Epigenetic factors in schizophrenia: mechanisms and experimental approaches.

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Epigenetic Factors in Schizophrenia - Mechanisms and Experimental Approaches

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Abstract

Schizophrenia is a chronic mental disorder that is still poorly understood despite decades of study. Many factors have been found to contribute to the pathogenesis, including neurodevelopmental disturbance, genetic risk, and environmental insult, but no single root cause has emerged. While evidence from twin studies suggests a strong heritable component, few individual loci have been identified in genomewide screens, suggesting a role for epigenetic effects. Rather, large numbers of weakly acting loci may cumulatively increase disease risk, including several mapping to epigenetic pathways. Here we discuss evidence for a epigenetic contribution to disease phenotype, mechanisms of epigenetic regulation, and the possible interaction of these pathways with current pharmacological treatment for schizophrenia. Finally, we describe the range of experimental tools currently available to study epigenetic effects associated with the disease.

Main Text

Schizophrenia occurs in about 1% of the population and is a highly debilitating socially and economically [1]. The aetiology is complex and poorly understood, and the interplay of genetic and environmental factors appears to be important in disease development. Symptoms defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) are classified as 'positive' or 'negative' [2]. Positive symptoms (e.g. hallucinations, delusions, and disorganised thought) arise from excess expression of normal function, while negative symptoms (e.g. reduced emotional expression, loss of the ability to experience pleasure, poverty of speech, social withdrawal, and catatonic immobility) reflect a decrease or flattening of normal emotional function. It is common for schizophrenia to be comorbid with other disorders such as depression and personality disorders however, adding to the difficulty of diagnosis.

Currently, clinical treatments rely mainly on antipsychotic compounds that act by inhibition of the type 2 dopamine receptor pathway [3]. These compounds primarily relieve the so-called negative symptoms. However, they are largely ineffective for relief of important symptoms such as attention and memory defects. Unfortunately, this leads to poor personal and social outcomes for schizophrenia [4].

Schizophrenia is associated with impaired performance across a range of measurable parameters, including neuron volume and number, synaptic connectivity, neurochemical balance, and sensory gating [5]. Post-mortem studies show dysregulation of the transcriptomes and proteomes in pre-frontal and temporal cortex oligodendrocytes [6-8]. Similarly, many key proteins responsible for effective neuronal function, including ion channels and neurotransmitter pathway enzymes, show expression changes in post mortem brain from schizophrenia patients [9]. Two general themes are central to most proposed models explaining schizophrenia pathology, the role of the synapse [10], and the early stages of neuronal development (differentiation and maturation) [11].

Genetics and epigenetics of schizophrenia

Schizophrenia shows a high degree of heritability (~ 64% based on family studies; [12]), leading to efforts to map causal loci using high-throughput genomics technologies. To date, this work has failed, in general, to highlight strong individual genetic risk factors. However, it has resulted in the discovery of large numbers of frequently occurring low risk variants, many mapping to early neurodevelopmental pathways. In particular, a genome-wide association study (GWAS) that included 36,989 cases and 113,075 individuals led to the identification of 128 common variants in 108 different loci, each contributing marginally to risk but cumulatively accounting for a modest disease risk (Odds Ratio ~ 1.3) [13]. Many of the identified genes are involved in glutamatergic neurotransmission, synaptic plasticity, or encode voltage-gated calcium channels, as well as the product of the *DRD2* gene (dopamine receptor D2), the main target of antipsychotic drugs. Furthermore, transcripts of these 108 genes show elevated expression patterns in fetal brain when compared to post-natal brain [14]. Similar studies have reported increased expression of schizophrenia risk genes during early brain development [15, 16]. These findings are consistent with a generalized neurodevelopmental role for many genes associated with risk of schizophrenia.

In contrast to these common risk factors, a number of schizophrenia-associated loci have been reported that are rare in the disease population. Copy number variants (CNV) mapping to eight loci were identified in a meta-analysis of 21,094 cases and 20,227 controls, including *NRXN1*, a neurexin involved in synapse formation and neurotransmission [17]. Other loss-of-function mutations have been reported

for the gene encoding histone H3 methyltransferase SETD1A [18]. These add to studies implicating epigenetic factors in schizophrenia, for example the finding that schizophrenia risk loci are more likely to be found proximal to DNA methylation quantitative trait loci [19]. A recent investigation that combined a transcription-wide association study (TWAS) with descriptions of gene expression, splicing, and chromatin activity, found 157 genes with transcriptional changes specifically associated with schizophrenia [20]. A significant proportion of these were strongly associated with nearby chromatin features, again supporting an important role for epigenetic mechanisms in the disease. Similarly, some variants associate significantly with disease but appear to be limited to individual pedigrees. A translocation found to segregate in a Scottish pedigree with mental disorders, including schizophrenia, resulted in disruption of the *DISC1* gene involved in synapse function and early neurodevelopment [21, 22]. However, *DISC1* was not identified as significant in the GWAS study mentioned above.

Mechanisms of epigenetic regulation

The above observations have switched research focus to to a search for epigenetic factors that might explain the discrepancy between the strong inheritance of schizophrenic disease and the lack of strong genetic markers. Epigenetics broadly refers to heritable changes in phenotype that are not encoded in the DNA sequence of the genome. These changes are therefore not permanent, but can be carried from parent to daughter cell (or from one generation to the next), by a form of molecular memory that regulates gene expression programmes. Several mechanisms that alter the chromatin environment surrounding the regulated genes underlie these effects (see below).

The level of mRNA and protein expression from a gene relies not only on features of the primary DNA sequence such as promotors and ribosome-binding sites. It is now clear that dynamic changes in the structure of the surrounding chromatin allows alteration in the activation, repression and general regulation of genes [23]. Several distinct forms of epigenetic regulation are known. These distinct mechanisms cross-talk with each other in ways that are not fully understood, and include: a) direct methylation of DNA; b) modification of the associated histone molecules by a range of chemical adducts (e.g. methylation, acetylation ubiquitination); c) exchange of histone molecules with related isoforms; d) regulation of access to DNA by manipulation of chromatin by nucleosome remodellers.

Direct methylation of DNA in mammals is mediated primarily by the DNMT (DNA methyltransferase) family that catalyzes transfer of methyl groups to the C5 position of CpG dinucleotides. Since levels of DNA methylation can be affected by dietary restriction, a link between periods of famine and increased incidence of schizophrenia has been proposed to be at least partly based on epigenetic phenomena [24, 25]. Gene expression can be influenced by methylation of the underlying DNA in a number of ways, for

example by preventing access to transcription factors, or by recruiting chromatin modifying enzymes. Much of the work towards epigenetically characterizing the schizophrenia epigenome has involved measuring direct DNA methylation through bisulphate sequencing and similar methods. For example, a recent study (41 cases, 46 controls) across four brain regions found many genome regions that displayed differentially methylated DNA [26], while a larger study (689 cases, 645 controls) using blood samples similarly identified many differentially methylated loci [27]. Other studies support the importance of DNA cytosine methylation in schizophrenia [28-30].

Post-translational modifications (PTMs) of histone proteins also have a significant effects on epigenetic regulation of neuropsychiatric disorder, and schizophrenia [31]. Each nucleosome comprises two copies each of four histone proteins (H2A, H2B, H3, H4). The N-terminal tails of H2A, H2B, H3 and H4 histones extend from the globular regions of the histone structure and are subject to extensive and dynamic modifications. Over 70 distinct histone amino acid modifications have been described, including methylation, phosphorylation, ubiquitination, acetylation and sumoylation [32]. They contribute to the regulation of gene expression in several ways, none completely understood. One mechanism involves the recruitment (or displacement) of transcription activators or repressors to the cognate gene through enhanced protein interaction affinity, while another mechanism involves chromatin conformational changes that increase the accessibility for positive and negative regulatory factors. Some PTMs at specific amino acids are associated with repression of gene expression (e.g. methylation of histone 3 at lysines 9 and 27), while others are associated with activation (e.g. acetylation at histone 3 at lysines 9 and 14). The observation that distinct combinations of histone PTMs are found in different transcriptional and genomic contexts led to the proposal that a 'histone code', capable of interpretation by the transcription machinery, governs the expression of associated genes [33].

These PTMs are regulated by a network of enzymes (e.g. histone methyltransferases and histone demethylases) that are often housed in multi-subunit protein complexes. For example, the Polycomb Repressor Complex (PRC1, PRC2) family of complexes mediate gene silencing via generation of trimethylated lysine 27 on histone H3 (H3K27me3) and ubiquitination of lysine 119 of histone H2A (H2AK119Ub). Similarly, the COMPASS family are associated with activating histone marks (H3K36me3). These complexes typically contain 5-10 protein subunits, including a core enzyme responsible for the histone modification reaction (e.g. EZH2 in PRC2; SETD1A in COMPASS). The functions of the additional proteins in these and similar complexes are under intense study, but typically include modulation of the core enzymatic activity, targeting of the complex to specific genomic loci, and mediating interactions with other chromatin and signaling proteins.

Another class of chromatin modification enzymes, termed 'remodellers' are capable of altering the structure of the chromatin itself in order to enhance or inhibit local gene expression. While different remodeling complexes employ different mechanisms, they share an ATPase-tranlocation function that can manipulate the histone-nucleosome architecture [34].

Relatively few reports have been published to date that specifically address the role of individual epigenetic pathways in schizophrenia. This can largely be attributed to the highly specialized technologies needed to study these effects, and to the difficulty in obtaining precious samples. Instead, the focus has been on assessing the levels of a small number of histone PTMs and the balance between histone acetylation and deacetylation. The use of peripheral blood mononuclear cells is one way to obtain suitable samples. Studies on histone PTM levels in schizophrenia found increased levels of the repressive histone mark H3K9me2 that was correlated with the age of onset [35], as well as resistance to treatment when this position was acetylated [36]. Other workers have focused on measuring the relative abundance of the enzymes responsible for generating these histone marks. Increased HDAC1 in schizophrenia patients has been noted in several studies, including those in the pre-frontal cortex [37] and the hippocampus and medial temporal lobe [38]. In keeping with this, over-expression of HDAC1 in mice is associated with behavioral abnormalities and working memory deficits [39]. HDAC2 has also been linked to schizophrenia, an effect conferred at least partly through regulation of the metabotropic glutamate receptor [40]. Finally, in an example of the emerging type of experiment now possible, analysis of enriched DNA motifs following chromatin profiling of pre-frontal cortex samples found evidence for the involvement of the MEF2C transcription factor in schizophrenia risk [41].

Environmental insult is now thought to be a major contributor to epigenetic change (Bale et al., 2010). Maternal behavior is associated with altered histone acetylation, in addition to increased DNA methylation [42]. Similarly, mice exposed to social defeat or isolation show increased levels of repressive histone marks, such as acetylation of histone H3 at lysine 4. Interestingly, these effects are reversible by administration of the antidepressant imipramine [43], while deacetylation of histones in the hippocampus blocks this effect [44]. In fact, histone deacetylation inhibitors show antidepressant effects in animal models of depression [45]. Immunological stress in the form of viral infection (pre- or post-natal) may also contribute to risk of schizophrenia [46].

The connection between schizophrenia risk and epigenetic effects has led to speculation that drugs acting on epigenetic pathways may be beneficial. For example, some studies in animal models suggest that the fear extinction response can be manipulated through modulation of histone deacetylase activity (HDAC) [47]. However, the exact relevance to schizophrenia, and issues concerning which brain regions are involved, and which specific HDAC isoforms should be targeted, make general conclusions problematic.

Experimental methods for investigating molecular epigenetic effects

The commercial availability of very high mass accuracy instruments in the last decade has led to improvements in quantitative proteomics studies, as well as increased confidence in molecular assignments when identifying proteins and PTMs. For example, acetylation and trimethylation of lysine, two critical epigenetic modifications on histones that are associated with different transcriptional outcome, differ by less than 0.04 Da. However these two modifications can be resolved using Orbitrap type instruments. In general, mass spectrometry analysis of histones can be described as 'top-down' (where the intact protein is ionized and analyzed), or 'bottom-up' (where the protein is first digested into peptides using a protease such as trypsin). Top-down approaches offer the possibility of a complete view of the individual histone molecule, including the exact state of PTMs present at a particular time. The drawbacks of the top-down approach are that it requires relatively specialized equipment and software, and that it needs large amounts of very pure sample [48]. A compromise approach, termed 'middle-down', involves analysis of long peptide fragments has shown considerable success [49]. Using the bottom-up approach, the very complex mixture of peptides (typically 1000's of distinct peptide molecules) produced following enzyme digestion are separated byonline reverse phase liquid chromatography (i.e. HPLC interfaced directly with the electrospray source on the mass spectrometer) [50, 51].

These approaches are particularly challenging for histone analysis for three reasons: First, the combinatorial arrangements of acetylation and methylation at distinct residues on a single peptide result in many isobaric molecular species. Second, histone proteolysis products tend to be hydrophilic, requiring adaptation of the HPLC gradients. Third, histones are enriched in the basic residues that are cleaved by tryptic enzymes and so produce small peptides that are difficult to analyze using mass spectrometry (and that lose valuable information concerning co-occurrence of neighboring histone marks). Generally, these obstacles are overcome through use of the enzyme Arg-C (which does not cleave after lysine residues), or by using alkylation chemistry [52]. The two main forms of alkylation of histones involve the use of propionic acid or acetic anhydride (the later generally in deuterated form to permit naturally occurring acetylation to be distinguished from the chemically introduced form) [50, 53]. A bonus of the chemical alkylation approach is that is alters the hydrophilic properties of many modified histone peptides, allowing chromatographic separation (and quantiation) of several isobaric peptide pairs that would otherwise be indistinguishable [51].

For many histone marks, relative abundance can therefore be compared when carefully controlled MS runs are carried out, by calculating the parent ion signal (MS1) for the corresponding peptide. This generally requires manual analysis of the mass spectrometry data using instrument-specific software to obtain extracted ion chromatographs (XIC), although recently developed programs such as MaxQuant [54] and Skyline [55] have considerably reduced the workload involved. In general, label-free methods can be employed, but where possible, metabolic labelling can be used to improve resolution [56]. Unfortunately, few cell culture models are available in schizophrenia research so metabolic labelling is usually not an option.

Characterization of the enzyme complexes mediating histone PTM deposition and removal also relies heavily on mass spectrometry. These studies range from analysis of the global chromatin proteome [57], to identification of the components of purified histone modifying protein complexes following affinity purification [58]. Recent developments include the use of affinity tagged peptides to isolate histone PTM 'readers' [59], and efforts to develop locus-specific recovery of histones using tagged nucleic acids or CRISPR-based reagents [60, 61]. Mass spectrometry also lends itself to the analysis of other epigenetic phenomena, for example the presence of histone variants. The histone 3 variants H3.1 and H3.3 for example can be distinguished from each other by a single amino acid difference in the N-terminal tail [62]. To date, these advanced approaches have not directly been applied to schizophrenia research. However, they are likely to offer exciting new viewpoints into the role of epigenetic mechanisms in the disease.

References

1. Kahn, R.S., et al., *Schizophrenia.* Nat Rev Dis Primers, 2015. **1**: p. 15067.
2. Renard, S.B., et al., *Unique and Overlapping Symptoms in Schizophrenia Spectrum and Dissociative Disorders in Relation to Models of Psychopathology: A Systematic Review.* Schizophr Bull, 2017. **43**(1): p. 108-121.
3. Miyamoto, S., et al., *Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents.* Mol Psychiatry, 2012. **17**(12): p. 1206-27.
4. Thornicroft, G., et al., *The personal impact of schizophrenia in Europe.* Schizophr Res, 2004. **69**(2-3): p. 125-32.
5. Olabi, B., et al., *Are there progressive brain changes in schizophrenia? A meta-analysis of structural magnetic resonance imaging studies.* Biol Psychiatry, 2011. **70**(1): p. 88-96.
6. Katsel, P., K.L. Davis, and V. Haroutunian, *Variations in myelin and oligodendrocyte-related gene expression across multiple brain regions in schizophrenia: a gene ontology study.* Schizophr Res, 2005. **79**(2-3): p. 157-73.
7. Martins-de-Souza, D., et al., *Alterations in oligodendrocyte proteins, calcium homeostasis and new potential markers in schizophrenia anterior temporal lobe are revealed by shotgun proteome analysis.* J Neural Transm (Vienna), 2009. **116**(3): p. 275-89.
8. Tkachev, D., et al., *Oligodendrocyte dysfunction in schizophrenia and bipolar disorder.* Lancet, 2003. **362**(9386): p. 798-805.
9. Sequeira, P.A., M.V. Martin, and M.P. Vawter, *The first decade and beyond of transcriptional profiling in schizophrenia.* Neurobiol Dis, 2012. **45**(1): p. 23-36.
10. Pocklington, A.J., M. O'Donovan, and M.J. Owen, *The synapse in schizophrenia.* Eur J Neurosci, 2014. **39**(7): p. 1059-67.
11. Birnbaum, R. and D.R. Weinberger, *Genetic insights into the neurodevelopmental origins of schizophrenia.* Nat Rev Neurosci, 2017. **18**(12): p. 727-740.
12. Lichtenstein, P., et al., *Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study.* Lancet, 2009. **373**(9659): p. 234-9.
13. Schizophrenia Working Group of the Psychiatric Genomics, C., *Biological insights from 108 schizophrenia-associated genetic loci.* Nature, 2014. **511**(7510): p. 421-7.

14. Birnbaum, R., et al., *Investigation of the prenatal expression patterns of 108 schizophrenia-associated genetic loci.* Biol Psychiatry, 2015. **77**(11): p. e43-51.
15. Jaffe, A.E., et al., *Mapping DNA methylation across development, genotype and schizophrenia in the human frontal cortex.* Nat Neurosci, 2016. **19**(1): p. 40-7.
16. Jenkins, A.K., et al., *Neurexin 1 (NRXN1) splice isoform expression during human neocortical development and aging.* Mol Psychiatry, 2016. **21**(5): p. 701-6.
17. Marshall, C.R., et al., *Contribution of copy number variants to schizophrenia from a genome-wide study of 41,321 subjects.* Nat Genet, 2017. **49**(1): p. 27-35.
18. Singh, T., et al., *Rare loss-of-function variants in SETD1A are associated with schizophrenia and developmental disorders.* Nat Neurosci, 2016. **19**(4): p. 571-7.
19. Hannon, E., et al., *Methylation QTLs in the developing brain and their enrichment in schizophrenia risk loci.* Nat Neurosci, 2016. **19**(1): p. 48-54.
20. Gusev, A., et al., *Transcriptome-wide association study of schizophrenia and chromatin activity yields mechanistic disease insights.* Nat Genet, 2018. **50**(4): p. 538-548.
21. Blackwood, D.H., et al., *Schizophrenia and affective disorders--cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family.* Am J Hum Genet, 2001. **69**(2): p. 428-33.
22. Millar, J.K., et al., *Disruption of two novel genes by a translocation co-segregating with schizophrenia.* Hum Mol Genet, 2000. **9**(9): p. 1415-23.
23. Margueron, R. and D. Reinberg, *Chromatin structure and the inheritance of epigenetic information.* Nat Rev Genet, 2010. **11**(4): p. 285-96.
24. Hoek, H.W., A.S. Brown, and E. Susser, *The Dutch famine and schizophrenia spectrum disorders.* Soc Psychiatry Psychiatr Epidemiol, 1998. **33**(8): p. 373-9.
25. St Clair, D., et al., *Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959-1961.* JAMA, 2005. **294**(5): p. 557-62.
26. Viana, J., et al., *Schizophrenia-associated methylomic variation: molecular signatures of disease and polygenic risk burden across multiple brain regions.* Hum Mol Genet, 2017. **26**(1): p. 210-225.
27. Montano, C., et al., *Association of DNA Methylation Differences With Schizophrenia in an Epigenome-Wide Association Study.* JAMA Psychiatry, 2016. **73**(5): p. 506-14.
28. Abdolmaleky, H.M., et al., *DNA hypermethylation of serotonin transporter gene promoter in drug naive patients with schizophrenia.* Schizophr Res, 2014. **152**(2-3): p. 373-80.
29. Ikegame, T., et al., *DNA methylation analysis of BDNF gene promoters in peripheral blood cells of schizophrenia patients.* Neurosci Res, 2013. **77**(4): p. 208-14.
30. Mill, J., et al., *Epigenomic profiling reveals DNA-methylation changes associated with major psychosis.* Am J Hum Genet, 2008. **82**(3): p. 696-711.
31. Thomas, E.A., *Histone Posttranslational Modifications in Schizophrenia.* Adv Exp Med Biol, 2017. **978**: p. 237-254.
32. Bannister, A.J. and T. Kouzarides, *Regulation of chromatin by histone modifications.* Cell Res, 2011. **21**(3): p. 381-95.
33. Strahl, B.D. and C.D. Allis, *The language of covalent histone modifications.* Nature, 2000. **403**(6765): p. 41-5.
34. Zentner, G.E. and S. Henikoff, *Regulation of nucleosome dynamics by histone modifications.* Nat Struct Mol Biol, 2013. **20**(3): p. 259-66.
35. Gavin, D.P., et al., *Dimethylated lysine 9 of histone 3 is elevated in schizophrenia and exhibits a divergent response to histone deacetylase inhibitors in lymphocyte cultures.* J Psychiatry Neurosci, 2009. **34**(3): p. 232-7.

36. Gavin, D.P., et al., *Reduced baseline acetylated histone 3 levels, and a blunted response to HDAC inhibition in lymphocyte cultures from schizophrenia subjects.* Schizophr Res, 2008. **103**(1-3): p. 330-2.
37. Sharma, R.P., D.R. Grayson, and D.P. Gavin, *Histone deactylase 1 expression is increased in the prefrontal cortex of schizophrenia subjects: analysis of the National Brain Databank microarray collection.* Schizophr Res, 2008. **98**(1-3): p. 111-7.
38. Benes, F.M., et al., *Regulation of the GABA cell phenotype in hippocampus of schizophrenics and bipolars.* Proc Natl Acad Sci U S A, 2007. **104**(24): p. 10164-9.
39. Jakovcevski, M., et al., *Prefrontal cortical dysfunction after overexpression of histone deacetylase 1.* Biol Psychiatry, 2013. **74**(9): p. 696-705.
40. Kurita, M., et al., *HDAC2 regulates atypical antipsychotic responses through the modulation of mGlu2 promoter activity.* Nat Neurosci, 2012. **15**(9): p. 1245-54.
41. Mitchell, A.C., et al., *MEF2C transcription factor is associated with the genetic and epigenetic risk architecture of schizophrenia and improves cognition in mice.* Mol Psychiatry, 2018. **23**(1): p. 123-132.
42. Monk, C., J. Spicer, and F.A. Champagne, *Linking prenatal maternal adversity to developmental outcomes in infants: the role of epigenetic pathways.* Dev Psychopathol, 2012. **24**(4): p. 1361-76.
43. Wilkinson, M.B., et al., *Imipramine treatment and resiliency exhibit similar chromatin regulation in the mouse nucleus accumbens in depression models.* J Neurosci, 2009. **29**(24): p. 7820-32.
44. Tsankova, N.M., et al., *Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action.* Nat Neurosci, 2006. **9**(4): p. 519-25.
45. Covington, H.E., 3rd, et al., *Antidepressant actions of histone deacetylase inhibitors.* J Neurosci, 2009. **29**(37): p. 11451-60.
46. Sorensen, H.J., et al., *Early developmental milestones and risk of schizophrenia: a 45-year follow-up of the Copenhagen Perinatal Cohort.* Schizophr Res, 2010. **118**(1-3): p. 41-7.
47. Bahari-Javan, S., et al., *HDAC1 regulates fear extinction in mice.* J Neurosci, 2012. **32**(15): p. 5062-73.
48. Tran, J.C., et al., *Mapping intact protein isoforms in discovery mode using top-down proteomics.* Nature, 2011. **480**(7376): p. 254-8.
49. Moradian, A., et al., *The top-down, middle-down, and bottom-up mass spectrometry approaches for characterization of histone variants and their post-translational modifications.* Proteomics, 2014. **14**(4-5): p. 489-97.
50. Garcia, B.A., et al., *Pervasive combinatorial modification of histone H3 in human cells.* Nat Methods, 2007. **4**(6): p. 487-9.
51. Soldi, M., M. Bremang, and T. Bonaldi, *Biochemical systems approaches for the analysis of histone modification readout.* Biochim Biophys Acta, 2014. **1839**(8): p. 657-68.
52. Bonaldi, T., J.T. Regula, and A. Imhof, *The use of mass spectrometry for the analysis of histone modifications.* Methods Enzymol, 2004. **377**: p. 111-30.
53. Smith, C.M., et al., *Heritable chromatin structure: mapping "memory" in histones H3 and H4.* Proc Natl Acad Sci U S A, 2002. **99 Suppl 4**: p. 16454-61.
54. Cox, J. and M. Mann, *MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification.* Nat Biotechnol, 2008. **26**(12): p. 1367-72.
55. MacLean, B., et al., *Skyline: an open source document editor for creating and analyzing targeted proteomics experiments.* Bioinformatics, 2010. **26**(7): p. 966-8.
56. Ramberger, E. and G. Dittmar, *Tissue Specific Labeling in Proteomics.* Proteomes, 2017. **5**(3).
57. Imhof, A. and T. Bonaldi, *"Chromatomics" the analysis of the chromatome.* Mol Biosyst, 2005. **1**(2): p. 112-6.

58. Oliviero, G., et al., *Dynamic Protein Interactions of the Polycomb Repressive Complex 2 during Differentiation of Pluripotent Cells.* Mol Cell Proteomics, 2016. **15**(11): p. 3450-3460.
59. Vermeulen, M., et al., *Quantitative interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers.* Cell, 2010. **142**(6): p. 967-80.
60. Dejardin, J. and R.E. Kingston, *Purification of proteins associated with specific genomic Loci.* Cell, 2009. **136**(1): p. 175-86.
61. Waldrip, Z.J., et al., *A CRISPR-based approach for proteomic analysis of a single genomic locus.* Epigenetics, 2014. **9**(9): p. 1207-11.
62. Loyola, A., et al., *PTMs on H3 variants before chromatin assembly potentiate their final epigenetic state.* Mol Cell, 2006. **24**(2): p. 309-16.