Chapter 8

Epigenetic and Schizophrenia

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Additional information is available at the end of the chapter

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Abstract

Schizophrenia is a complex psychiatric disorder characterised by the presence of positive, negative and cognitive symptoms that lack a unifying neuropathology. The absence of consistently replicated genetic effects, together with evidence for lasting changes in gene expression after environmental exposures, suggests a role of epigenetic mechanisms. In this chapter, we will focus on these mechanisms, such as DNA methylation, hydroxymethylation, histone modifications or non-coding RNA, as key mechanisms through which environmental factors interact with individual's genetic constitution which affect the risk of psychotic conditions throughout life. Due to the advances experienced in recent years, it is to be expected that in the next decades, an increasing amount of data will provide us with a more complete landscape of the contribution of epigenetics to the development of mental disorders such as schizophrenia.

Keywords: schizophrenia, epigenetic, DNA methylation, histone modifications, human brain

1. Introduction

Schizophrenia is a complex illness characterised by different types of positive, negative and cognitive symptoms that affect all aspects of mental activity. This disorder has a world prevalence of 1%, but it is higher in first-degree relatives as well as in monozygotic twins.

Although one of the main repository of scientific publications (the US National Library of Medicine, accessible from www.pubmed.com website) compiles, on average, more than 6000 original research papers per year, we only have fragmented knowledge about the aetiology, development and accurate diagnosis of schizophrenia.

What are the causes of schizophrenia? Does the genetic background of the individuals play a role in predisposition, onset and progression of this disorder? Or are the environment and its

different types of pressures (stress, nutrition, diseases, etc.) responsible for the development of this disorder? For decades, the nature versus nurture debate absorbed efforts from scientist either from biological sciences to psychiatry professionals. However, recent advances indicate that this debate could be finally overcome. In fact, nature and nurture interact in different ways, and we can imagine this situation as a cycle. In a dynamically changing environment, stimuli should enter the cell where this information is 'interpreted' and a plethora of biological mechanisms are able to produce a molecular response. Usually, this molecular response implies expression of genes that, in due course, will generate an organic response to confront the initial stimuli. And the cycle begins again (**Figure 1**), allowing to the cell to maintain its homeostasis.

Currently, we can see the intimacy of this interaction at a molecular level and we can understand how a single cell (whether an epithelial cell or a neuron) is able to adapt to an environment that is in a constant change, whether they be minimum changes (for example, a new cellular interaction) or significant changes (from a pH change to microbe attacks or environmental catastrophes).

Certainly, cells do not carry a gene for every possible response against environmental stimuli; we therefore need another 'way of reading' the genetic information or, even more, another layer of information. In fact, genetic information tends to be rigid: it is hard to change it, and when it happens (what we usually know as mutations) it biological meaning is comprised from cell death to cell survival, through a wide range of decisions that clash with the dynamics of environment changes. Although this rigidity is key to passing all this information from generation to generation, it does not seem to be useful when the cell needs a quick, dynamic response to its medium.

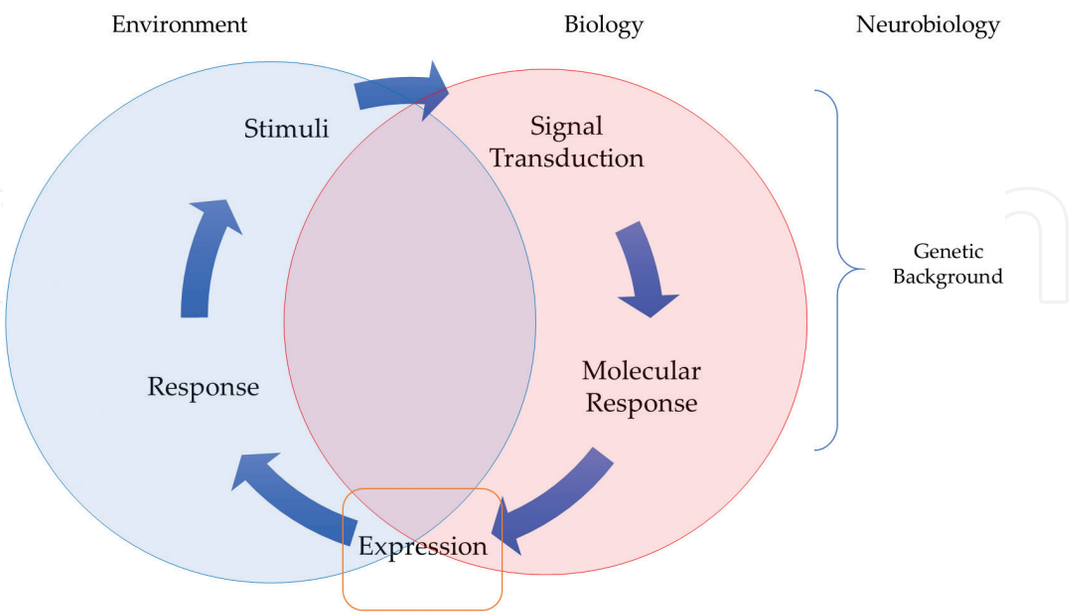

Figure 1. Interactions nature-nurture. Genetic and environment constantly interact to assure the maintaining of a constant internal medium for every cell, despite facing several types of stimuli.

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In 1942, Conrad Waddington coined the term 'Epigenetic' to name the plasticity of genomes when facing environmental changes through his metaphor of marbles (representing cells in its developmental process) rolling down a hill, through valleys and forks (representing the environment) that affect the cellular fate (**Figure 2**) [1].

Since 1942, we have developed a more precise definition of epigenetic:

> *Epigenetic comprises all the molecular mechanisms that affect gene expression without changing the DNA sequence.*

As we will see throughout this chapter, epigenetics is both a new layer of information and a new way of reading the genetic information, leading to a gain in actual plasticity of the rigid genetic information.

In this sense, epigenetics could shed some light on elusive points of the aetiology, and general pathology of mental disorders, especially in schizophrenia, where interactions nature-nurture seems to be key to its onset [2].

1.1. Is epigenetic that important? A new layer of information

We can imagine epigenetic as a new layer of information that regulates the differential reading of genetic information, or the access to it. In turn, this regulation of reading or access to the genetic information is also regulated, as is common in living cells, by epigenetic mechanisms, too. Therefore, we can distinguish:

a. Mechanisms implied in differential reading of the genetic information: these mechanisms are responsible to selectively guiding the genetic expression according to minor (and sharply defined) atomic modifications of DNA. When these modifications are located in the right places along the DNA sequence, all the genetic expression can increase or decrease, thereby allowing the cells to response when facing dynamic changes. In this category, we could indicate the DNA methylation and hydroxymethylation.

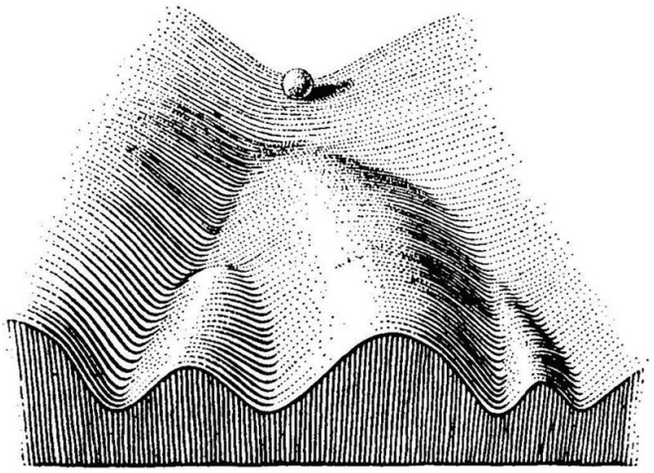

Figure 2. The epigenetic landscape by Conrad Waddington. With this metaphor, Waddington tries to illustrate how environment can influence the cells' fate during its developmental process.

b. Mechanisms implies in allowing (or avoiding) access to the genetic information: these subtle mechanisms encompass molecular changes that physically allow or avoid the access to DNA sequences or specific regions. In this category, we could indicate the post-translational histone modifications and the role of chromatin remodelling complexes.

c. Mechanisms that regulate epigenetic changes: the presence of feedback loops is common in living cells and epigenetic changes are also regulated in this way. There are several types of molecules (in particular, some types of RNA as we will see later in this chapter) that can regulate the epigenetic landscape in a healthy cell or be affected in an unhealthy cell.

The role of genetic background has been widely demonstrated in relation to the onset of schizophrenia. However, this factor is not unique as a causal one, and the individual needs to be in contact with several kinds of environmental factors to develop this mental disorder. In other words, the genetic information should interact with an environment to generate a schizophrenic phenotype, which may position epigenetic changes as key to understanding the molecular basis of this pathology.

As we can see, epigenetic changes could be subtle (such as specific atomic changes on a DNA base, or molecular changes in its associated proteins), or they can imply an actual remodelling of huge portions of chromatin, but always differently depending on the tissue, even more, depending on cell type. However, to understand how epigenetic mechanisms could trigger a mental disorder, we need to understand all the epigenetic changes as a whole, generating a framework instead of interpreting fragmented data. We call this framework the neuroepigenome. This new field implies efforts from several branches of Science in order to get a complete view of the epigenetic modifications of the nervous system which, in due course, will leads to a better understanding of pathological conditions.

Throughout the next sections, we will discuss the neuroepigenome from simpler modifications (such as DNA methylation) to the more extensive changes (such as the remodelling of chromatin), focusing on its role and recent advances regarding molecular pathology of schizophrenia. Given this field is still under frantic research, we will try to offer the most established (and replicated) facts, while leaving open doors to new developments and knowledge.

2. Differential reading of genetic information: DNA methylation and hydroxymethylation

The existence of DNA methylation was firstly proposed by Hotchkiss in 1948. More precisely, in those experiments, Hotchkiss was able to separate a modified cytosine by using chromatography. It was called 5-methylcytosine (5mc) and it was hypothesised that this form could be commonly present in nature. However, its role in regulation of genetic expression was not unveiled until the decade of the 1980s [3].

What is currently known as 'DNA methylation' is, in fact, cytosine methylation, and it consists of the transferring of a methyl group to 5′ carbon of cytosine, to generate 5mc. This modification is extremely active during embryo development, but it is relatively slower in

differentiated cells, and generates a characteristic pattern of methylation distributed along the genome. This pattern is not part of a random distribution but a tightly regulated distribution.

DNA methylation is frequently observed in cytosines adjacent to guanines, in the so-called CpG sites. This dinucleotide is mainly enriched at promoter sequences where it is repeated and grouped generating what is known as 'CpG island'. These 'islands' comprise around 1000 base pairs of the promoter region, with a high degree of conservation between species, becoming clear hotspots for methylation. Given the role of gene promoters, it was proposed that methylation acts as a regulator of genetic expression and this was the more replicated finding throughout research in life sciences. However, methylation was also observed in gene bodies, introns and intergenic sequences, with other functions that still remain elusive. On the other hand, it was also described methylation of non-CpG sites in murine cellular models or human stem cells, however its role is currently unclear, and it is under extensive research [4].

DNA methylation is a tightly regulated process and we can distinguish several types of enzymes that catalyse the necessary steps either to write or to erase this addition. The DNA methyltransferase (DNMT) catalysed the addition of methyl groups to cytosines. Three members of this DNMT family were described (DNMT1, DNMT3a and DNMT3b) and, despite its similarities, they have unique functions. DNMT1 catalyses the addition of methyl groups to the nascent DNA chain during replication, maintaining the methylation pattern of the cell lineage. However, DNMT3a and DNMT3b do not show specificity for hemimethylated sequences, so it was proposed that these enzymes are responsible for *de novo* methylation. However, how these enzymes target specific DNA regions or sequences are still unknown. There is also described a third isoform, called DNMT3L, that lacks its catalytic domain and it is mainly expressed during early development and in germinal cells. Although without its own catalytic function, this isoform could be associated to DNMT3a and DNMT3b to promote their methyltransferase activity.

On the other hand, the erasing of DNA methylation patterns could be a passive or active process. In mammals, DNA methylation in the form of 5mC can be actively reversed to unmodified cytosine (C) through TET dioxygenase-mediated oxidation of 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC), followed by replication-dependent dilution or thymine DNA glycosylase (TDG)-dependent base excision repair [5]. Instead, as a passive process, it was proposed that several chemical changes (deamination, oxidation) could generate a modified cytosine that activates the base excision repair (BER) system that eventually replaces that cytosine.

However, 5hmc it is also a modification that may have more biological function, different from an intermediary product of demethylation. In fact, high expression of this modification is found in brain tissue and during embryogenesis as well as in stem cells. It was proposed that this modification could influence the genome structuration during early phases of development. In fact, it was described that oxidation of 5mc to 5-hmc only takes place in paternal (but not in maternal) pronucleous [6, 7]. Regarding brain tissue, Wang et al. [8] reported a positive correlation between 5-hmc levels and cerebellum development in humans. Given that the previous methods to interrogate 5mc were unable to distinguish from 5hmc, this modification has only recently been assessed as an actual epigenetic marker. Therefore, many of its biological functions are currently under research.

2.1. DNA methylation as a research tool: main findings in schizophrenia

The development of methylation-specific polymerase chain reaction (MSP) allowed us to interrogate specific DNA sequences to assess its methylation status. In this technique, DNA is treated with sodium bisulfite that deaminates unmethylated cytosines to generate thymine (uracil). This conversion does not occur when there is a methyl group at the 5′ position of cytosine, which remains as a cytosine. This change allows researchers to generate specific primers for PCR amplification, in order to discriminate methylated from unmethylated alleles. This technique was a useful tool to understand the pattern of methylation in different gene regions. However, it was mainly applied to research promoter sequences trying to find correlations with gene expressions. In general, it was accepted that high levels of promoter's methylation mean decreased expression, whereas lower levels mean increased genetic expression. Of course, this is just a general rule and it is not applied to all genes. However, with this general idea, brain tissue of schizophrenic patients was evaluated. Although interesting, the main results are controversial. The base of this controversy relies on the usage of MSP, that only allows to interrogate some selected CpG sites, which leads to a fragmented information if we want to extrapolate (and correlate) to genetic expression. Keeping this situation in mind will facilitate us to understand some controversial findings in the next paragraphs.

Initial reports try to shed some light on the molecular mechanism of schizophrenia by using MSP and focused on the methylation changes of single genes. In this sense, in schizophrenic brain tissue, a higher level of methylation of GABA promoter regions that leads to lower levels of GABA mRNA was observed [9]. A similar finding was observed regarding *RELN* gene, that codifies reelin, a secreted protein involved in neurodevelopment with putative roles in schizophrenia onset [10].

Several other genes also showed changes of methylation pattern in schizophrenia, as those related to glutamate and serotonin signalling. For instance, hypermethylation of glutamate transporter genes or serotonin receptor genes (leading to a lower protein expression) was observed in schizophrenic brain [11, 12], but not in other studies [13].

On the other hand, specific changes of gene methylation carried out in specific anatomical regions could represent a more concrete regulatory event. For instance, hypomethylation of *COMT* gene (that leads to higher levels of COMT mRNA) may contribute to dopamine degradation in frontal lobes of schizophrenic patients [14], although it is a result that has not been replicated in other studies [13].

Finally, in a recent study, Alelú-Paz et al. [15] compared the DNA methylation pattern across the human genome in several normal and schizophrenic brain areas that have previously been linked to neuropathological features of schizophrenia, such as dorsolateral prefrontal cortex (DLPFC), hippocampus and the anterior cingulate cortex (ACC), reported several genes associated with cognitive impairment characteristic of schizophrenia, such as *LIF*, *PRKCE* or *CNTNAP2*.

We may indicate several studies related to single-gene changes in brain samples. However, currently scientists are more interested in understanding the epigenetic changes as a whole and, in fact, the recent development of Epigenetic-Wide Association Studies (EWAS) allow researchers to interrogate several thousand methylated sequences in a single chip. Even more, this technique is not restricted to gene promoter but to all genome, including gene bodies,

introns and intergenic regions, leading to a more complete scenario of methylation changes. It is relatively easy to assess around 450,000 selected sequences in a single chip. And the number of interrogated regions is rapidly increasing. In fact, by using this technique with schizophrenic brain tissues, it was demonstrated that changes in methylation occur along several portions of genes of glutamate transporters as well as dopamine and serotonin receptors, among others. In blood cells, this technique was also applied to study discordant monozygotic twins, where researchers observed several differentially methylated regions (DMR) between twins, in genes related to cell death, survival and cell movement [16].

By using EWAS technique, researchers are able to find several candidates as predisposition marks or putative biomarkers but, despite its higher resolution, EWAS generates a huge amount of information and the limits of its usage in diagnosis, treatment or prognosis of schizophrenia remains still elusive. It is still a novel field of investigation and its findings will need several years to clearly reveal molecular aspects of schizophrenia. In this sense, EWAS confounding factors are still under research. For instance, antipsychotics (as clozapine or sulpiride), smoking, age and several other environmental factors may also affect methylation and be detected by applying this approach.

An additional problem for using this technique is sample selection. Unfortunately, several EWAS were (and still are) carried out in peripheral blood or even in saliva samples [17]. More than a decade ago, it was completely demonstrated that epigenetic patterns are cell specific, so findings from tissues other than brain are controversial in their correlation to schizophrenia [17]. Even more, different anatomical zones (or cells) of brain may contain its specific epigenetic signature, leading to complications in the interpretation of results [15, 18]. Also, statistical analysis is key to ensure validity of results, therefore scientist need to be aware of these points to trust the technique and its results.

3. Accessing to the genetic information: histone modifications

If we were able to extend all the DNA molecule that lies in the nucleus, we would have a line measuring 2 m. In addition, all this information should be packed into a space measuring around 5 μm (1 μm is the millionth part of the meter). DNA condensation is key to ensure that all the genetic information is protected while is not in use, but it also need to assure that the required information could be accessible according to cells' needs. This condensation is achieved via several highly regulated steps, which begin with the formation of a DNA:protein complex. In doing this, a group of proteins called 'histones' are the key. Five histones have been described: H1, H2a, H2b, H3 and H4. Two molecules of every histone (except H1) are grouped to form a kind of flattened disk around which DNA is spun (approximately) two turns: this complex of protein and DNA is known as 'nucleosome' and it is the minimal structure implicated in DNA condensation. Histone H1 is located 'under' every nucleosome to keep them in place, and around 50 base pairs ahead another nucleosome is formed [19]. This sequence is repeated along the DNA molecule. In successive steps, groups of nucleosomes are gathered to form a highly condensed molecule. Finally, the mitotic chromosome represents the higher level of condensation of a DNA molecule.

This condensation is necessary to pack the DNA in the limited space of nuclei, but also to 'hide' genetic information, that may not be potentially necessary for the homeostasis of cells. Being more precise, it is well-known that a part of genetic information should not be physically accessible (for instance, the condensation of one chromosome X in males): this part of the genome constitutes what is called heterochromatin.

However, another part of genetic information should be accessible according to cell's needs which constitute what is called euchromatin. Although accessible, euchromatin information is still packed, so how this information is reached in a condensed molecule? As we previously indicate, eight molecules of histones form the protein core of nucleosome. As with every protein, histones have an N-terminal portion that is called the 'histones' tails' and are subject of different kinds of modifications: the post-translational modifications of histones. When a post-translational modification occurs, it implies a structural modification that is communicated as a nucleosome's opening or closing. Under these circumstances, genetic information is more accessible (or not) to transcription factors, which eventually promotes the gene expression.

Among these modifications we can find methylation, acetylation, phosphorylation, ubiquitination and others. These modifications can occur on many different residues, some of them harbouring more than one type of modification (i.e., residues can be either acetylated or methylated). Likewise, we can find different types of modifications on different amino acid residues. To complicate this picture, each modification carries implicit information that may indicate an opening (or closing) of nucleosomes, affecting the access of transcription factors to genetic information. Even more, modifications are different (and independent) in every tail of every histone. In the next paragraphs, we will describe some of the better-established histone modifications and indicate the most replicated role in regulation of gene expression. However, we need to take into account that the 'final conclusion' of all these modifications is, in fact, a balance between 'opening' and 'closing' signals, that eventually will lead to allow (or not) transcription factors to generate a mRNA.

3.1. Histone acetylation

One of the best-studied histone modifications is acetylation. It consists in the transfer of an acetyl group from acetyl coenzyme A to a specific histone lysine. This action is modulated by two enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs): increases in former activity promote acetylation and the corresponding increase in gene transcription and increases in HDAC activity, which involves removing the acetyl group from histones, results in a repression of gene expression.

3.2. Histone phosphorylation

Histone phosphorylation is restricted to tyrosine (Y), serine (S) and threonine (T) residues. It has been described eight characterised phosphorylation sites on histones H2A, H2B, H3 and H4, which have been linked to specific cognate kinases. Although we still do not know in depth the role of histone phosphorylation, probably is important in the interpretation of combinatorial post-translational modifications which together regulate various biological processes, including gene transcription and DNA repair.

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3.3. Histone methylation

Acting S-adenosyl-l-methionine as the methyl donor, the methylation of histones is carried out, mainly, by lysine methyltransferases (KMTs) and, to a lesser extent, by protein arginine (R) methyltransferases (PRMTs). More than 50 different histone methyltransferases have been described in humans, including SET1, MLL, SMYD3, ESET, G9a, SETDB or EZH2, that catalyse methylation of H3 at K4, K9 and K27 in mammalian cells.

On the contrary, histone demethylation, that is, the removal of methyl groups via histone lysine demethylases (KDMs) is, at the very least, a controversial topic. The discovery of KDMs suggests that this mechanism is not a permanent modification. To date, two classes of enzymes have been described: the amine oxidase-type lysine-specific demethylases 1 and 2 (KDM1A and KDM1B) and the Jumonji C (JmjC) domain-containing histone demethylases.

3.4. Histone ubiquitination

Clearly, the less well studied post-translational modifications. We know this mechanism acts by the addition of ubiquitin molecule to specific lysine residues on histone tails, which requires the sequential activities of ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2) and ubiquitin ligase enzymes (E3). It is important to note that histones are the most abundantly monoubiquitinated conjugates in the nucleus of mammalian cells, including ubiquitination at K119 on histone H2A, K34 and K120 on histone H2B, all of them associated with the transcriptional control of gene expression and the DNA damage response, including transcriptional reprogramming and DNA repair.

A summary of the aforementioned modification is shown in **Figure 3**.

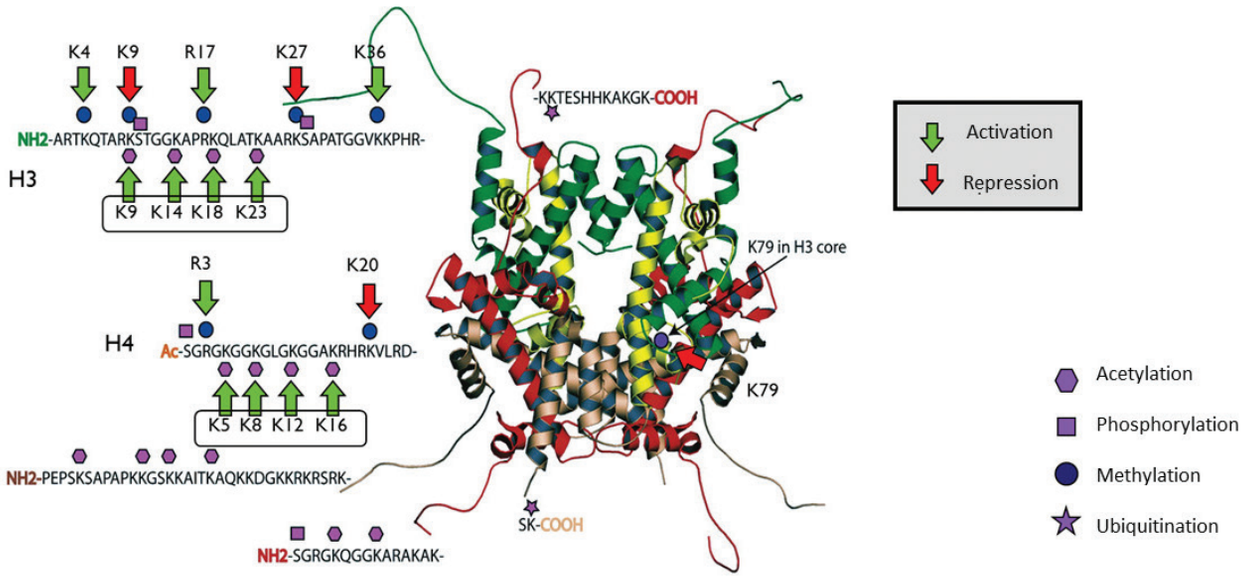

Figure 3. Main histone modifications and their roles regarding gene expression. The complexity of this information may facilitate gene expression or repression according to environmental (and internal) signals. The final combination of every modification will generate the proper response.

3.5. Histone modifications and molecular pathology of schizophrenia

Given their complexity, histone modifications were mainly studied in pathologies with well-characterised cellular and animal models. Unfortunately, the role of histone modifications is not clear for the development of mental disorders, although we find some post-mortem brain tissue studies that can be established as an interesting starting point.

Huang and Akbarian reported shifts in the prefrontal cortex chromatin surrounding GAD1 promoter accompanied by a decrease in GAD1 mRNA [20], whilst the latter author suggested a correlation between methylation of histone H3 at arginine 17 and down-regulation of several metabolic genes in schizophrenia [21].

Chase et al. [22] employed fresh-frozen parietal cortex post-mortem tissue, found significant increased levels of H3K9me2 in parietal cortical samples from patients with schizophrenia when compared to healthy controls, suggesting that initial inactivation of gene promoter activity at various schizophrenia candidate genes can result in gradual entrenchment of the heterochromatin state as a result of disease chronicity and disuse.

Other authors, focused on the role of interneurons in the pathophysiology of the disorders, suggested that acetylation of H3K9K14 correlated with gene expression levels for several schizophrenia-related genes, including GAD1, considered to be among the most frequently replicated findings in schizophrenia post-mortem brain [23].

Kurita and colleagues found a relationship between long treatment with antipsychotics and down-regulation of GRM2, a metabotropic glutamate 2 receptor, through decreased histone acetylation at its promoter region in the human frontal cortex, which could represent a promising new target for schizophrenia treatment [24].

Finally, in a recent paper, Schroeder et al. [25] reported decreased HDAC2 transcript in the dorsolateral prefrontal cortex of schizophrenia patients which represents a set of targets with demonstrated therapeutic relevance.

It is easy to see that we still need to understand more in depth the dynamic changes of histones during the development of mental disorders. Maybe, the main obstacle is not having proper models either animal or cellular. In this sense, the rapid development of techniques that implies stem cells (and induced pluripotent stem cells) may represent an option in the next years.

4. Regulation of genetic information by RNA molecules

A few decades ago, the huge amount of apparently useless DNA sequences lead scientist to coin the term ‘junk sequences’. Nowadays, we know that a clear majority of the genome is transcribed, but only 2% generates proteins. So, a third epigenetic mechanism includes several activities carried out by different types of RNA molecules that were called ‘non-coding RNA’ (ncRNA) to differentiate from coding RNA that generates proteins.

The ncRNAs are classified according to their length: short non-coding RNAs (sncRNA) (length < 30 nucleotides and <200 nucleotides in general) and long non-coding RNAs (lncRNA) (length > 200 nucleotides). Among sncRNA we can find the so-called short interfering RNA (siRNA), microRNAs (miRNA) and Piwi-interacting RNA (piRNA).

Short non-coding RNAs are widely known, as well as the processes carried out to generate them. For a more extensive review of these molecular aspects, see Ref. [26]. In general, their functions are related to protein silencing by interfering the mRNA processing. Long non-coding RNA, as the name indicates, do not generates proteins, although their processing may be closer (or similar) to the normal mRNA. Furthermore, the length could easily reach more than 2000 nucleotides, they could be distributed either at nucleus or cytoplasm and their origins belong to introns or to transposable elements. Recently, it was observed a new type of lncRNA, which has its origin at the intergenic regions. This molecule was called long intergenic non-coding RNA (lincRNA).

In general, both types of transcripts are generated as a response to face environmental changes or as intermediary in other cellular processes. Even more, DNA methylation or histone modifications are targets of (or responsible for) their actions. For instance, some sncRNA are transcribed according to the methylation pattern of the specific gene (either at promoters or bodies), generating a siRNA that silence a target protein. On the other hand, the X chromosome silencing is mainly mediated by a 17 kb lncRNA that acts as an intermediary of histone methylations that generates 'repressive' marks (H3K27me3).

Although interesting and powerful, these mechanisms are poorly understood with regard to mental disorders and there is a clear lack of information. Regarding schizophrenia, there are relatively few reports that describe a general landscape or putative functions of non-coding RNA in development, onset, prognosis or diagnosis of this mental disorder. This is partly because this area is currently under active research (but mainly in oncology where ncRNA roles are better established). This also leads to generate only fragmented data with regard to other diseases that are different from cancer. Given this picture, we can summary some findings:

- Some miRNA showed association with schizophrenia risk in Chinese populations [27] although some controversy during its replication was generated [28].
- miRNAs were proposed as biomarkers of schizophrenia by assessing them in blood samples [29].
- Furthermore, miRNA seem to be dysregulated even in progenitor cells from schizophrenic patients [30].
- By using microarrays, lncRNAs were assessed in blood cells of schizophrenic patients and researchers found that downregulation of two lncRNA (from 40,000 tested lncRNA) were associated to better treatment outcomes and symptoms when patients were evaluated by using the Positive and Negative Syndrome Scale (PANSS) [31].
- A recent report proposed to lncRNAs as key elements in the dynamic of neurostructure and, in consequence, may have putative roles in schizophrenia development [32].

As we previously indicate, this are only selected items, and we hope that we will soon get a more complete view of the role of RNA molecules in schizophrenia development.

5. Epigenetics in the development of treatments: feasibility and challenges

Throughout this chapter, we summarised the main epigenetic findings focused on schizophrenic patients. Do these findings open any door to treatment development? Certainly, it is hard to predict the actual utility of epigenetic findings in drugs or treatment development and we may need to observe what happens in another pathology, mainly cancer. Nervi et al. [33], provide us with a more concrete point of view of clinical trials of epigenetic treatments for solid tumours.

With regard to schizophrenia, HDACs inhibitors are promising drug targets. In this sense, it was observed that HDACs expression may be associated to schizophrenia-like phenotypes [34] and that also their mRNA could be differentially expressed (and reduced) in prefrontal cortex of schizophrenic patients [25]. Valproic acid is a well-characterised HDAC inhibitor and it may be used in combination with clozapine (a demethylating agent) in order to promote chromatin remodelling, and eventually, to correct genetic expression deficits [35]. On the other hand, the role of HDACs isoform-specific inhibitors is still under frantic research. For instance, it was proposed that HDAC2 inhibition may help to treat symptoms in schizophrenia [24, 36]. Finally, methylating and demethylating agents lack specificity and its usage is restricted to research in cell models.

Despite the aforementioned, we must keep in mind that the possibility of treatment development arises from an extensive knowledge of the related epigenetic mechanisms about the pathology. In other words, firstly, we need the knowledge of basic processes and then we can develop successful treatments. Unfortunately, this could take time, and, in schizophrenia, we are at the beginning of a new research era, and still lack information about the epigenetic mechanisms triggered along its onset and progression. Therefore, in the next decade we will see an increase in our knowledge. The success of this approach will rely on sample selection, statistical accuracy and generated hypotheses by responsible and committed scientist aimed to obtain reliable results instead adding 'noise' to research in mental disorders [37].

6. Conclusions

Epigenetic mechanisms add a new layer of information that improves the cell plasticity against environmental changes. These processes are key to maintaining cellular health while frequently also being disorganised during the development of illness. Although there is no change to DNA sequence, these mechanisms play several roles in reading or providing access to the genetic information. Given this feature, epigenetic mechanisms were proposed as new targets to understand how a genetic background interacts with an environment to develop

schizophrenia. The first results obtained were promising, showing changes in methylation pattern of specific genes. However, this was only a piece of information and scientists are currently working in the development and application of more powerful techniques, such as Epigenetic-wide association studies. On the other hand, there is still an almost unexplored field regarding of role of histone modifications in the development of mental disorders. Great efforts are being made to unravel their role, although there is still a clear lack of information. We could indicate the same regarding other mechanisms as chromatin remodelling, therefore it is to be expected that in the next decades, an increasing amount of data will provide us with a more complete landscape of the contribution of epigenetics to the development of mental disorders as schizophrenia.

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