well. Whether this HDAC inhibition accounts for any therapeutic actions as an anti-convulsant or mood stabilizer or for any toxic effects, such as teratogenicity, is not yet known.

HDAC inhibitors may not only block deacetylation, but they may also activate demethylation, with the consequence that both actions activate genes.⁵³ This seems to occur with valproate, for example.^{54,55} By some mysterious mechanism unrelated to HDAC inhibition, the antipsychotics clozapine and sulpiride but not haloperidol or olanzapine may activate brain DNA demethylation.⁵⁶ The tricyclic antidepressant amitriptyline also seems to reduce DNA methylation, but not by HDAC inhibition.⁵⁵ Whether any of these epigenetic actions of various psychotropics are epiphenomena or are linked to any therapeutic actions or to any side effects of these drugs is not known since these drugs all have many additional and better understood pharmacologic properties.

To unravel all of this, selective agents have been developed and tested in animals with some very interesting results that point the way to how such agents might be tested in psychiatric disorders once they are cleared for testing in man. In animals, selective HDAC inhibitors may exert antidepressant actions, increase the levels of the neurotrophic factor called brain

derived neurotrophic factor, and be neuroprotective in models of stroke, Huntington's Disease, Alzheimer's Disease, Parkinson's Disease, and other conditions.^{4,53} HDAC inhibitors activate certain genes, but it is not clear yet which one or ones of these are critical for these potential therapeutic actions. Furthermore, the known HDAC inhibitors do more than block histone deacetylation. They also block the deacetylation of lots of proteins, from cytoskeletal proteins, to metabolic enzymes, to transcription factors, so all of their actions may not be epigenetic.^{4,53}

HDAC inhibitors can also enhance long term memory formation, possibly by activating critical genes.⁴ However, since methylation can also enhance other forms of memory,⁴ and this process silences genes, memory is obviously a complex proposition. Nevertheless, HDAC inhibitors not only seem to regulate long term memory formation, especially for spatial memories, but also the extinction of certain memories, which is itself a form of learning (ie, learning to forget), as in extinction of fear conditioning.⁴ There is even evidence that HDAC inhibitors can restore memories that have been forgotten, and perhaps relatedly, might be able to re-open critical periods of plasticity in the brain, allowing the re-emergence of brain plasticity after neurodevelopment.⁴ These are all very exciting possibilities and suggest that HDAC inhibitors have vast therapeutic potential in the future if they can be proven to be safe. Obviously, much more research must be done prior to introducing such agents into man, but HDAC inhibitors are warming up to try and fool the course of mother nature in psychiatric disorders.

RNA INTERFERENCE

Now for something out of *Star Wars* to fool mother nature. DNA is transcribed into RNA and RNA builds neurons because it is the template for all the proteins that a cell makes.⁵⁷ Cells have a natural defense system to zap viruses and other invaders from outer space by a process known as RNA interference (RNAi).⁴⁷ Now scientists are trying to hijack this defense system to zap RNA that comes from unwanted genes. Small pieces of specially designed RNA can be made which are programmed to interfere with the RNA that comes from defective genes. They are called micro RNA and small interfering RNA (siRNA). When these siRNAs are administered to a cell, that cell cannot make the defective protein.

This is an exciting new approach but has

many problems for use in psychiatric disorders. The brain is a very difficult place to penetrate and RNA is destroyed if given orally. Even if RNA is given intravenously and even if it gets into the brain this way, it may not last very long. Scientists are working with various, hopefully safe virus transporters for delivery instead of administering naked siRNA.

This all assumes that we know the gene that we are targeting and that is far from the case yet for any major psychiatric disorder. Also, it is likely that the RNA from many genes may have to be targeted simultaneously for this to work very well in psychiatric conditions. Nevertheless, this approach is making headway in some conditions where the target is a virus (such as HIV) or where the target is a single well known gene product (as in Huntington's Disease). Hopefully, some day this approach may become a useful one in psychiatry.

CONCLUSION

Epigenetic mechanisms can be therapeutic targets in psychiatry. Sometimes, unwanted genes may need to be silenced. This could occur by promoting methylation of gene promoters and histones. Currently that may be possible by enhancing the availability of methyl donors such as by administering L-methyl folate to restore epigenetic balance. This theoretically has the potential to correct deficiencies in methyl donor availability when they happen and when they are due to environmental depletion or inborn errors of metabolism. In the distant future, this may become possible by interfering with the translation of RNA from defective genes into unwanted proteins with iRNA. At other times, genes may need to be activated. HDAC inhibitors can activate genes, show promise in animal models of psychiatric disorders, and include some already known psychotropic drugs that have HDAC inhibitory properties. The future is bright and even though you cannot choose your parents, you may be able to fool mother nature with epigenetic mechanisms to treat the symptoms or reduce the chances of getting a psychiatric disorder. **CNS**

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