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Review

Chronic Pain: Emerging Evidence for the Involvement of Epigenetics

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Epigenetic processes, such as histone modifications and DNA methylation, have been associated with many neural functions including synaptic plasticity, learning, and memory. Here, we critically examine emerging evidence linking epigenetic mechanisms to the development or maintenance of chronic pain states. Although in its infancy, research in this area potentially unifies several pathophysiological processes underpinning abnormal pain processing and opens up a different avenue for the development of novel analgesics.

Introduction

Chronic pain is a major public health problem, with epidemiological studies reporting about one fifth of the general population to be affected both in the USA and Europe (Breivik et al., 2006). The condition is debilitating and causes not only considerable personal suffering but also enormous socioeconomic costs, estimated to reach an annual 60 billion U.S. dollars in lost productivity. It is a figure that only stands to increase with the aging populations of the Western world (Krueger and Stone, 2008).

In addition to these bleak statistics, pharmacological management of chronic pain conditions has seen only limited progress in the last decades. Despite the seemingly bewildering array of nonprescription analgesics being advertised and sold in dedicated drugstore aisles, treatment of pain is still very much dominated by two classical medications: opioids and nonsteroidal anti-inflammatory drugs. Only a handful of compounds acting on novel, distinct molecular targets have emerged since the 1960s, for instance gabapentinoids, TprVI agonists, or cannabinoids (Kissin, 2010). Many of these painkillers have serious side effects, such as neurotoxicity (e.g., TrpVI agonists) and addictive properties (e.g., opioids and cannabinoids). More importantly, many chronic conditions, such as neuropathic pain, still cannot be effectively treated in the majority of patients, at least not over sufficiently long periods of time.

Meanwhile basic science has made good progress over the years, and key neurobiological mechanisms central to the generation of chronic pain have been identified. Here we will initially outline several of these mechanisms where, as we go on to describe, there is emerging evidence for an important controlling role of epigenetic processes.

Pain Mechanisms

Sensitization of the pain signaling system is a key process in chronic pain states. Such sensitization, and also tonic activation, can be induced by mediators generated and released at different levels of the neuroaxis (Figure 1). One important source of such mediators is peripheral tissue affected by injury or disease, since local anesthetic treatment of these tissues gives at least temporary relief to most chronic pain patients (e.g., Rowbotham et al., 1996). The cellular source of these peripheral mediators is not for the most part known, but considerable preclinical and more limited clinical evidence suggests that immune cells play a pivotal role. Thus both resident cells (including mast cells, dendritic cells, and resident macrophages) and recruited cells (most prominently circulating macrophages, neutrophils, and T cells) are known to be the source of proalgesic factors including prostanoids, the cytokines TNFα and IL-1β, nerve growth factor (NGF), and a number of chemokines including CCL2, CCL3, and CXCL5 (Binshtok et al., 2008; Dawes et al., 2011; Rittner et al., 2005; Verri et al., 2006; Zhang et al., 2005). The importance of immune cells has been tested with strategies to reduce their total number, their recruitment, or their activation, and while these techniques are probably often suboptimal, they have produced clear evidence for the role of different cell types. Thus, stabilizing mast cells with compound 48/80 (Ribeiro et al., 2000), reducing chemotaxis of neutrophils (Ting et al., 2008), depleting circulating macrophages with clondronate (Barclay et al., 2007), and using T cell-deficient mice (Kleinschnitz et al., 2006) all reduce pain-related behavior in a variety of models. Interestingly, these studies did not just examine inflammatory pain models (e.g., following zymosan or carrageenan administration) but also neuropathic ones, such as peripheral nerve ligation. Indeed, nerve injury is almost always associated with a strong immune response—a fact neglected in the literature, which tends to focus on the consequences of neuronal damage.

Once peripheral pain mediators have been released as just described, they activate and sensitize the terminals of nociceptors, making them spontaneously active and more readily activated. The detailed molecular mechanisms underlying this process are still being unravelled (Basbaum et al., 2009). One cardinal route seems to be altered neurotrophic support, in the form of increased factors such as NGF from inflamed tissues, which will act on a subpopulation of DRG neurons to effect release of further neurotrophins such as BDNF (Malcangio et al., 2000; Fukuoka et al., 2001).

In line with all this evidence, anti-inflammatory drugs such as steroids are effective in reducing pain in many circumstances, especially when applied locally (Wong et al., 2010). However, their adverse side effects such as weight gain, high blood pressure, and increased risk of osteoporosis or diabetes render them unsuitable for long-term analgesic therapy. Alternative strategies

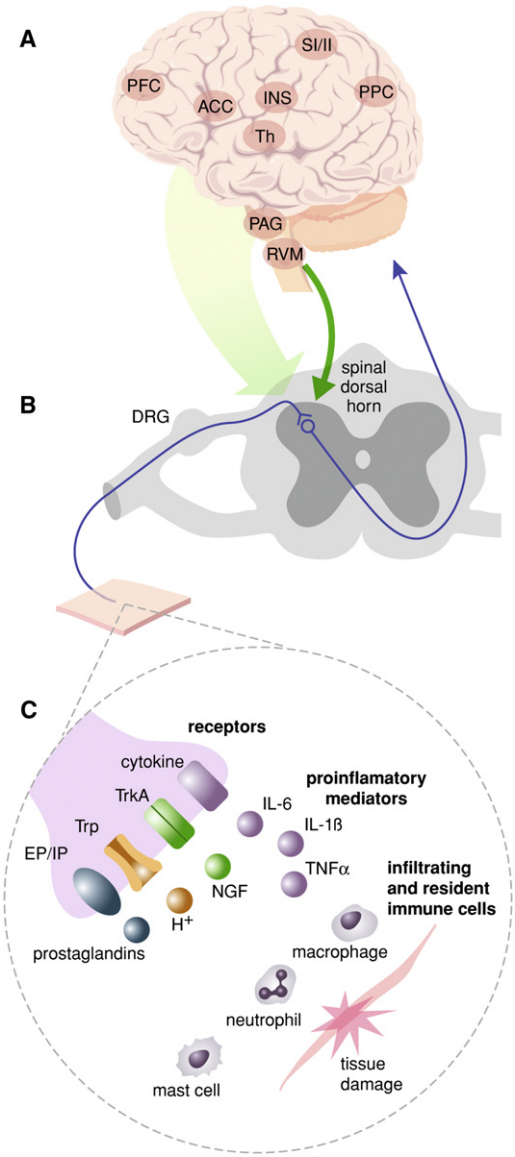

Figure 1. Schematic Representation of the Key Processes Thought to Underlie Chronic Pain States

(A) Changes in brain function: a network of cortical and subcortical areas is involved in processing nociceptive signals and the sensation of pain (among others: prefrontal cortex [PFC], sensory motor cortices [SI/SII], posterior parietal cortex [PPC], anterior cingulate cortex [ACC], insula [INS], thalamus [Th], periaqueductal gray [PAG], and rostral ventromedial medulla [RVM]). In chronic pain patients, many of these display profound changes in fMRI bold signal, interconnectivity, and top-down modulation of ascending spinal signals.

(B) Abnormal amplification of pain signals in DRG and spinal cord neurons: sensory neurons display hyperexcitability as a result of altered neurotrophic support and extensive changes in the expression of relevant genes, most notably ion channels and nociceptors. Second-order cells exhibit central sensitization as a result of several processes including immune and glial cell recruitment in the CNS.

(C) Peripheral inflammation and sensitization of nociceptors: tissue damage activates and recruits immune cells (e.g., mast cells, macrophages and neutrophils). These cells will release or stimulate the production of a variety of cytokines (e.g., IL-6, IL-1β, TNFα) and proinflammatory mediators (e.g., NGF and prostaglandins). This will activate or modulate the action of receptors on the sensory nerve terminals (e.g., the TrkA, cytokine, and prostaglandin receptors [EP/IP] are activated and Trp channels can be modulated). This process will result in sensitization of the nociceptive neuron.

which modulate the inflammatory response, and particularly the proalgesic components, would therefore be of considerable potential benefit in the treatment of chronic pain.

A second locus for amplification of pain-related signals occurs within the central nervous system, a process called central sensitization by analogy with its peripheral counterpart. The best studied forms are in the spinal cord, where projection neurons that carry sensory information to the brain become more responsive to both noxious and innocuous inputs. Central sensitization is a form of synaptic plasticity and is precipitated by repetitive activity in nociceptors and depends critically on recruitment of NMDA receptors (Dickenson and Sullivan, 1987; Woolf, 2011). However, multiple mechanisms appear to participate, including factors released from nonneuronal and immune cells in the spinal cord (Clark and Malcangio, 2011; Guo and Schluesener, 2007; Marchand et al., 2005). It seems likely that analogous forms of synaptic plasticity will operate at other CNS sites involved in pain processing.

Another feature of persistent pain states is dramatically altered gene expression in nociceptors, with at least 10% of the transcriptome being dysregulated in traumatic injury models of neuropathic pain. The change appears to affect a very broad range of genes: the receptors expressed by nociceptors (e.g., TrpV1, TrpA1, GABA-B1, 5-HT_{3A}), ion channels regulating nociceptor excitability (e.g., $Na_V1.8$), and transmitters and modulators released centrally (e.g., substance P, BDNF, neuropeptide Y) all display abnormal expression levels (e.g., see Lacroix-Fralish et al., 2006; Maratou et al., 2009; Lacroix-Fralish et al., 2011 for meta-analysis).

Research has started to examine the functional role of some of these genes more specifically. For instance, the reduced expression of the μ-opioid receptor in neuropathic conditions appears to contribute to the limited efficacy of opiates in these states (Lee et al., 2011; Porreca et al., 1998). Similarly, the increased expression (and activity-dependent release) of BDNF has been proposed to drive some of the central hyperexcitability seen in inflammatory conditions (Pezet et al., 2002). And, the altered expression of particular potassium channel subunits appears to contribute to nociceptor hyperexcitability (Chien et al., 2007), as does the downregulation of postsynaptic genes, such as GAD65, GAD67, and GABA-B1 in spinal inhibitory systems (Meisner et al., 2010; Wang et al., 2011). Nonetheless, we currently have a fragmentary understanding of the reasons for and coordination behind the extensive amount of transcriptional change.

In addition to peripheral and spinal mechanisms, fMRI studies of the past several years have uncovered a rather dramatic change in higher brain function in chronic pain patients. These experiments have shown an alteration in the cortical representation of somatotopic areas generating pain, a shift in their connectivity, and dynamic changes in gray and white matter density (Apkarian et al., 2004; Tracey, 2011; Tracey and Mantyh, 2007; Seminowicz et al., 2011). There is also evidence suggesting that the brains of chronic pain patients exert altered descending control on the spinal cord (Brooks and Tracey, 2005), and this is supported by preclinical work (De Felice et al., 2011). The cause

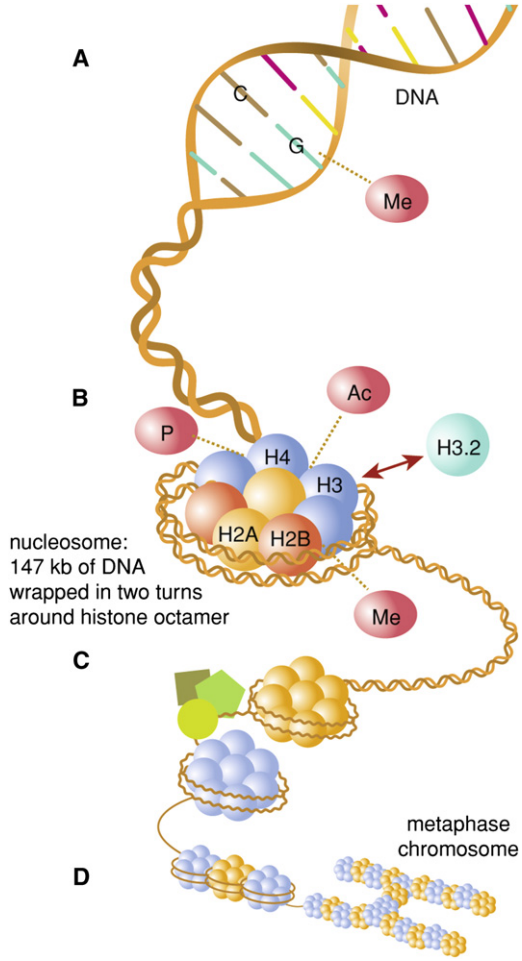

Figure 2. Schematic Representation of Epigenetic Processes and General Chromatin Structure

(A) DNA methylation was traditionally considered mainly in the context of CpG islands and is known to have silencing functions (e.g., silencing of the inactive X chromosome). More recent evidence indicates that methylation can also occur in non-CpG context, may sometimes increase transcription, and is highly variable between tissues and individuals—particularly around CpG island shores (Irizarry et al., 2009; Lister et al., 2009). Another contentious point is the stability and reversibility of methyl marks in adult mammalian cells, since it is unclear whether and to what extent active demethylation occurs. Several putative mechanisms have now been proposed (Ma et al., 2009; Ito et al., 2011; He et al., 2011).

(B) Histone variants and modifications: DNA is wrapped around a histone octamer consistent of a histone H3, H4 tetramer and two histone H2A, H2B dimers. The lysine residues of histones can be modified, e.g., by phosphorylation (P), acetylation (Ac) and methylation (Me), and this changes chromatin conformation (Bannister and Kouzarides, 2011). Different histone variants exist (e.g., H3.2) that have distinct posttranslational modification patterns, occur at different stages of neuronal development (Piña and Suau, 1987), and hence affect chromatin function.

(C) Chromatin remodeling complexes: chromatin conformation can also be changed through protein complexes whose actions are fuelled by ATP hydrolysis (Hargreaves and Crabtree, 2011).

(D) Nucleosomes are further condensed into chromatin fibers and packaged into chromosomes to fit inside the nucleus. The structure of chromatin and chromosomes is highly dynamic and varies with cell cycle.

of many of these cortical changes remains mostly speculative, as does the specific influence they each exert on the pain experience. However, they are likely to be of some functional significance, given that many of the current effective psychological treatments for chronic pain conditions target the brain. For instance, researchers have found that cognitive behavioral therapy can relieve lower back pain (Lamb et al., 2010).

Evidence is starting to emerge supporting the involvement of epigenetic mechanisms at multiple loci relevant to pain processing. Here we will provide a brief introduction to epigenetic mechanisms before examining their role in peripheral inflammatory processes, their role in nociceptive gene regulation, and their possible role in plasticity and cortical pain mechanisms.

Epigenetics and Pain

The term epigenetics refers to processes that lead to stable and/or heritable changes in gene function without any concomitant DNA sequence changes. Examples include DNA methylation, histone modification, and chromatin remodeling (see Figure 2 for more detail). The proteins supporting these mechanisms can be broadly classified into writers, readers, and erasers (Table 1), depending on whether they add an epigenetic mark, are recruited by a particular mark, or remove a mark. Research in this area has also started to examine certain transcription factors that impact these epigenetic writers or readers, for instance the RE1-silencing transcription factor (REST), which recruits HDAC1, HDAC2, and MeCP2 and will be discussed in more detail in the following.

Over the past ten years, our understanding of epigenetics has significantly increased as a result of many seminal studies, such as the discovery of histone demethylases (Shi et al., 2004; Tsukada et al., 2006) and work on the genome-wide distribution of acetylation and methylation marks in human cell lines (Barski et al., 2007; Ernst et al., 2011; Lister et al., 2009; Wang et al., 2008). Many of the results are continuously added to databases such as Ensembl and UCSC (http://www.ensembl.org, http://www.genome.ucsc.edu), and efforts are underway to sequence the epigenome to create DNA methylation and histone modification maps for as many different cell types as possible (Nature, 2010).

There has also been a surge in research investigating epigenetic mechanisms in the nervous system with a significant literature on memory and synaptic plasticity (for review, see Guan et al., 2009; Peleg et al., 2010; Day and Sweatt, 2011) and the emergence of a whole new field dubbed "behavioral epigenetics" (Szyf and Meaney, 2008; Weaver et al., 2004). In chronic pain, three main areas of epigenetic control can be identified based on the work to date and will be discussed below.

Epigenetic Regulation of Peripheral Inflammation

As explained previously, the importance of inflammatory mediators in the establishment of many pain conditions is well recognized. Equally, there is quite a thorough literature on epigenetic influences in the inflammatory process (for review, see Selvi et al., 2010). Histone deacetylase (HDAC) inhibitors—compounds that prevent the removal of acetyl groups from histones—can ameliorate symptoms in a number of animal models of inflammatory diseases, such as arthritis, colitis, and hepatitis (Chung et al., 2003; Glauben et al., 2006; Leoni et al., 2005). Moreover, significant clinical benefits of an HDAC inhibitor have been observed against both arthritic and painful components of juvenile idiopathic arthritis, albeit in an open-label trial

Table 1. Summary of Epigenetic Writers, Readers, and Erasers

	Writers	*Readers*	*Erasers*
DNA methylation	DNMT1 DNMT3A DNMT3B	MeCP2 MBD1-4	not clear - only putative targets so far: - MBD2 (Bhattacharya et al., 1999) - TET enzymes leading to iterative oxydation resulting in eventual removal of methyl-cytosine (Ito et al., 2011, He et al., 2011)
Histone acetylation	***Histone acetyltransferases (HATs)*** GCN5/PCAF > H3K9/K14/K18 GNAT related (e.g.,HAT1, TFIIIC) > H4K5/K12 Myst family (e.g.,TIP60, HBO1) > H4, H3K14 CBP/p300 family > H3K14/K18 H4K5/K8 H2A, H2B TAF250 family > H3K20 SRC family (e.g., SRC1, TIF2) > H3K27	***Bromodomain proteins*** e.g., most HATs BET family (Brd2, Brd4, Bdf1) Brg-1	***Histone deacetylases (HDACs)*** class I (HDAC1, HDAC2, HDAC3, HDAC8) class IIa (HDAC4, HDAC5, HDAC7, HDAC9) class IIb (HDAC6, HDAC10) sirtuins (SIRT1 - SIRT7) class IV (HDC11)
Histone methylation	***Lysine methyltransferases (KMTs)*** KMT1A - KMT1F (e.g., G9a, GLP) > H3K9 MLL family (e.g.,MLL1, hSET1A) > H3K4 KMT3A - KMT3C (e.g., NSD1) > H3K36 DOT1 > H3K79 KMT5A, KMT5B (e.g., SUV420H1) > H3K20 KMT6/ EZH2 > H3K27 KMT7/ SET7&9 > H3K4 KMT8/ RIZ1 > H3K9	***Royal family*** - chromo-domain proteins, e.g., HP-1 like, polycomb like, CHD like - tudor-domain proteins, e.g., SMN ***PHD proteins*** e.g., CBD, ING2, DNMT3L, PHF6	***Lysine demethylases (KDMs)*** LSD1/ KDM1 JHDM/Jumonji (e.g., JHDM1A/B, JHDM2A/B, JHDM3A-D, JARID1A-D, UTX)
Histone phosphorylation	***Serine/Threonine Kinases*** e.g., MST, AMPK > H2B Haspin, VRK, Aurora B > H3 PKCα, PKCβ, MSK1/2, JNK > H3	***14-3-3 proteins*** seven isoforms: theta, gamma, zeta, eta, epsilon, beta, mu	***Protein Phosphatases*** e.g., Serine/ Threonine protein phosphatases (PPP2CA, PPP2CB, PPP1CC) Protein phosphatase 1D Eye-absent homologues (EYA1-3)

Large families of proteins have been identified that add the various epigenetic marks (writers), remove them (erasers), and bind them to exert downstream effects (readers). This table does not provide an exhaustive list, and many issues are still under debate such as the existence of active DNA demethylation (Bhattacharya et al., 1999; Ito et al., 2011; He et al., 2011). In the case of histone writer molecules, there tends to be quite a clear preference for particular lysine residues, the identity of which is also indicated here (e.g., H3K9 indicates preferential action at lysine residue 9 of histone 3). Current evidence suggests that the same preference does not exist for histone erasers. It is important to bear in mind that many of these molecules do not exclusively act on histones or even in the nucleus, but that they are also capable of modifying cytoplasmic proteins (e.g. tubulin; for review, see Sadoul et al., 2011). Hence drugs targeting their function, such as HDAC inhibitors, can also affect nonepigenetic processes.

(Vojinovic et al., 2011). The effects of these compounds are believed to be mediated in part through suppression of cytokines, with their administration having been shown to reduce expression of many crucial proinflammatory mediators, including IL-1β and TNFα (Leoni et al., 2002). In turn, binding of these same proinflammatory factors to their receptors can also harness epigenetic processes. Thus, interleukin and TNFα receptor activation results in H4 hyperacetylation of many other inflammatory promoters through the action of the transcription factor NF-κB and its subunits p50 and p65 (Ito et al., 2000; Rahman et al., 2002). Similarly, H3k4 methylation via methyltransferase SET7/9 can affect recruitment of NF-κB to proinflammatory genes (Li et al., 2008). The peripheral mechanisms underpinning chronic inflammatory pain states are controlled by these same mediators (Marchand et al., 2005) and involve action of both glial and neuronal NF-κB (Fu et al., 2010; Niederberger and Geisslinger, 2008), making it likely that similar epigenetic processes are at play.

Epigenetic Gene Regulation in Pain Processing

Three epigenetic factors have so far been uncovered that can influence expression of nociceptive genes in chronic pain states. These are histone acetylation, DNA methylation, and REST. Pharmacological interference with the process of histone acetylation can affect pain behavior, with both systemic and intrathecal administration of HDAC inhibitors having analgesic effects in models of inflammatory pain (Chiechio et al., 2010; Bai et al., 2010). In one study, this effect was shown to be mediated by expression changes of the mGluR2 receptor in both DRG and spinal cord (Chiechio et al., 2009). Conversely, a pathological pain state may be able to induce changes in histone acetylation at relevant pronociceptive genes. Injection of an inflammatory agent (complete Freund's adjuvant, CFA) into the paws of rats

was shown to lead to transcriptional downregulation of GAD65 in the dorsal raphé nucleus coupled with hypoacetylation at its promoter. The same was true after spinal nerve ligation, which is used to mimic a neuropathic pain state (Zhang et al., 2011).

Similar influences on expression could be shown in the case of DNA methylation and its reader molecule MeCP2. The methyl binding protein MeCP2 has been shown to promote abnormal upregulation of a group of genes in inflammatory pain conditions. In rats, its usually repressive function appears to be curtailed through phosphorylation after injection of CFA into the ankle joint (Géranton et al., 2007). This mechanism was shown to be partly dependent on intact descending serotonergic input into the spinal dorsal horn (Géranton et al., 2008). Further supporting this role for MeCP2 are studies demonstrating altered pain thresholds as a result of reduced MeCP2 expression levels. This can be observed in conditional knockout mice, as well as individuals with Rett's syndrome—a disease caused by mutations within the MeCP2 locus (Samaco et al., 2008). Lastly, two recent reports have emerged as the first to directly measure changes in DNA methylation at genes associated with chronic pain conditions. Tajerian et al. (2011) found that intervertebral disc degeneration, and the chronic pain associated with it, correlates with increases in methylation at the SPARC gene promoter in both mice and humans. However, since the extracellular matrix protein SPARC is involved in both disc degeneration and the resulting lower back pain, it is not obvious which is more relevant and whether this is a true "pain target." In contrast, the second paper describes the promoter of the endothelin-1 B [ET(B)] receptor, which was found to be methylated only in biopsies obtained from painful human oral cancers, but not from nonpainful oral dysplasias (Viet et al., 2011). Moreover, rescuing ET(B) receptor expression in a mouse model of oral cancer could attenuate pain behavior, providing further evidence for the existence of methylation-mediated promoter regulation of a nociceptive gene.

Finally, there is evidence for the involvement of REST in chronic neuropathy. REST is a transcription factor that recognizes a specific promoter sequence (RE-1 element) present in nearly 2,000 genes with primarily neuronal function (Bruce et al., 2004). REST recruits a host of chromatin modifiers, either directly or through interaction with Co-REST and Sin3 complexes, to exert repressive action on its target genes. The list includes the G9a methyltransferase, histone deacetylases (HDAC1, HDAC2), demethylases LSD1 and JARID1C, and the DNA methyl binding protein MeCP2 (Buckley et al., 2010). Partial sciatic nerve ligation, a model of neuropathic pain, resulted in a long-lasting increase in expression of this repressive transcription factor in mouse DRG (Uchida et al., 2010a). Using chromatin immunoprecipitation (ChIP, see Figure 3), it could further be shown that REST promoter binding is directly responsible for reduced expression of several genes known to be relevant for nociceptive processing in the DRG, including the μ-opioid receptor, the sodium channel $Na_v1.8$, and the potassium channel $K_v4.3$. Accordingly, knockdown of REST using RNA interference was shown to protect against this abnormal downregulation and consequently rescue some of the injury-induced phenotype on both electrophysiological and behavioral measures (Uchida et al., 2010a, 2010b).

Epigenetic Involvement in Plasticity and Cortical Pain Processing

As mentioned previously, there is quite a substantial literature on the involvement of epigenetic processes in the regulation of memory and synaptic plasticity (for review, see Day and Sweatt, 2011). To briefly summarize some of the most salient pieces of evidence: HDAC2 overexpression has significant effects on spine density, synaptic function, and memory consolidation (Guan et al., 2009); a sizable number of CpG-rich regions in the genome show rapid DNA methylation changes as a result of intense hippocampal neuronal activity (Guo et al., 2011); and associative learning in animals has repeatedly been shown to affect histone marks. Thus, young mice were seen to display changes in H4K12 acetylation in the hippocampus after contextual fear conditioning in contrast to their aging counterparts (Peleg et al., 2010). Memory formation was also reported to induce changes in histone phosphorylation (e.g., Chwang et al., 2007) and methylation (e.g., at the BDNF promoter, Gupta et al., 2010). Finally, it was demonstrated that learning can be aided or disrupted by interfering with histone marks on a molecular level and that induction of long-term potentiation (LTP) can be altered by administration of HDAC inhibitors (Levenson et al., 2004).

It is possible that similar epigenetic mechanisms are at play in chronic pain conditions, as neural plasticity is vital to the encoding of noxious stimuli in both spinal cord and brain. Central sensitization of spinal neurons relies on molecular processes very similar to those underlying associative learning, in particular the formation of LTP (Ji et al., 2003). Both forms of plasticity crucially involve NDMA receptor function, protein kinase pathways, CREB activation, and can be influenced by BDNF release. In the hippocampus, those signaling pathways have now all been shown to be epigenetically regulated, and in turn control or influence epigenetic processes (Chwang et al., 2007; Koshibu et al., 2009; Lubin et al., 2008). In the brain, multiple areas undergo changes in neural connectivity as a result of chronic pain, including the anterior cingulate cortex and the amygdala (Gonçalves et al., 2008; Li et al., 2009; Wu et al., 2005). Most recently, cortical epigenetic processes have been hypothesized to be modulators of chronic back pain to account for shifts in event-related EEG peaks over relevant brain regions (Vossen et al., 2010).

In summary, direct evidence that epigenetic mechanisms could be involved in the development and/or maintenance of chronic pain conditions is only just beginning to surface, and the field is in its infancy. Yet the current research already indicates that this new direction has promise and presents an opportunity to identify new treatments for chronic pain. There are also a number of questions that arise from this new knowledge and will be discussed in the following section.

Epigenetics and Chronic Pain—Critical Questions

The first and most obvious question is whether epigenetic marks contribute to the altered transcriptional control observed in chronic pain states. For instance, histone modifications and consequent changes in chromatin structure and recruitment of transcription factor complexes could be hypothesized to be possible mechanisms through which widespread gene

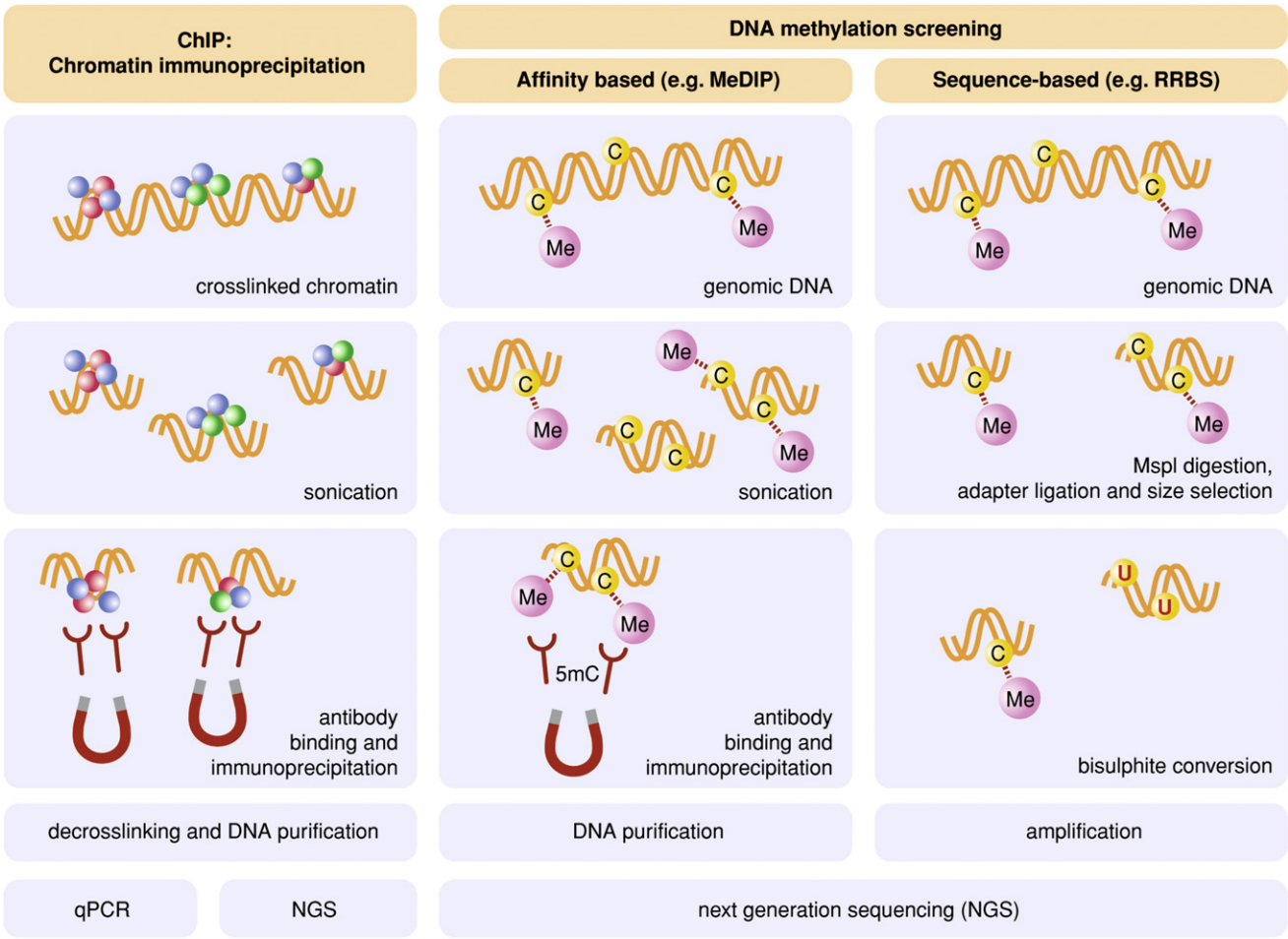

Figure 3. Techniques Used to Probe Epigenetic Mechanisms

In ChIP, an antibody is used on sheared, crosslinked chromatin to select modifications of interest and determine gene-specific (qPCR) or genome-wide (NGS) enrichment. The latter is commonly referred to as "ChIP-seq." Antibody availability and specificity is one of the main difficulties with this technique (Egelhofer et al., 2011). In addition, great care has to be taken to include appropriate controls: 10% input chromatin, total histone ChIP to control for nucleosome density and negative control primers for qPCR.

DNA methylation can be probed with (1) affinity-based methods like MeDIP where an antibody against 5-methyl-cytosine (5mC) is used to pull down methylated regions of DNA. If followed by next generation sequencing (NGS), this is referred to as MeDIP-seq. Alternatively, there are sequence-specific methods (2), like reduced representation bisulfite sequencing (RRBS), which include bisulfite conversion. For genome-wide studies, DNA methylation methods have been compared (e.g., by Bock et al., 2010). RRBS was found to have the best resolution, accuracy, and robustness. However, it is important to bear in mind that sequence-specific methods cannot distinguish between 5mc and 5-hydroxymethyl-cytosine (5hmC). If not probed with 5hmC-specific antibodies using MeDIP, conversion of 5mC to 5hmC can thus be mistaken for the disappearance of methyl-sites (for a review on 5hmC and its potential functions, see Branco et al., 2012).

expression changes are implemented and coordinated (see Figure 4). In particular, the three-dimensional aspect of chromatin conformation and evidence for extensive histone cross-talk (for review, see Bannister and Kouzarides, 2011) could explain how seemingly varied sets of genes are regulated in tandem. In individual cases, there is already evidence that changes in transcription are correlated with changes in acetylation (mGluR2 and GAD65; Chiechio et al., 2009; Zhang et al., 2011), but evidence for clear causal directionality is still lacking. Hence, rigorous animal studies will be required to move beyond correlational data and establish a timeline of events. Moreover, it is likely that expression changes at multiple genes will be the cause of most complex chronic pain syndromes. Hence, patterns of modifications across loci will have to be determined with genome-wide techniques such as ChIP-seq and MeDIP-seq (Figure 3).

Another issue is whether epigenetic mechanisms are equally important in the different cell types involved in the nociceptive pathway. It is well known that DNA methylation and histone modification patterns are very cell type specific, which not only has implications for scientific hypotheses, but also raises several methodological issues. Genome-wide methylation studies examining complex neurological phenotypes in human are currently conducted using blood samples, and information on how the data obtained in this way correlate with more disease-relevant tissues is only just beginning to be addressed (Dempster et al., 2011). In the case of chronic pain, where prominent transcriptional changes are occurring in spinal cord and DRG,

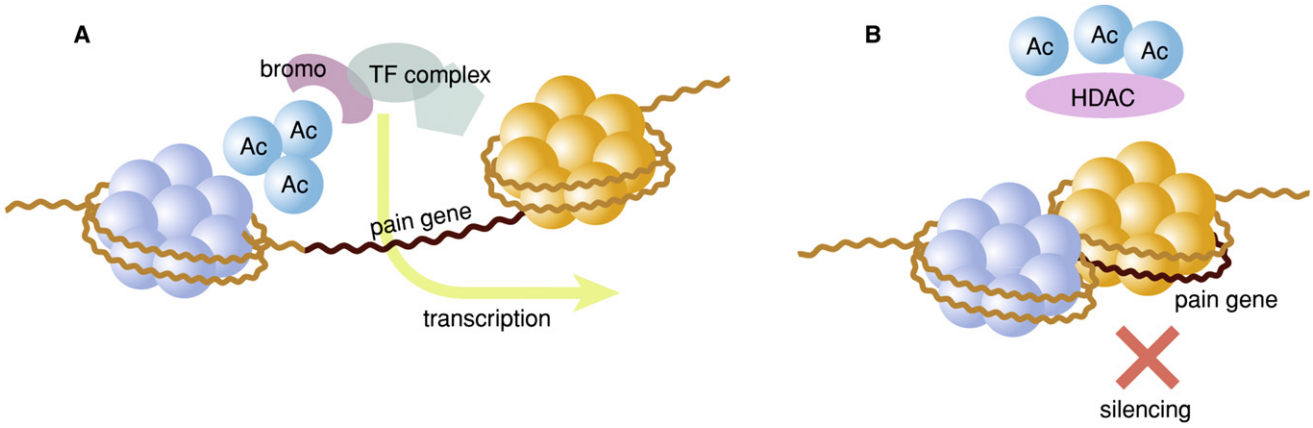

Figure 4. Schematic Illustrating One Possible Mechanism by which Epigenetic Processes Could Impact Development of Chronic Pain
(A) Acetylation at relevant nociceptive genes (Pain Gene, PG) could lead to a more open chromatin conformation through the negative charge of acetyl groups and the recruitment of transcription factor complexes (TF complex) containing bromodomain (bromo) readers. This would then lead to increased transcription of the genes in question, as is indeed observed in chronic pain states, where a large number of loci show abnormal upregulation (Lacroix-Fralish et al., 2011).
(B) In contrast, in a nonpain state, HDACs may be present to deacetylate the nociceptive promoters thus leaving the region in a heterochromatic, silenced state.

assaying relevant human tissue will be problematic. Animal models will be a necessary reference point, especially in the case of the very heterogeneous cell populations in spinal dorsal horn and brain. In these instances, it is now technically possible to conduct ChIP and DNA methylation studies after selecting fluorescently tagged cell types with flow cytometry (Guo et al., 2011; Jiang et al., 2008).

In addition to basic research, there is the potential for the study of epigenetics to reveal novel drug targets for the treatment of pain. It is already clear from only a few experiments that HDAC inhibitors might be an interesting group of drugs to explore. Currently, these compounds display limited selectivity or, more precisely, a selectivity bias toward the ubiquitously expressed class I HDACs (Bradner et al., 2010). As a consequence, if given systemically, many adverse side effects, such as fatigue, nausea, and diarrhea, can occur. Nevertheless, two HDAC inhibitors (vorinostat and romidepsin) are already approved for use in the clinic against T cell lymphoma, and many more are being trialed as chemotherapeutic agents (Lemoine and Younes, 2010). Because of their relevance in the fight against cancer, development of more selective and hence more tolerable HDAC inhibitors is a high priority not only for research but also for the pharmaceutical industry. In the pain field, drugs specifically targeting class IIa HDACs would be of particular interest. This class is expressed less widely, and some evidence suggests that one of its members (HDAC4) is implicated in pain processing. Thus, Rajan et al. (2009) reported that knockout of the HDAC4 deacetylase domain in mice decreases their thermal sensitivity on a hot plate (Rajan et al., 2009).

It will also be of great interest to test the effects of other groups of compounds, for instance, those interfering with the actions of histone acetyltransferases or lysine methyltransferases. These enzymes add acetyl or methyl groups to histones (as well as other proteins) and tend to be more selective in the residues they modify, potentially making them better targets for drug development than deacetyl- or demethylases (Copeland et al., 2009; Dekker and Haisma, 2009). Finally, many of the epigenetic "reader proteins" (Table 1) could be viable drug targets, since their precise involvement is likely to be disease-process and situation specific. Hence, a deeper knowledge of epigenetic processes in chronic pain is needed to gauge their therapeutic potential.

Conclusion

The currently available data suggest that epigenetic mechanisms may be important contributors to chronic pain states. Descriptive studies, for instance examination of genome-wide histone acetylation or methylation in various models of chronic pain, will be useful and are certainly feasible. Causal interactions may take longer to establish, but a wide variety of compounds, targeting specific epigenetic proteins, are being developed and will greatly facilitate this effort. Such studies present an opportunity to test a new unifying hypothesis, namely, that epigenetic mechanisms regulate and coordinate the diverse transcriptional alterations that have been observed in chronic pain states. They also have the potential to provide much needed progress in the treatment of chronic pain, opening up new avenues for drug development.

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