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Invited review

Epigenetic mechanisms of drug addiction

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ABSTRACT

Drug addiction involves potentially life-long behavioral abnormalities that are caused in vulnerable individuals by repeated exposure to a drug of abuse. The persistence of these behavioral changes suggests that long-lasting changes in gene expression, within particular regions of the brain, may contribute importantly to the addiction phenotype. Work over the past decade has demonstrated a crucial role for epigenetic mechanisms in driving lasting changes in gene expression in diverse tissues, including brain. This has prompted recent research aimed at characterizing the influence of epigenetic regulatory events in mediating the lasting effects of drugs of abuse on the brain in animal models of drug addiction. This review provides a progress report of this still early work in the field. As will be seen, there is robust evidence that repeated exposure to drugs of abuse induces changes within the brain's reward regions in three major modes of epigenetic regulation—histone modifications such as acetylation and methylation, DNA methylation, and non-coding RNAs. In several instances, it has been possible to demonstrate directly the contribution of such epigenetic changes to addiction-related behavioral abnormalities. Studies of epigenetic mechanisms of addiction are also providing an unprecedented view of the range of genes and non-genic regions that are affected by repeated drug exposure and the precise molecular basis of that regulation. Work is now needed to validate key aspects of this work in human addiction and evaluate the possibility of mining this information to develop new diagnostic tests and more effective treatments for addiction syndromes.

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1. Introduction

Drug addiction can be viewed as maladaptive neural plasticity that occurs in vulnerable individuals in response to repeated exposure to a drug of abuse. That vulnerability is determined roughly half by genetic factors (although few specific causative genes have as yet been identified) and half by non-genetic factors which include environmental exposures as well as stochastic events during development. Once formed, in turn, addiction can drive life-long behavioral abnormalities.

These features of addiction suggest an important role for epigenetic mechanisms. The term epigenetics has several definitions; this review utilizes a broad one, which defines epigenetics as a series of biochemical processes through which changes in gene expression are achieved throughout the lifecycle of an organism without a change in DNA sequence (Jaenisch and Bird, 2003). Epigenetics can thus be viewed as the vehicle through which environment interacts with an individual's genome to determine all aspects of function, in health and disease. A subset of epigenetic changes are very stable, which makes them ideal mediators both of addiction vulnerability and of drug-induced brain maladaptations that underlie an addiction syndrome.

Within this context, there are three general roles that epigenetic mechanisms likely play in addiction (Tsankova et al., 2007; Robison and Nestler, 2011). First, repeated exposure to a drug of abuse in adolescence or adulthood causes addiction in vulnerable individuals by inducing stable changes in gene expression through epigenetic regulation of those specific genes. Such epigenetic regulation involves alterations in the steady state expression levels of a set of genes as well as changes in other genes' inducibility—both sensitization (priming) and desensitization—without a change in steady state expression. Regulation of gene inducibility can be seen as "latent" in that it would not be apparent by analysis of mRNA or protein levels. Epigenetic regulation of genes also alters the expression of splice isoforms of a gene, which is usually not apparent from traditional microarray analyses of expressed mRNAs. Second, epigenetic regulation mediates changes in steady state gene expression or inducibility of genes that occur throughout an individual's lifetime in response to a host of environmental exposures, which help determine that individual's vulnerability to drug exposure and addiction later in life (Hiroi and Agatsuma, 2005). Third, there is the possibility that drugs or other environmental exposures induce epigenetic changes in sperm or ova, which are

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then passed on to offspring and alter their vulnerability to addiction. Such trans-generational epigenetic inheritance of addiction vulnerability remains controversial.

Finally, because the large majority of investigations of epigenetic mechanisms have been carried out on cultured cells or peripheral tissues, studies of epigenetic regulation in addiction models will teach the field fundamental principles about epigenetics in the developing and adult nervous system. In this way, such work provides the first ever look at mechanisms of transcriptional regulation in brain, and will likely have enormous impact on the field.

2. Overview of mechanisms of epigenetic regulation

The 3 billion nucleotides of DNA in a mammalian genome would be ~2 m long if stretched out linearly, yet fits within a microscopic cell nucleus due to its extraordinary degree of organization and compaction in chromatin—nuclear material composed of DNA, histones, and non-histone proteins (Jaenisch and Bird, 2003). The fundamental unit of chromatin is the nucleosome, which consists of ~147 base pairs of DNA wrapped around a core histone octamer (~1.65 turns). Each octamer contains two copies each of the histones H2A, H2B, H3, and H4 (Fig. 1A). Epigenetic mechanisms control the spacing of nucleosomes and the degree to which they are condensed, which thereby determines gene activity. In simplified terms, chromatin exists in an inactivated, condensed state (heterochromatin), which does not allow transcription of genes, and in an activated, open state (euchromatin), which allows individual genes to be transcribed (Fig. 2). In reality, chromatin exists in many states in between these two extremes. Regulation of the state of chromatin around specific genes, as well as in non-genic regions, is controlled by complex biochemical processes, involving diverse types of post-translational modifications of histones, methylation of DNA itself, large families of chromatin remodeling proteins, and non-coding RNAs, which are described briefly here.

2.1. Histones

The best characterized chromatin remodeling mechanism in brain is the post-translational, covalent modification of histones at distinct amino acid residues on their N-terminal tails (Jenuwein and Allis, 2001). Such modifications include acetylation, ubiquitination, or SUMOylation at lysine (K) residues, methylation at lysine or arginine (R) residues, phosphorylation at serine (S) or threonine (T) residues, and ADP-ribosylation at glutamate (E) residues (e.g., Fig. 1B). Acetylation generally promotes decondensation of chromatin and increases gene activity by negating the positive charge of K residues in histone tails and increases spacing between nucleosomes. In contrast, histone methylation can either promote or repress gene activity, depending on the residue undergoing methylation. Phosphorylation of histones is also associated with chromatin inhibition or activation. The roles of histone ubiquitylation, SUMOylation, and ADP ribosylation are less well understood. The diversity of histone modifications supports the "histone code hypothesis," which posits that the sum of modifications at a particular gene defines a specific epigenetic state of gene activation or silencing (Jenuwein and Allis, 2001). However, as will be seen, such codes are likely to be highly complex and have yet to be identified.

The enzymes that mediate these various covalent modifications of histones can be understood as "writers" and "erasers," respectively. For example, histone acetyltransferases (HATs) catalyze acetylation and histone deacetylases (HDACs) catalyze deacetylation, while histone methyltransferases (HMTs) catalyze methylation and histone demethylases (HDMs) catalyze demethylation. The specificity of numerous HATs and HDACs for specific K residues remains incompletely understood. In contrast, distinct HMTs and HDMs control the methylation of specific K and R residues and even the valence of methylation, i.e., mono-, di-, or tri-methylated states. The functional consequences of histone modifications are mediated partly through "readers"—proteins that bind to specific modified residues and effect transcriptional change (Jenuwein and Allis, 2001; Jaenisch and Bird, 2003). For example, different families of chromatin remodeling proteins, which use ATP-derived energy to alter nucleosome spacing and condensation, recognize specific forms of modified histones and enhance or repress the activity of nearby genes. Ultimately, hundreds of proteins are thought to be recruited to a gene in concert with its activation or repression, again emphasizing the extraordinary complexity of epigenetic mechanisms.

2.2. DNA methylation

DNA methylation occurs with the addition of a methyl group to the C5 position of cytosine (5-mC) predominantly at CpG sites (Klose and Bird, 2006). It plays a pivotal role in cell differentiation, imprinting, and X chromosome inactivation. DNA methylation

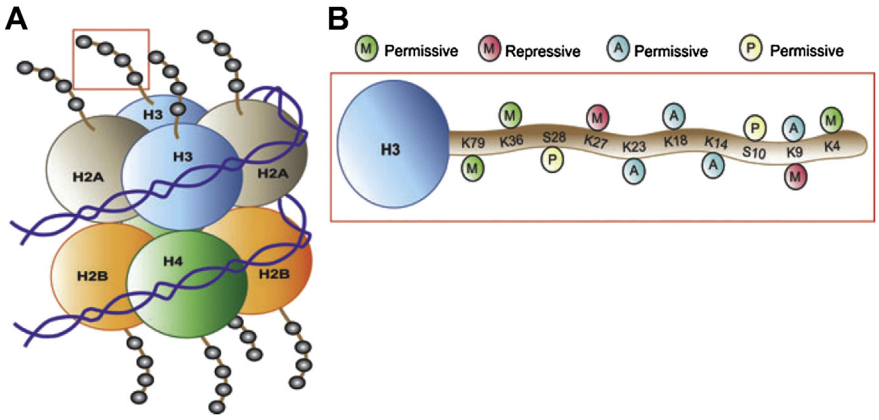

Fig. 1. Scheme of post-translational modifications of histones. (A) The nucleosome is the functional unit of chromatin, composed of 147 bp of DNA wrapped around a core octamer of histone proteins (two copies each of H2A, H2B, H3, and H4). The N-terminal tails of these histones face outward from the nucleosome. (B) Combinations of acetylation, phosphorylation, methylation, etc., on histone tails (here, H3 is depicted) alter chromatin compaction and regulate gene expression. Histone modifications that weaken the interaction between histones and DNA or that promote the recruitment of transcriptional activating complexes (e.g., H3 acetylation at K23, K18, K14, and K9, as well as methylation at K79, K36, and K4 or phosphorylation at S28 and S10) correlate with permissive gene expression. Histone deacetylation, which strengthens histone:DNA contacts, or histone methylation on K27 or K9, which recruits repressive complexes to chromatin, promote a state of transcriptional repression. Adapted from Tsankova et al. (2007) and Maze and Nestler (2011) with permission.