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Chromatin Remodeling: A Novel Mechanism of Psychotropic Drug Action

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Abstract

Regulation of gene expression is known to contribute to the long-term adaptations underlying the effects of psychotropic drugs, including the actions of antidepressants and drugs of abuse in behavioral models. However, the precise molecular events that are required for modification of chromatin and that underlie gene repression or activation have not been elucidated. Recent reports, including the article by Cassel et al. in this issue, address this question and demonstrate that psychotropic drugs modify specific methyl-CpG-binding proteins that control histone acetylation and gene expression.

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Introduction

The actions of most drugs used for the treatment of psychiatric illnesses require long-term treatment indicating that neuronal adaptations in specific brain regions are required for a therapeutic response. In addition, the development of dependence and tolerance to drugs of abuse also occurs over time and requires cellular adaptations, albeit in a different set of neural circuits and brain regions. One area of intensive research aimed at identifying these long-term adaptations is the regulation of gene expression changes that are induced by psychotropic drugs. There are numerous investigations reporting altered gene expression in response to different types of psychiatric medications (e.g., antidepressants and antipsychotics) and drugs of abuse (e.g., psychostimulants and opiates), demonstrating the plasticity of neural systems at a molecular level (Duman et al., 1997; Duman et al., 2000; Manji et al., 2001; Nestler, 2004). In many cases the relevance of a specific gene to a particular drug action or behavior has been shown using targeted approaches (i.e., mutant mice, viral vectors, RNAi), demonstrating the functional significance of altered gene expression.

The binding sites and acute actions of psychotropic drugs occur primarily at neurotransmitter receptors or transporters located on cell membranes of pre- and postsynaptic terminals, and the pathways leading from these sites to the regulation of gene expression in the nucleus represent another area of intensive research. Much of the work has focused on intracellular signal transduction cascades and transcription factors that bind to specific gene promoter elements, but the molecular mechanisms that control the expression or repression of genes, referred to as epigenetic mechanisms, have been understudied and underappreciated. Recent progress in cellular and molecular biology has highlighted the importance of chromatin remodeling in controlling rates of gene transcription, revealing a complex set of mechanisms for regulating the accessibility of genes to the transcriptional machinery that is responsible for RNA synthesis and gene expression. A

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recent article in this issue (Cassel et al., 2006), as well as other related reports (Kumar et al., 2005; Tsankova et al., 2006), demonstrate that antidepressants and drugs of abuse regulate chromatin remodeling and the molecular machinery associated with this process.

Mechanisms of Chromatin Remodeling

The structural unit of chromatin is the nucleosome, which is composed of 147 bp of DNA wrapped around a histone octamer made up of 4 pairs of the basic histone proteins (H2A, H2B, H3 and H4). DNA that is tightly coiled in nucleosomes is inaccessible to DNA binding proteins such as transcription factors, co-factors and RNA polymerases that comprise the gene transcription machinery (Figure 1) (see reviews by (Colvis et al., 2005; Jenuwein and Allis, 2001; Turner, 2002). Access of DNA in the nucleosome occurs via enzymatic remodeling mechanisms involving a complex association of proteins. The amino acid residues in the C-terminal tails of histones are targets for a wide range of post-translational modifying enzymes that contribute to either tighter coiling or relaxation of the DNA-nucleosome complex. In general, increased histone acetylation or hyperacetylation by histone acetylases/acetyltransferases (HATs) is associated with DNA relaxation and elevated transcriptional activity and gene expression, while decreased acetylation or hypoacetylation which is brought about by histone deacteylases (HDACs) and methyl CpG binding proteins such as MeCP2 leads to tighter DNA coiling and gene repression or silencing (Figure 1). MeCP2 is the prototypical member of a family of methyl-CpG binding domain (MBD) proteins that have been linked with transcriptional silencing. MeCP2 has two functional domains, a methyl-CpG binding domain and a transcription repressor domain. It binds with specificity to methylated DNA and then serves to attract chromatin-remodeling machinery consisting of HDACs, the repressor Sin3A and histone methyltransferases that then cause gene silencing. While the actions of HATs and

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HDACs modify histones at the biochemical level another class of chromatin remodeling enzymes, the SWI/SNF family of genes, alter nucleosome structure by utilizing energy from ATP hydrolysis and cause local changes in DNA. This combination of biochemical and structural remodeling of chromatin plays a pivotal role in precise regulation of eukaryotic gene transcription.

Regulation of Chromatin Remodeling by Psychotropic Drugs

The article by Zwiller and colleagues in this issue provides evidence that psychotropic drugs regulate different forms of methyl-CpG-binding proteins in brains of experimental animals. They report that chronic administration of either a 5-HT selective reuptake inhibitor (i.e., fluoxetine) or a psychostimulant (i.e., cocaine) increases the expression of MeCP2, as well as another methyl-CpG-binding protein, MBD1 in forebrain regions of adult rat. These effects were most pronounced in the striatum, with similar but milder effects in the frontal cortex and dentate gyrus of the hippocampus. The induction of these methyl-CpG DNA binding proteins suggests that there is a repression of gene expression in response to fluoxetine or cocaine treatments. This possibility is supported by analysis of histone modifications, and as predicted the results demonstrate that acetylation of histone H3 is decreased, indicative of a state of more tightly coiled DNA that may not be as actively transcribed. Although the numbers of HDAC1 and HDAC2 positive cells were not altered, there was an increase in the intensity of immunoreactivity for HDAC2, the more abundant form in the brain regions examined. In addition, levels of HDAC2 mRNA were significantly increased by both fluoxetine and cocaine, indicating that the regulation of histone acetylation occurs via increased expression and recruitment of HDAC to the DNA complex.

The results of this study provide the first evidence that psychotropic drugs can influence chromatin remodeling via the expression of methyl-CpG-binding proteins, and represent an exciting

new level of understanding of the actions of antidepressants and drugs of abuse. As discussed by the authors, evidence of epigenetic alterations in post-mitotic neurons demonstrates the capability for regulating the interpretation of the DNA methylation code that is determined during development. The effects are also dependent on chronic drug administration, although other studies have reported rapid chromatin remodeling effects of drugs that act at dopamine receptors in the striatum (Li et al., 2004). The results also indicate that monoamine neurotransmitter signaling can influence chromatin remodeling, and the role of 5-HT is supported by negative results with selective dopamine and norepinephrine reuptake inhibitors. However, it will be important to further delineate the actions of fluoxetine and cocaine on chromatin remodeling as there are clearly functional differences between the selective 5-HT selective reuptake inhibitors which represent the drug class that is most widely used for the treatment of depression, and psychostimulants such as cocaine that have strong abuse potential. The similar actions of fluoxetine and cocaine on MeCP2, MBD1 and histone acetylation leave many questions unanswered.

A key question that is not addressed in this study, and that could help elucidate the specific effects of these different classes of psychotropic drugs, is what gene or sets of genes are regulated by cocaine and fluoxetine administration? Although the results suggest that gene repression takes place when MeCP2 and MBD1 are increased, there is no evidence to support this conclusion. In fact, recent studies from another laboratory demonstrate opposite effects on histone acetylation, and increased, not decreased expression of specific target genes, brain derived neurotrophic factor (BDNF) and Cdk5, two genes that have been implicated in the actions of chronic antidepressant and/or psychostimulant administration (Kumar et al., 2005; Tsankova et al., 2006). These latter studies use a different approach, chromatin immunoprecipitation (ChIP) combined with PCR analysis to amplify specific gene promoters that are bound to acetylated histones. They find that

chronic antidepressant administration increases H3 acetylation in the hippocampus and find a corresponding decrease in the expression of HDAC5, with no change in levels of HDAC2. These effects could contribute to the induction of BDNF expression in the hippocampus by antidepressant treatment and could oppose the actions of chronic social stress on BDNF and chromatin remodeling. This study also reports the novel finding that chronic social defeat causes a long-lasting and apparently irreversible increase in histone dimethylation that contributes to repression of BDNF expression, and the authors speculate that this, or similar effects could contribute to chronic depression in humans (Tsankova et al., 2006). In another study from the same group, chronic cocaine administration was found to increase the hyperacetylation of histone H3 at the BDNF and Cdk5 promoters in the striatum (Kumar et al., 2005). The discrepancy between these studies and Cassel paper are further complicated by a report that MeCP2 binds to and represses BDNF gene expression in rodent brain under basal conditions (Chen et al., 2003). This further underscores the need to investigate the regulation of specific genes to determine the functional relevance of altered methyl-CpG binding proteins and histone acetylation. In addition, double-labeling approaches (immunohistochemistry combined with in situ hybridization) can be used to analyze gene expression changes in the same cells that exhibit elevated MeCP2 to address the issue of cell-specificity, as well as gene repression or activation.

There are several possible explanations for the differences observed in these studies. There are methodological issues, including the drugs used (fluoxetine vs. imipramine), dosing paradigms (e.g., length of drug administration), and species (rat vs. mouse), as well as type of analysis (expression levels of chromatin binding proteins and enzymes vs. ChIP analysis of bound promoters). It is also possible that there are cell specific effects, as the Cassel study provides evidence that the induction of MeCP2 occurs largely in GABAergic interneurons. Yet another

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possibility is that there are multiple sites for histone acetylation that could be differentially regulated in concert and in a gene-specific manner, further highlighting the requirement for analysis of the regulation of specific gene targets.

Another interesting issue to consider is the stability of these epigenetic events. Although all of these studies demonstrate that changes in histone modifications and chromatin remodeling require repeated treatments over several days or weeks, and are also long-lasting (Tsankova et al., 2006), the repressed state of BDNF gene expression can be quickly reversed by neuronal depolarization via phosphorylation of MeCP2 (Chen et al., 2003). This demonstrates that chromatin remodeling can undergo rapid remodeling in response to neuronal stimulation, as well as long-term regulation by psychotropic drugs, and indicates that additional mechanisms or events may be recruited that can influence stability.

Future Directions and Conclusions

The fact that the histone code (Jenuwein and Allis, 2001) and chromatin remodeling is altered by antidepressants and drugs of abuse provides a platform for further experimentation to seek out the functional consequences of drug-induced chromatin modifications. The recent explosion in technology and molecular tools allows us to conduct elegant high-resolution studies to address fundamental questions about this process. For example, it is now possible to conduct gene expression profiling in the specific cell types where altered MeCP2 and H3 acetylation are observed by combining immunohistochemistry with laser microdissection, RNA amplification and microarray analysis. Another question that remains unanswered is what intracellular signaling cascades activate specific sets of chromatin/histone modifiers in a manner that leads to recruitment and regulation of specific transcription factors and target genes? There is one study demonstrating a role for the

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cAMP-protein kinase A cascade in the regulation of histone modification by dopaminergic agents (Li et al., 2004), but there are likely to be multiple additional signaling cascades that underlie the actions of psychotropic drugs. The advent of ChIP-chip (tandem combination of chromatin immunoprecipitation with microarray chips) technology can enable one to study these transcription factor driven gene networks and correlate existing or parallel gene expression data sets. Although ChIP-chip analysis is a very popular and powerful tool it is currently being applied only to cell lines and has not yet been utilized to investigate chromatin remodeling and gene expression changes in brain tissue.

The recent progress in understanding transcriptional events at the chromatin level has led to an appreciation of how a well-coordinated series of biochemical modifications of histones can result in precise physico-chemical alterations of the DNA can then result in repression or activation of the gene expression. The discovery that antidepressants and drugs of abuse can influence this process represents an exciting step in elucidating the molecular actions of these psychotropic agents that have been previously characterized by virtue of their pharmacology, neurotransmitter modulation, receptor activation and signaling cascades. On the other hand this is not entirely surprising, as previous studies have implicated chromatin remodeling either indirectly or directly in the actions of these agents. For example, a primary transcriptional target of antidepressants and drugs of abuse is CREB (cAMP response element binding protein), which interacts with another protein, CBP (CREB binding protein) that possesses intrinsic HAT activity. In addition, a drug used for the treatment of bipolar disorder, valproic acid, is an HDAC inhibitor, and is thought to exert its therapeutic response, at least in part via modification of histones (Phiel et al., 2001). The possibility of targeting specific chromatin remodeling players for drug development is supported by studies demonstrating that modification of histone acetylation, by administration of inhibitors or over expression of specific

HDACs, alters behavior in models of depression and drug preference (Kumar et al., 2005; Tsankova et al., 2006). One particularly interesting target is HDAC5, which is decreased by antidepressant treatment and when over-expressed was found to block the behavioral effects of antidepressants (Tsankova et al., 2006).

However, further eludicaton of the wide-range of histone modifications and the ensuing consequences on gene expression will be necessary before the potential for drug development can be realized. Phosphorylation and ubiquitination, are some other examples, in addition to acetylation and methylation, of the rapidly expanding list of modifications. There are also numerous yet to be answered questions pertaining to the stability, reversibility, global and local frequency, synchrony and partnerships involved in the modulation of the nucleosome and chromatin remodeling. The current and related recent papers are exciting leads that will stimulate further studies to characterize the molecular alterations underlying chromatin remodeling and the regulation of these events by psychotropic drugs.

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Figure 1. Psychotropic Drug-Induced Chromatin Remodeling. Shown in schematic form is the sequence of nuclear events that occur in response to chronic administration of antidepressants or drugs of abuse. Acting via intracellular signaling cascades, these pharmacological agents exert nuclear effects by activating chromatin-remodeling enzymes and associated binding partners that either compact or relax nucleosomal DNA. Methylation and deacetylation of histone proteins (represented by colored spheres) is generally associated with gene repression while demethylation and acetylation leads to increased gene expression. The resultant changes in gene expression then contribute to the pharmacological actions of these agents. MeCP2, methyl CpG binding protein 2; MBD, methyl-CpG binding domain proteins; HAT, histone acetyltransferase; HDAC, histone deacetylase; TF, transcription factor; RNA pol, RNA polymerase.

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