Published in final edited form as:

J Pain. 2013 June ; 14(6): 549–557. doi:10.1016/j.jpain.2013.01.772.

Epigenetics: A Promising Paradigm for Better Understanding and Managing Pain

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Abstract

Epigenetic regulation of gene expression is a rapidly growing area of research. Considering the longevity and plasticity of neurons, the studies on epigenetic pathways in the nervous system should be of special interest for both the epigeneticists and the neuroscientists. Activation or inactivation of different epigenetic pathways becomes more pronounced when the cells experience rapid changes in their environment, and such changes can be easily achieved by injury and inflammation, resulting in pain perception or distortion of pain perception (e.g. hyperalgesia). Therefore, in this regard, field of pain is at advantage to study the epigenetic pathways. More importantly, understanding pain from epigenetics point of view would provide a new paradigm for developing drugs or strategies for pain management. In this review, we introduce basic concepts of epigenetics, including chromatin dynamics, histone modifications, DNA methylation, and RNA induced gene silencing. In addition, we provide evidence from published studies suggesting wide implication of different epigenetic pathways within pain pathways.

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Dedication

We wish to dedicate this work to the memory of our parents, Elpidio Cruciani, John Lomber, and Eduardo Urrutia, who through their own personal experience with aging and disease made us reinforced our commitment to understanding and treating pain. This article is, therefore, meant to serve as a testimony that their teaching continues inspiring our work.

Disclosures

Dr. Urrutia is funded by NIH grant R01 DK52913, The Role of Zinc Finger Genes in Pancreatic Cell Growth, Mayo Clinic Center for Cell Signaling in Gastroenterology Grant P30 DK84567 and Translational Epigenomics Program, Center for Individualize Medicine (CIM), Mayo Clinic. Dr. Ou is funded by NIH/NIAAA (AA020103) and Brain & Behavior Research Foundation (formerly NARSAD). No conflict of interest exists with any of the authors.

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Keywords

Pain; Epigenetic; Chromatin; Histone modification; Transcription factor; DNAmethylation; Opioid receptors

1. Introduction

The regulation of gene expression through chromatin modifications and remodeling underlies the phenomenon of epigenetics. Epigenetic mechanisms confer pluripotent progenitor cells that possess identical genomic DNA with the ability to differentiate into morphologically and functionally distinct populations, such as neurons, astrocytes, and Schwann cells. This wide range of differentiation originates from modulating genome expression in different manners that are inheritable with each somatic cell division.

Beyond determination of cell fate, epigenetic regulation is of special interest in the nervous system due to the longevity of neurons. Unlike other organs in the body, the regeneration rate of neurons in both central and peripheral nervous system is remarkably low, suggesting that neurons are designed to survive for decades, possibly even the perpetuity of the organism. In order to survive such a long period of time, neurons must possess the greatest capacity for adaptation to changes in the environment, including physical and psychological stress. Given the inherent stability of DNA, such flexibility is most likely the result from epigenetic regulation of genes.

Easily detectable phenotypes that reflect the wide range of epigenetic mechanisms in the neurons are observed in extreme conditions such as development, where the cells experience rapid environmental changes and chemical stress. Likewise, injury and inflammation cause such environmental changes to the innervating neurons that perceive pain. So far, the focus of pain related research has been mainly on genomics and pharmacogenomics^{38, 44, 45}. Yet, recently, increasing number of investigators have started to realize the importance of epigenetic mechanisms that link the phenotype and genotype related to pain⁷¹. Those studies on epigenetics of pain are reviewed in this manuscript as initial evidence showing participation of epigenetic mechanisms in pain state.

Understanding the mechanism of how injury and inflammation induce changes in gene expression leading to changes in pain perception (e.g. analgesia or allodynia), would allow us to develop better therapeutic strategies. Our goal in this review is to introduce the basic concepts of epigenetics, and to review direct evidence of epigenetic mechanisms involved in pain signal. Epigenetic drugs that are already available for other diseases will be discussed briefly as well. In this review we will: 1) briefly discuss the achievement and limitation of pain genetics, 2) discuss basic aspects of molecular mechanisms that are important for understanding gene regulation, 3) examine existing evidence of epigenetic mechanisms' contribution to pain pathways by reviewing transcriptional regulation of opioid receptors, and 4) mention some epigenetic drugs that may be used for intervention.

2. Genetics and pharmacogenomics in pain and analgesia

Pain has an impact on the patient's life at several levels. In addition to the suffering, pain, especially chronic pain, can impair the individual's ability, quality of life, mood, and sleep, which can result in difficulties in personal and social life. Even though objective assessment of pain has been challenging, many self report scales have been developed in order to standardize the measurements, and have been utilized successfully both in clinical practice and research²⁵. The inter-individual variability in pain response and analgesic response to pharmacological agents, and variability in analgesic response patterns in different stains of

mice have indicated genetic basis of pain perception^{11, 59, 60}. Following such observation, significant efforts have been made to identify genes and polymorphisms that are involved in specific pain phenotype, and to develop tailored therapy for each patient^{14, 75}.

Numerous genes and single nucleotide polymorphisms (SNPs) have been identified to affect pain perception and response to analgesic drugs. However, accumulating data have been showing some controversial results on the genetic associations with pain related phenotype⁷⁴. For example, the most prevalent single nucleotide polymorphism (SNP) on human μ -opioid receptor (*ORPM1A*118G, asn40asp, rs1799971) was initially reported to show increased binding affinity for β -endorphin and to cause subsequent increase in G-protein coupled potassium channel activation, compared to the wild type². Yet, more recent independent study found no significant difference in ligand binding affinity, including that of β -endorphin, and in the activation of intracellular signaling cascade, between cells expressing the mutant and the wild type *ORPM1*¹. Similarly, catechol-O-methyltransferase (COMT) val158met SNP (rs4680) was reported to be associated with higher pain sensitivity by some groups, while others found the same SNP to have no association with pain perception^{39, 89}. Such inconsistencies from genetic studies suggest possible existence of additional, DNA sequence independent, factors that play a role in pain phenotypes. Such pathways, proteins, and mechanisms are essential topics in the field of epigenetics.

3. Fundamental Concepts of Epigenetics

Chromatin Dynamics Forms the Basis of Epigenetics

Chromatin is the structural conformation of DNA in association with assembly proteins. The nucleosome is the fundamental repeating unit of chromatin, consisting of about 140 base pairs of DNA wrapped around a histone octamer (consisting two copies of H2A, H2B, H3 and H4). Chromatin is dynamic, often switching between two higher order chromatin structures: euchromatin and heterochromatin.

Euchromatin is de-condensed chromatin, consists largely of coding sequences that are “poised” for transcription⁶³. The transcriptional activity of the genes in euchromatin is determined by recruitment of transcription factors and other nucleosome-remodeling complexes, which may be either repressors or activators. On the other hand, heterochromatin is a highly compacted chromatin structure where genes are silenced. Heterochromatin is known to define centromeres, facilitating chromosome segregation during mitosis and meiosis. Also, heterochromatin is formed at telomeres, ensuring stability of genome. Histone tail deacetylation and methylation of specific lysine residues recruit heterochromatin-associated proteins (e.g. HP1) which establish DNA methylation and the formation of heterochromatin^{61, 66}.

The nucleosome is also dynamic with post-translational histone modifications, subunit variation, DNA methylation, and small non-coding RNA interaction. All these modifications on nucleosome are interrelated to each other and regulate euchromatin and heterochromatin formation in an orchestrated manner³². In other words, the complexity achieved by combinations of all the different modifications mentioned above provides multidimensional layers to the readout of DNA and results in different patterns of gene expression or silencing from the same genome. Therefore, in order to gain a complete understanding of the epigenetic regulation of gene networks, all the basic paradigms must be incorporated into an integrated picture.

The Histone Code and Subcode Hypothesis: Codifying Gene Activation and/or Silencing

Histones are small, basic proteins that are extremely conserved throughout evolution. The first 20 or so amino acids of histones, known as the histone tail, are very highly conserved,

and they structurally extend from the nucleosomal disk, which allows access of post translational modification enzymes⁵⁴. In the histone tail, serine (S), threonine (T), and tyrosine (Y) residues can undergo phosphorylation, and other residues, such as lysine (K) and arginine (R), can be methylated, acetylated, ubiquitinated, and sumoylated⁷⁶. Furthermore, lysine residues have potential to be mono-, di-, or tri-methylated⁴². These histone modifications are known as “marks” and provide specific docking sites for many chromatin-associated proteins. The Histone Code hypothesis predicts that the type, location, and combination of histone marks determine recruitment of a specific chromatin-associated proteins or transcription factors, and subsequently, determine whether the gene would be expressed or silenced under a particular set of circumstances³².

The histone marks can be created, recognized, and reversed by different proteins: writers, readers, and erasers respectively⁸⁴. The writers include HAT (histone acetyltransferases), histone kinases, and HMT (histone methyltransferases)^{56, 73}. Marks imprinted by writers are recognized by the reader proteins containing domains engineered to recognize particular mark, i.e. chromodomain for methylation and bromodomain for acetylation^{34, 43}. The modifications can also be reversed by eraser proteins, such as HDAC (histone deacetylases), phosphatases, LSD (lysine specific demethylases), and Jumonji domain-containing proteins. The histone subcode hypothesis predicts that some of these chromatin-associated proteins may also be post-translationally modified to create an additional layer of the chromatin code, a subcode, that determines transcriptional activity of the target genes⁵¹. This hypothesis was supported by changes in localization of pan-nuclear HP1 γ , which becomes exclusively euchromatic with phosphorylation of Ser 83 residue, suggesting that p-Ser 83-HP1 γ may adopt different modes for silencing the target gene compared to non-phosphorylated HP1 γ ⁵¹. Together, the histone code and histone subcode hypotheses show how post-translational modifications of different nuclear proteins precipitate differential protein complex recruitment, gene expression, and chromatin dynamics.

Nucleosome Remodeling Machines and Histone Variants

In addition to histone modifications, chromatin structures can be altered by recruitment of nucleosome remodeling complexes. Nucleosome remodeling machines, such as SWI/SNF, NuRD (nucleosome remodeling and deacetylation) and CHRAC (chromatin accessibility complex), can move nucleosomes along DNA in ATP-dependent manner^{47, 77, 88}. Several of these molecular machines have been found to be conserved from yeast to human^{49, 82}. The SWI/SNF family of nucleosome remodeling complexes transiently alters the structure of nucleosome, exposing DNA⁶⁷. Similarly, FACT (facilitates chromatin transcription) initiates nucleosome release from DNA, and allows transcriptional elongation by RNA Pol II⁶⁴. These nucleosome remodeling complexes work in concert with histone modification complexes such as SAGA (Spt-Ada-Gcn5-acetyltransferase) to regulate gene transcription¹⁹.

Other nucleosome remodeling complexes, such as the SWR1 complex, are involved in exchange of histones and histone variants⁵⁷. There are a number of histone variants that may occupy a nucleosome to yield an effect on transcriptional activity²². For instance, histone H3 has three variants in mammals, H3.1, H3.2, and H3.3, which differ from each other by one to four amino acids in the tail⁵². These small differences allow the variants to have unique patterns of modifications and different affinities to the binding factors. Together, the combinatorial effect between histone variants and their participation in the Histone Code, known as the histone “barcode,” introduces another level of complexity to transcriptional regulation²². The mechanisms and timing of histone variant exchange and synthesis, and interrelationship between the chromatin dynamics and nucleosome remodeling is an active area of research.

Sequence Specific Recruitment of Chromatin Associated Protein Complexes

In order for the nucleosome remodeling complexes and histone modification complexes to function properly in regulating gene transcription, they must be recruited to the correct location at the correct time. In this regard, DNA sequences, especially the promoter sequences, play a significant role. Promoter footprinting and Electrophoretic Mobility Shift Assays (EMSAs) were utilized to identify sequence specific transcription factors ³¹. Subsequent investigations on these sequence-specific transcription factors revealed DNA binding domains and transcriptional regulatory domains, indicating that these proteins serve as adaptor proteins between DNA and chromatin-associated protein complexes ⁵⁰. For example, the Sp1/KLF (Kruppel-Like Factor) family was established based on their similarities in the DNA binding domain, which is comprised of three zinc fingers. Some of the members of this transcription factor family were able to compete with each other for the same promoter, and due to the variations in their regulatory domain, promoter occupancy of a specific family member resulted in distinct levels of transcription ⁵⁰. Proteins that interact with transcription factors to promote transcription are called co-activators, while proteins that repress transcription are called co-repressors. These non-histone chromatin proteins function either as co-activators or co-repressors via distinct mechanisms, as mediators of histone modifications, such as acetylation, methylation, and ubiquitination.

DNA Methylation

In addition to the basis of sequence specific recruitment of transcription factors, DNA can also function as a template for modifications. Unlike histone modifications which can occur in great variability, from methylation to ubiquitination, DNA modification is limited to methylation of cytosine residues. DNA methylation was the first type of epigenetic changes to be studied as a mechanism for the inactivation of tumor suppressors. DNA methylation occurs on di-nucleotide CpGs by DNMTs (DNA methyltransferases), and the methylation is enriched at non-coding regions ¹². DNA methylation is closely related to heterochromatin formation and chromatin silencing. DNMTs interact with HP1 and the SUV39H1 histone methyltransferase, which functions to establish tri-methylation of histone H3-K9, and induce heterochromatin formation and gene silencing ^{16, 72}. DNA methylation-mediated gene silencing has physiological significance. First, hypermethylation of one of the two parental alleles of a gene is a process of genomic imprinting that ensures monoallelic expression of genes ⁵. Second, hypermethylation of repetitive genomic sequences prevent chromosomal instability and translocations ³⁵.

DNA methylation has been classified into two types, namely de-novo and maintenance methylation ¹². Human cells utilize three DNA methylases, DNMT1, DNMT3a, and DNMT3b. DNMT3a and 3b function to establish embryonic methylation patterns, while DNMT1 works at the replication forks during DNA replication to maintain the methylation patterns in semi-conservative way. However, DNMT1 appears to be inefficient at maintaining the methylation of many CpG dense regions. Therefore, the de-novo activities of DNMT3a and DNMT3b are also necessary in somatic cells in order to reestablish the methylation patterns ⁶. The mechanism of DNA methylation inheritance is more obvious than many other epigenetic regulators and the mark appears to be very stable. However, the presence of de-novo DNMTs suggests that certain amount of flexibility to this mechanism may also exist.

RNA directed gene silencing

Beside DNA and histone modifications, RNA interference (RNAi) machinery has been linked to post-transcriptional gene silencing and heterochromatin formation. RNAi machinery, including proteins like Dicer, Argonaute, and RdRP (RNA dependent RNA polymerase), breaks down double stranded RNA (dsRNA) into small RNA molecules,

named microRNA (miRNA), to interact and degrade the mRNA of homologous genes and inhibit translation¹³. Studies in *S. Pombe* have shown that mutations of any proteins in the RNAi machinery lead to defects in chromosome segregation due to unstable centromeric heterochromatin^{23, 83}. This evidence suggests the role of miRNA in heterochromatin formation, in addition to post-transcriptional gene silencing. Similar evidence has been found in mammals. For example, mouse embryonic stem cells with depletion in a component of RNAi machinery, Dicer, showed significant decreases in the formation of centromeric heterochromatin and failed to differentiate³³. In human-chicken hybrid cell line, loss of Dicer resulted in abnormal localization of heterochromatin proteins, depletion of H3-K9 methylation, accumulation of abnormal mitotic cells with premature sister chromatid separation, and ultimately cell death¹⁵. Therefore, these studies indicate an active participation of RNAs in chromatin remodeling and gene silencing. In summary, there are multiple layers of regulatory mechanisms that are functionally interrelated for target gene silencing or activation.

4. Pain Research: Opening the Door to Epigenetics

Field of pain is at a great advantage to study the epigenetic pathways. The wealth of information gained from previous molecular studies, microarray studies, and pain genetics provide a robust ground for further epigenetic investigations to come^{38, 45, 58, 81}. First, molecular studies have shown that injury and inflammation induce release of proteins, such as glucocorticoid, nerve growth factor (NGF), and substance P (SP)^{69, 9, 86}. These proteins bind to specific receptors to activate intracellular signal pathways and intra-nuclear transcription regulatory pathways for gene networks. Second, microarray data provides extensive list of target genes to be studied. In combination with the molecular studies, this information allow us to concentrate on exploring epigenetic mechanisms, answering questions like, which complexes appear under which circumstances, how do different complexes or pathways interact with each other, and what are the target gene networks for each mechanism. Third, the data from pain genetics provide an additional list of genes to be studied, and some SNP information can be incorporated into DNA methylation studies. Among pain related genes, opioid receptors are one of a few examples where substantial amount of investigations on transcriptional regulation have been done. Therefore, this opens up a great opportunity for investigators to study the effect of pain on gene transcription. In this section, we will use transcriptional regulation of a opioid receptor (ORPM1) as a model to illustrate how different aspects of epigenetic mechanisms we have discussed in the previous section can come together to regulated transcription of single gene.

Opioid receptors are G protein-coupled receptors, activated in response to opioids. They play critical roles in regulation of the pain experience, stress response, and action of analgesic opioid drugs. Therefore, understanding the expression patterns of these genes during pain state is important for generating strategies for pain management. There are four subtypes of opioid receptors, which are initially determined by their ligand binding characteristics; μ opioid receptors (OPRMs), δ opioid receptors (OPRDs), κ opioid receptors (OPRKs), and nociceptin receptors (ORLs)⁸⁵. Among these receptors, OPRMs have been identified to play a critical role in morphine response, tolerance development, and physical dependence³⁷. Mechanisms of transcriptional regulation of OPRMs have been described in a number of recent studies. As expected, there appears to be several layers of mechanisms that work together (i.e. DNA methylation, histone modification, and miRNA induced repression)^{28, 85}.

A number of sequence specific transcription factors, including Sox18, Sp1, PCBP, CREB, PU.1, AP1, and NF- κ B have been identified to bind promoter region of *ORPM1*, and to alter the transcription activity^{3, 26, 27, 30, 40, 41, 48}. PU.1 was identified to be transcriptional

repressor while others were activators. Most of these transcription factors are known to bind to specific DNA sequence and recruit chromatin-associated protein complexes to induce histone modifications and/or DNA modifications. For example, Sp1 and CREB have been shown to interact with p300 and/or CBP, histone acetyltransferases (HATs), usually resulting in transcriptional activation of the target gene^{65, 80}. Questions addressing how these transcription factors activities are regulated, the existence of competition or cooperativity between transcription factors, and in what circumstances these transcription factors gain access to the target gene promoters remain to be investigated. More importantly, identities of epigenetic regulatory complexes (i.e. histone mark writers, readers, and erasers) that are recruited specifically to the *OPRM1* promoter by these transcription factors are crucial information to further our understanding of epigenetic gene regulations in pain pathways.

Some of the chromatin associated proteins on the *OPRM1* promoter have been identified by ChIP assays²⁸. In this study, the authors identified transcription factor Sp1 binding, recruitment of nucleosome remodeling proteins Brg1 and BAF155, dissociation of transcriptional corepressors HDAC1, HDAC3, mSin3A, and MeCP2 on this promoter, and subsequent activation of transcription during neuronal cell differentiation. Brg1 is the ATPase subunit and BAF155 (Brg1-associated factor, 155kD) is a ubiquitous component of the nucleosome remodeling SWI/SNF complex. Other studies have indicated direct interaction between Brg1 and Sp1 to activate target gene expression⁵⁵. Similar mechanisms of SWI/SNF complex induced recruitment of RNA polII may apply to *OPRM1* regulation. The mSin3A is known to form complex with HDAC proteins to induce histone deacetylation, which usually result in transcriptional repression²⁰. Concordantly, the loss of mSin3A, HDAC1 and HDAC3 from the promoter region resulted in decrease of H3 and H4 acetylation²⁸. Additional changes in histone modification, such as increase in H3K4me2 and decrease in H3K9me2, have been observed with transcriptional activation²⁸. This result indicates involvement of more chromatin associated proteins which have not been identified yet (i.e. MLL complex for H3K4 methylation, G9a and Suv39 H3K9 methyltransferases, and JmJc-domain containing H3K9 demethylases).

In addition to histone modifications, nucleosome remodeling, and transcription factor interactions mentioned above, DNA methylation also plays a critical role in *OPRM1* transcription. First, when the gene was activated by neuronal cell differentiation, the promoter region became de-methylated and MeCP2 (methyl CpG binding protein 2) level decreased²⁸. Following study showed that disruption of MeCP2 expression or addition of an artificial demethylation agent, 5-Aza-C, resulted in activation of *OPRM1* gene, suggesting the DNA methylation on the promoter region was actively repressing the gene²⁹. Second, a SNP in *OPRM1* (A118G, rs1799971) was shown to introduce new CpG-methylation site, resulting in enhanced methylation of *OPRM1* DNA, which leads to reduced expression of the protein^{2, 62}. This result is a good example showing how genetic studies can be re-evaluated from the epigenetics point of view. Many of the proteins introduced so far in relation to *OPRM1* transcription are rather large and work in multi-component complexes. How all these complexes and proteins can come together in restricted space around the promoter region (~800bp) is an intriguing question to be investigated.

Two miRNA species have been identified for regulation of *Oprm* transcription. First, miRNA23b, cloned from mouse brain cortex and hippocampus, was found to interact with 3'UTR of *Oprm* mRNA to decrease the polysome-mRNA association rate, and to ultimately result in decreased translation^{10, 46, 87}. Second, let-7 family miRNAs, which includes nine distinct, mature 22-nucleotide sequences with only one to four nucleotide differences from the canonical let-7a, were also found to down regulate translation by interacting with 3'UTR of *Oprm* mRNA²⁴. Questions like under what circumstances these miRNAs become active,

do these miRNAs interact with any other mRNAs, and is there interaction between these two miRNA mechanisms, remain to be investigated.

As we have reviewed here, evidence for epigenetic regulation of pain pathways is emerging from all directions. Transcription of the *OPRM1* is regulated by histone modifications, nucleosome remodeling, sequence specific transcription factor binding, DNA methylation, and miRNA interactions, all at the same time. So far, the studies presented here have investigated each individual aspect of epigenetic mechanisms in isolation. With all the information we gained from individual studies, the importance of understanding how all of the mechanisms are orchestrated in a cell in response to injury or pain is apparent.

4. Epigenetic mechanisms and interventions in pain management

Important advantage for bringing epigenetic drugs into therapeutics is in the reversible nature of epigenetic pathways⁷⁸. Unlike genetic mutations, if a pain phenotype is caused by epigenetic phenomena, such as DNA methylation or histone modifications, they can be chemically reversed. Such involvements of epigenetic mechanisms in pain have been already implicated and accordingly some therapeutic strategies for pain management have been suggested^{4, 7, 18}. However, as implicated above with the opioid receptor, transcription is regulated by complex multi-layered pathways which involve proteins with multiple target genes and substrate. Therefore, understanding the epigenetic pathway is critical to apply epigenetic drugs for intervention. In this section, we will briefly review some of the available epigenetic drugs and how they may be applicable for pain management.

Histone deacetylase inhibitors (HDACis) have been evaluated as therapeutic agents in several conditions including cancer, autism, neuropathic and inflammatory pain^{8, 68, 70}. HDACis induce increase in acetylated histones by blocking histone deacetylase activity of HDAC proteins. In general, histone acetylation has been associated with transcriptional activation. Recent study has investigated the potential of HDACis as analgesic drugs. Based on studies reporting the upregulation of mGlu2 in DRG can induce analgesia and transcription of mGlu2 is regulated by histone acetylation, the authors tested two HDACis, MS-275 (benzamine derivative) and SAHA (suberoylanilide hydroamic acid) in a mouse model of persistent inflammatory pain⁷. With 5-day treatment, both HDACis showed analgesic effect on the mice and increased mGlu2 expression in DRG without changing the expression of mGlu1a, mGlu4, or mGlu5 receptors. Another study showed that treatment with a HDACi, Trichostatin A, in a mouse model of endometriosis induced transient decrease in the capsaicin activated type-1 cation channel (TRPV1) and significant improvement in response to a noxious thermal stimulus, suggesting its role as an analgesic drug⁵³.

While these evidences appear promising for practical applications, with current knowledge, clinical use of these agents is still limited. As we have previously discussed, HDAC proteins have multiple target genes. Therefore, it is evident that HDACis will not only inhibit deacetylation of desired chromatin, but also that of other chromatin which may result in severe side effect. Drugs that affect DNA methyltransferase activities, such as glucosamine and valproic acid, would present similar specificity problem. Thus, in order to understand the mechanism and regard possible application of these drugs, changes in transcription of all genes, rather than a single target gene, need to be considered. Development of more specific HDACis for each HDAC family members, and perhaps, combined with gene therapy, development of a target gene specific HDACis will be extremely beneficial for therapeutics.

The siRNAs, as possible epigenetic drugs, stand on a slightly different ground from other epigenetic interventions. Unlike HDACis, siRNA sequence can be designed for a specific target gene. A number of siRNA treatments, targeting NR1 and NR2 subunits of NMDA

receptors, RTPV1 channels, BDNF, and other pain related genes, in animal models have been successful for alleviating pain^{17, 21, 36, 79}. Some of these may become one of the first epigenetic drugs that become applicable for clinical trials in pain management.

5. Concluding remarks

Epigenetic regulation of gene expression is a rapidly growing area of research. As epigenetic mechanisms, including DNA methylation, histone modifications, RNAi, and chromatin dynamics, are theoretically reversible, there exists considerable confidence as to therapeutic possibilities. The fundamental concepts and general principles described in this review are only a brief summary of what is known today. Our goal is to expose the readers to these concepts so that pain-related phenotypes can be investigated from the epigenetic point of view.

Many fundamental questions connecting the concepts of epigenetics and pain remain to be answered. For example, in the innervating nociceptive neurons, which epigenetic pathways are activated or repressed during injury and inflammation? What is the epigenetic basis of peripheral sensitization and pain centralization (e.g. hyperalgesia and allodynia)? How does inflammation at the end of a periphery neuron affect gene expression in the connected neurons of the central nervous system? What is the epigenetic basis of neuropathic pain? How do the experience, stress, and memory of pain affect patterns of gene expression in the brain, and how can they be reversed? Do emotional pain and physical pain differ or coincide in terms of changes in gene expression? Does pain relief follow the same epigenetic pathways that generate pain? The questions regarding the epigenetics of pain range from basic molecular biology to psychology and even to philosophy. We anticipate that investigations in this field of research will reveal new paradigms for understanding the pathology of pain, and for developing better pain management strategies.

References

1. Beyer A, Koch T, Schroder H, Schulz S, Holtt V. Effect of the $\alpha 118g$ polymorphism on binding affinity, potency and agonist-mediated endocytosis, desensitization, and resensitization of the human μ -opioid receptor. *J Neurochem.* 2004; 89:553–560. [PubMed: 15086512]
2. Bond C, LaForge KS, Tian M, Melia D, Zhang S, Borg L, Gong J, Schluger J, Strong JA, Leal SM, Tischfield JA, Kreek MJ, Yu L. Single-nucleotide polymorphism in the human μ opioid receptor gene alters beta-endorphin binding and activity: Possible implications for opiate addiction. *Proc Natl Acad Sci U S A.* 1998; 95:9608–9613. [PubMed: 9689128]
3. Borner C, Holtt V, Kraus J. Involvement of activator protein-1 in transcriptional regulation of the human μ -opioid receptor gene. *Mol Pharmacol.* 2002; 61:800–805. [PubMed: 11901219]
4. Buchheit T, Van de Ven T, Shaw A. Epigenetics and the transition from acute to chronic pain. *Pain Med.* 13:1474–1490. [PubMed: 22978429]
5. Caspary T, Cleary MA, Baker CC, Guan XJ, Tilghman SM. Multiple mechanisms regulate imprinting of the mouse distal chromosome 7 gene cluster. *Mol Cell Biol.* 1998; 18:3466–3474. [PubMed: 9584186]
6. Cheng X, Blumenthal RM. Mammalian DNA methyltransferases: A structural perspective. *Structure.* 2008; 16:341–350. [PubMed: 18334209]
7. Chiechio S, Zammataro M, Morales ME, Busceti CL, Drago F, Gereau RWt, Copani A, Nicoletti F. Epigenetic modulation of $mglu2$ receptors by histone deacetylase inhibitors in the treatment of inflammatory pain. *Mol Pharmacol.* 2009; 75:1014–1020. [PubMed: 19255242]
8. Doebering A, Geisslinger G, Lotsch J. Epigenetics in pain and analgesia: An imminent research field. *Eur J Pain.* 15:11–16. [PubMed: 20584621]
9. Donnerer J, Schuligoi R, Stein C. Increased content and transport of substance p and calcitonin gene-related peptide in sensory nerves innervating inflamed tissue: Evidence for a regulatory function of nerve growth factor in vivo. *Neuroscience.* 1992; 49:693–698. [PubMed: 1380138]

10. Dostie J, Mourelatos Z, Yang M, Sharma A, Dreyfuss G. Numerous micrnps in neuronal cells containing novel micrnas. *RNA*. 2003; 9:180–186. [PubMed: 12554860]
11. Elmer GI, Pieper JO, Negus SS, Woods JH. Genetic variance in nociception and its relationship to the potency of morphine-induced analgesia in thermal and chemical tests. *Pain*. 1998; 75:129–140. [PubMed: 9539682]
12. Esteller M. Epigenetics in cancer. *N Engl J Med*. 2008; 358:1148–1159. [PubMed: 18337604]
13. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded rna in caenorhabditis elegans. *Nature*. 1998; 391:806–811. [PubMed: 9486653]
14. Flores CM, Mogil JS. The pharmacogenetics of analgesia: Toward a genetically-based approach to pain management. *Pharmacogenomics*. 2001; 2:177–194. [PubMed: 11535108]
15. Fukagawa T, Nogami M, Yoshikawa M, Ikeno M, Okazaki T, Takami Y, Nakayama T, Oshimura M. Dicer is essential for formation of the heterochromatin structure in vertebrate cells. *Nat Cell Biol*. 2004; 6:784–791. [PubMed: 15247924]
16. Fuks F, Hurd PJ, Deplus R, Kouzarides T. The DNA methyltransferases associate with hp1 and the suv39h1 histone methyltransferase. *Nucleic Acids Res*. 2003; 31:2305–2312. [PubMed: 12711675]
17. Garraway SM, Xu Q, Inturrisi CE. Design and evaluation of small interfering rnas that target expression of the n-methyl-d-aspartate receptor nr1 subunit gene in the spinal cord dorsal horn. *J Pharmacol Exp Ther*. 2007; 322:982–988. [PubMed: 17551091]
18. Geranton SM. Targeting epigenetic mechanisms for pain relief. *Curr Opin Pharmacol*. 12:35–41. [PubMed: 22056026]
19. Grant PA, Schieltz D, Pray-Grant MG, Steger DJ, Reese JC, Yates JR 3rd, Workman JL. A subset of taf(ii)s are integral components of the saga complex required for nucleosome acetylation and transcriptional stimulation. *Cell*. 1998; 94:45–53. [PubMed: 9674426]
20. Grzenda A, Lomberk G, Zhang JS, Urrutia R. Sin3: Master scaffold and transcriptional corepressor. *Biochim Biophys Acta*. 2009; 1789:443–450. [PubMed: 19505602]
21. Guo W, Robbins MT, Wei F, Zou S, Dubner R, Ren K. Supraspinal brain-derived neurotrophic factor signaling: A novel mechanism for descending pain facilitation. *J Neurosci*. 2006; 26:126–137. [PubMed: 16399679]
22. Hake SB, Allis CD. Histone h3 variants and their potential role in indexing mammalian genomes: The “H3 barcode hypothesis”. *Proc Natl Acad Sci U S A*. 2006; 103:6428–6435. [PubMed: 16571659]
23. Hall IM, Shankaranarayana GD, Noma K, Ayoub N, Cohen A, Grewal SI. Establishment and maintenance of a heterochromatin domain. *Science*. 2002; 297:2232–2237. [PubMed: 12215653]
24. He Y, Yang C, Kirkmire CM, Wang ZJ. Regulation of opioid tolerance by let-7 family microRNA targeting the mu opioid receptor. *J Neurosci*. 30:10251–10258. [PubMed: 20668208]
25. Hjermstad MJ, Fayers PM, Haugen DF, Caraceni A, Hanks GW, Loge JH, Fainsinger R, Aass N, Kaasa S. Studies comparing numerical rating scales, verbal rating scales, and visual analogue scales for assessment of pain intensity in adults: A systematic literature review. *J Pain Symptom Manage*. 41:1073–1093. [PubMed: 21621130]
26. Hwang CK, Wu X, Wang G, Kim CS, Loh HH. Mouse mu opioid receptor distal promoter transcriptional regulation by sox proteins. *J Biol Chem*. 2003; 278:3742–3750. [PubMed: 12446692]
27. Hwang CK, Kim CS, Choi HS, McKercher SR, Loh HH. Transcriptional regulation of mouse mu opioid receptor gene by pu.1. *J Biol Chem*. 2004; 279:19764–19774. [PubMed: 14998994]
28. Hwang CK, Kim CS, Kim do K, Law PY, Wei LN, Loh HH. Up-regulation of the mu-opioid receptor gene is mediated through chromatin remodeling and transcriptional factors in differentiated neuronal cells. *Mol Pharmacol*. 2010; 78:58–68. [PubMed: 20385708]
29. Hwang CK, Song KY, Kim CS, Choi HS, Guo XH, Law PY, Wei LN, Loh HH. Epigenetic programming of mu-opioid receptor gene in mouse brain is regulated by mecp2 and brg1 chromatin remodelling factor. *J Cell Mol Med*. 2009; 13:3591–3615. [PubMed: 19602036]

30. Im HJ, Smirnov D, Yuhi T, Raghavan S, Olsson JE, Muscat GE, Koopman P, Loh HH. Transcriptional modulation of mouse mu-opioid receptor distal promoter activity by sox18. *Mol Pharmacol*. 2001; 59:1486–1496. [PubMed: 11353810]
31. Istrail S, Davidson EH. Logic functions of the genomic cis-regulatory code. *Proc Natl Acad Sci U S A*. 2005; 102:4954–4959. [PubMed: 15788531]
32. Jenuwein T, Allis CD. Translating the histone code. *Science*. 2001; 293:1074–1080. [PubMed: 11498575]
33. Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, Livingston DM, Rajewsky K. Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. *Genes Dev*. 2005; 19:489–501. [PubMed: 15713842]
34. Kanno T, Kanno Y, Siegel RM, Jang MK, Lenardo MJ, Ozato K. Selective recognition of acetylated histones by bromodomain proteins visualized in living cells. *Mol Cell*. 2004; 13:33–43. [PubMed: 14731392]
35. Karpf AR, Matsui S. Genetic disruption of cytosine DNA methyltransferase enzymes induces chromosomal instability in human cancer cells. *Cancer Res*. 2005; 65:8635–8639. [PubMed: 16204030]
36. Kasama S, Kawakubo M, Suzuki T, Nishizawa T, Ishida A, Nakayama J. Rna interference-mediated knock-down of transient receptor potential vanilloid 1 prevents forepaw inflammatory hyperalgesia in rat. *Eur J Neurosci*. 2007; 25:2956–2963. [PubMed: 17509082]
37. Kieffer BL, Evans CJ. Opioid tolerance-in search of the holy grail. *Cell*. 2002; 108:587–590. [PubMed: 11893329]
38. Kim H, Clark D, Dionne RA. Genetic contributions to clinical pain and analgesia: Avoiding pitfalls in genetic research. *J Pain*. 2009; 10:663–693. [PubMed: 19559388]
39. Kim H, Neubert JK, San Miguel A, Xu K, Krishnaraju RK, Iadarola MJ, Goldman D, Dionne RA. Genetic influence on variability in human acute experimental pain sensitivity associated with gender, ethnicity and psychological temperament. *Pain*. 2004; 109:488–496. [PubMed: 15157710]
40. Kim SS, Pandey KK, Choi HS, Kim SY, Law PY, Wei LN, Loh HH. Poly(c) binding protein family is a transcription factor in mu-opioid receptor gene expression. *Mol Pharmacol*. 2005; 68:729–736. [PubMed: 15933215]
41. Kraus J, Borner C, Giannini E, Holtt V. The role of nuclear factor kappaB in tumor necrosis factor-regulated transcription of the human mu-opioid receptor gene. *Mol Pharmacol*. 2003; 64:876–884. [PubMed: 14500744]
42. Kustatscher G, Ladurner AG. Modular paths to ‘decoding’ and ‘wiping’ histone lysine methylation. *Curr Opin Chem Biol*. 2007; 11:628–635. [PubMed: 17988933]
43. Lachner M, O’Carroll D, Rea S, Mechtler K, Jenuwein T. Methylation of histone h3 lysine 9 creates a binding site for hp1 proteins. *Nature*. 2001; 410:116–120. [PubMed: 11242053]
44. Lacroix-Fralish ML, Mogil JS. Progress in genetic studies of pain and analgesia. *Annu Rev Pharmacol Toxicol*. 2009; 49:97–121. [PubMed: 18834308]
45. LaCroix-Fralish ML, Austin JS, Zheng FY, Levitin DJ, Mogil JS. Patterns of pain: Meta-analysis of microarray studies of pain. *Pain*. 152:1888–1898. [PubMed: 21561713]
46. Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M, Lin C, Socci ND, Hermida L, Fulci V, Chiaretti S, Foa R, Schliwka J, Fuchs U, Novosel A, Muller RU, Schermer B, Bissels U, Inman J, Phan Q, Chien M, Weir DB, Choksi R, De Vita G, Frezzetti D, Trompeter HI, Hornung V, Teng G, Hartmann G, Palkovits M, Di Lauro R, Wernet P, Macino G, Rogler CE, Nagle JW, Ju J, Papavasiliou FN, Benzing T, Lichter P, Tam W, Brownstein MJ, Bosio A, Borkhardt A, Russo JJ, Sander C, Zavolan M, Tuschl T. A mammalian microRNA expression atlas based on small rna library sequencing. *Cell*. 2007; 129:1401–1414. [PubMed: 17604727]
47. Langst G, Bonte EJ, Corona DF, Becker PB. Nucleosome movement by chrac and iswi without disruption or trans-displacement of the histone octamer. *Cell*. 1999; 97:843–852. [PubMed: 10399913]
48. Lee PW, Lee YM. Transcriptional regulation of mu opioid receptor gene by camp pathway. *Mol Pharmacol*. 2003; 64:1410–1418. [PubMed: 14645671]

49. LeRoy G, Orphanides G, Lane WS, Reinberg D. Requirement of rsf and fact for transcription of chromatin templates in vitro. *Science*. 1998; 282:1900–1904. [PubMed: 9836642]
50. Lomberg G, Urrutia R. The family feud: Turning off sp1 by sp1-like klf proteins. *Biochem J*. 2005; 392:1–11. [PubMed: 16266294]
51. Lomberg G, Bensi D, Fernandez-Zapico ME, Urrutia R. Evidence for the existence of an hp1-mediated subcode within the histone code. *Nat Cell Biol*. 2006; 8:407–415. [PubMed: 16531993]
52. Loyola A, Almouzni G. Marking histone h3 variants: How, when and why? *Trends Biochem Sci*. 2007; 32:425–433. [PubMed: 17764953]
53. Lu Y, Nie J, Liu X, Zheng Y, Guo SW. Trichostatin a, a histone deacetylase inhibitor, reduces lesion growth and hyperalgesia in experimentally induced endometriosis in mice. *Hum Reprod*. 25:1014–1025. [PubMed: 20118114]
54. Luger K, Richmond TJ. The histone tails of the nucleosome. *Curr Opin Genet Dev*. 1998; 8:140–146. [PubMed: 9610403]
55. Ma Z, Chang MJ, Shah R, Adamski J, Zhao X, Benveniste EN. Brg-1 is required for maximal transcription of the human matrix metalloproteinase-2 gene. *J Biol Chem*. 2004; 279:46326–46334. [PubMed: 15317818]
56. Marmorstein R, Trievel RC. Histone modifying enzymes: Structures, mechanisms, and specificities. *Biochim Biophys Acta*. 2009; 1789:58–68. [PubMed: 18722564]
57. Mizuguchi G, Shen X, Landry J, Wu WH, Sen S, Wu C. Atp-driven exchange of histone h2az variant catalyzed by swr1 chromatin remodeling complex. *Science*. 2004; 303:343–348. [PubMed: 14645854]
58. Mogil JS. Pain genetics: Past, present and future. *Trends Genet*. 28:258–266. [PubMed: 22464640]
59. Mogil JS, Kest B, Sadowski B, Belknap JK. Differential genetic mediation of sensitivity to morphine in genetic models of opiate antinociception: Influence of nociceptive assay. *J Pharmacol Exp Ther*. 1996; 276:532–544. [PubMed: 8632319]
60. Muralidharan A, Smith MT. Pain, analgesia and genetics. *J Pharm Pharmacol*. 63:1387–1400. [PubMed: 21988420]
61. Nakayama J, Rice JC, Strahl BD, Allis CD, Grewal SI. Role of histone h3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science*. 2001; 292:110–113. [PubMed: 11283354]
62. Oertel BG, Doebering A, Roskam B, Kettner M, Hackmann N, Ferreira N, Schmidt PH, Lotsch J. Genetic-epigenetic interaction modulates mu-opioid receptor regulation. *Hum Mol Genet*. 21:4751–4760. [PubMed: 22875838]
63. Orphanides G, Reinberg D. A unified theory of gene expression. *Cell*. 2002; 108:439–451. [PubMed: 11909516]
64. Orphanides G, LeRoy G, Chang CH, Luse DS, Reinberg D. Fact, a factor that facilitates transcript elongation through nucleosomes. *Cell*. 1998; 92:105–116. [PubMed: 9489704]
65. Owen GI, Richer JK, Tung L, Takimoto G, Horwitz KB. Progesterone regulates transcription of the p21(waf1) cyclin- dependent kinase inhibitor gene through sp1 and cbp/p300. *J Biol Chem*. 1998; 273:10696–10701. [PubMed: 9553133]
66. Peters AH, Mermoud JE, O'Carroll D, Pagani M, Schweizer D, Brockdorff N, Jenuwein T. Histone h3 lysine 9 methylation is an epigenetic imprint of facultative heterochromatin. *Nat Genet*. 2002; 30:77–80. [PubMed: 11740497]
67. Peterson CL, Workman JL. Promoter targeting and chromatin remodeling by the swi/snf complex. *Curr Opin Genet Dev*. 2000; 10:187–192. [PubMed: 10753786]
68. Rodriguez-Menendez V, Tremolizzo L, Cavaletti G. Targeting cancer and neuropathy with histone deacetylase inhibitors: Two birds with one stone? *Curr Cancer Drug Targets*. 2008; 8:266–274. [PubMed: 18537550]
69. Sahbaie P, Shi X, Guo TZ, Qiao Y, Yeomans DC, Kingery WS, Clark JD. Role of substance p signaling in enhanced nociceptive sensitization and local cytokine production after incision. *Pain*. 2009; 145:341–349. [PubMed: 19660865]
70. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. *Carcinogenesis*. 31:27–36. [PubMed: 19752007]

71. Sibille KT, Witek-Janusek L, Mathews HL, Fillingim RB. Telomeres and epigenetics: Potential relevance to chronic pain. *Pain*. 153:1789–1793. [PubMed: 22770844]
72. Smallwood A, Esteve PO, Pradhan S, Carey M. Functional cooperation between hp1 and dnmt1 mediates gene silencing. *Genes Dev*. 2007; 21:1169–1178. [PubMed: 17470536]
73. Smith BC, Denu JM. Chemical mechanisms of histone lysine and arginine modifications. *Biochim Biophys Acta*. 2009; 1789:45–57. [PubMed: 18603028]
74. Smith MT, Muralidharan A. Pharmacogenetics of pain and analgesia. *Clin Genet*. 82:321–330. [PubMed: 22779698]
75. Somogyi AA, Barratt DT, Collier JK. Pharmacogenetics of opioids. *Clin Pharmacol Ther*. 2007; 81:429–444. [PubMed: 17339873]
76. Strahl BD, Allis CD. The language of covalent histone modifications. *Nature*. 2000; 403:41–45. [PubMed: 10638745]
77. Sudarsanam P, Winston F. The swi/snf family nucleosome-remodeling complexes and transcriptional control. *Trends Genet*. 2000; 16:345–351. [PubMed: 10904263]
78. Szyf M. Epigenetics, DNA methylation, and chromatin modifying drugs. *Annu Rev Pharmacol Toxicol*. 2009; 49:243–263. [PubMed: 18851683]
79. Tan PH, Yang LC, Shih HC, Lan KC, Cheng JT. Gene knockdown with intrathecal sirna of nmda receptor nr2b subunit reduces formalin-induced nociception in the rat. *Gene Ther*. 2005; 12:59–66. [PubMed: 15470478]
80. Thompson PR, Kurooka H, Nakatani Y, Cole PA. Transcriptional coactivator protein p300. Kinetic characterization of its histone acetyltransferase activity. *J Biol Chem*. 2001; 276:33721–33729. [PubMed: 11445580]
81. Tremblay J, Hamet P. Genetics of pain, opioids, and opioid responsiveness. *Metabolism*. 59(Suppl 1):S5–8. [PubMed: 20837195]
82. Tsukiyama T, Palmer J, Landel CC, Shiloach J, Wu C. Characterization of the imitation switch subfamily of atp-dependent chromatin-remodeling factors in *saccharomyces cerevisiae*. *Genes Dev*. 1999; 13:686–697. [PubMed: 10090725]
83. Volpe TA, Kidner C, Hall IM, Teng G, Grewal SI, Martienssen RA. Regulation of heterochromatic silencing and histone h3 lysine-9 methylation by rna. *Science*. 2002; 297:1833–1837. [PubMed: 12193640]
84. Wang Y, Fischle W, Cheung W, Jacobs S, Khorasanizadeh S, Allis CD. Beyond the double helix: Writing and reading the histone code. *Novartis Found Symp*. 2004; 259:3–17. discussion 17–21, 163–169. [PubMed: 15171244]
85. Wei LN, Loh HH. Transcriptional and epigenetic regulation of opioid receptor genes: Present and future. *Annu Rev Pharmacol Toxicol*. 51:75–97. [PubMed: 20868272]
86. Woolf CJ, Safieh-Garabedian B, Ma QP, Crilly P, Winter J. Nerve growth factor contributes to the generation of inflammatory sensory hypersensitivity. *Neuroscience*. 1994; 62:327–331. [PubMed: 7530342]
87. Wu Q, Law PY, Wei LN, Loh HH. Post-transcriptional regulation of mouse mu opioid receptor (mor1) via its 3' untranslated region: A role for microRNA23b. *FASEB J*. 2008; 22:4085–4095. [PubMed: 18716031]
88. Xue Y, Wong J, Moreno GT, Young MK, Cote J, Wang W. Nurd, a novel complex with both atp-dependent chromatin-remodeling and histone deacetylase activities. *Mol Cell*. 1998; 2:851–861. [PubMed: 9885572]
89. Zubietta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu Y, Koeppe RA, Stohler CS, Goldman D. Comt val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. *Science*. 2003; 299:1240–1243. [PubMed: 12595695]

Perspective

This article provides a brief overview of epigenetic pathways for gene regulation and highlights their involvement in pain. Our goal is to expose the readers to these concepts so that pain-related phenotypes can be investigated from the epigenetic point of view.