Spring 2021 – Epigenetics and Systems Biology
Lecture Outline (Systems Biology)
Michael K. Skinner – Biol 476/576
Weeks 5, 6 and 7 (February 2021)

Epigenetics (History / Molecular Processes/ Genomics)

- Definitions and History
- Molecular Factors (DNA Methylation, Histone Modification, Chromatin Structure, ncRNA)
- Epigenetics Technology and Genomics

Required Reading

Holliday R. Epigenetics: a historical overview. Epigenetics. 2006 Apr-Jun;1(2):76-80.

Books (Reserve in Library)

Kevin V. Morris (2012) Non-coding RNAs and Epigenetic Regulation of Gene Expression: Drivers of Natural Selection. Caister Academic Press.

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Literature

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Lee J-H, Xiong F, Li W. Enhancer RNAs in cancer: regulation, mechanisms and therapeutic potential. RNA Biol. 2020 Nov;17(11):1550-1559.
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Review

Epigenetics

A Historical Overview

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ABSTRACT

In the first half of the twentieth century, developmental biology and genetics were separate disciplines. The word epigenetics was coined by Waddington to link the two fields. Epigenetics could be broadly defined as the sum of all those mechanisms necessary for the unfolding of the genetic programme for development. Several decades later specific mechanisms were proposed in which information was superimposed on DNA sequences. In particular, it was suggested that 5-methyl cytosine had a role in controlling gene expression, and also that the pattern of methylation was heritable. These predictions are now supported by a large body of evidence which shows that methylation is strongly associated with gene silencing in a variety of biological contexts. There are now also many examples of epigenetic inheritance through the germ line There are several other important epigenetic mechanisms involving chromatin and histone modifications, and also the expanding field of regulatory RNAs. The human epigenome project will unravel the pattern of DNA methylation in different tissues, and will this determine whether the regulation of gene expression is at the level of DNA or chromatin, or both.

INHERITANCE AND DEVELOPMENT

In the nineteenth century the leading biologists considered inheritance and development to be one and the same problem. The genius of Gregor Mendel was to realize, and then to demonstrate, that inheritance could be studied on its own, without including development. In a scholarly review and discussion of nearly 70 pages, Sandler and Sandler[1] explain that this was the major reason why Mendel's work was ignored by the leading biologists of his day. When it was finally re-discovered thirty five years later, the science of genetics subsequently flourished. Again, the problem of development was sidelined, and it is remarkable that one of the pioneers of the new genetics, Thomas Hunt Morgan, was by background an embryologist, but his laboratory did not study *Drosophila* development. It was only in his books that he re-visited embryology.

Whilst the science of genetics was making rapid progress, embryologists and developmental biologists were using methods and procedures that took little account of genes and gene action. Towards the middle of the twentieth century, there were a few leading biologists who realized that genetics and developmental biology were indeed related and should eventually come together in a common discipline. One was Conrad Waddington, who was knowledgeable in both fields of research. He took the Greek word epigenesis, a theory of development which proposed that the early embryo was undifferentiated, and changed it to epigenetics.[2] He was the Buchanan Professor of Genetics at Edinburgh University, and he also set up an Epigenetics Research Unit supported by the Medical Research Council for some years. Epigenetics could be broadly defined as the unfolding of the genetic program for development, but to Waddington, epigenetics was not very different from embryology. For example, his book *The Epigenetics of Birds* is largely an account of the development of the chick.[3] He also coined the term epigenotype, which was defined as "The total developmental system consisting of interrelated developmental pathways through which the adult form of the an organism is realized."[2] This is so broad that it is not very useful, and I will return to a more specific definition of the epigenotype later on.

The another leading biologist interested in both genetics and development was Ernst Hadorn in Zurich. Many of his studies were on mutations that affect *Drosophila* development, and he also wrote a book *Developmental Genetics and Lethal Factors*.[4] He also worked for many years on the remarkable properties of the imaginal discs of *Drosophila*. These are regions of embryonic tissue that are present in fly larvae. Each disc will later

develop into a specific adult structure: two for each wing, two for antennae, and so on. The disc cells are completely undifferentiated, but it can be said that they are determined to differentiate later on. Hadorn and his colleagues grew disc tissue in the abdomen of adult flies, and passaged it from fly to fly. When the disc tissue was treated with the hormone ecdysone, it differentiated into the appropriate adult structure. In other words, the determined state was heritable, sometimes for hundreds of cell divisions. However, from time to time the disc changed from one determined state to another, for example from a leg to a wing. This event was called transdetermination, and in innumerable studies it was shown that transdetermination followed certain pathways. For example, disc A could change into disc B, and B to C, but A never changed directly into C. This remarkable experimental system (reviewed in ref. 5) has not been exploited in modern experimental studies. Everything that is known about it comes from Hadorn's laboratory years ago.

Waddington and Hadorn were not the only important biologists who wanted to make connections between genetics and development. Another was Richard Goldschmidt, but his views were quite controversial (see ref. 6). Others, such as Julian Huxley[7] and J.B.S. Haldane, certainly understood the importance of the relationship, but the latter was particularly interested in the the biochemistry of gene activity. In this area there had been the early insights of Garrod, who realized that some inherited defects in man blocked specific steps in metabolic pathways.[8] This interpretation was ignored for many years, until Haldane became involved in the genetics of pigment formation in plants, and Ephrussi and Beadle attempted similar studies in *Drosophila*. Finally, Beadle and Tatum started to isolate biochemical mutants and their effects on metabolic pathways in Neurospora. Their work was very successful and culminated in the concept of one gene-one enzyme,[9] which was eventually verified in the 1950s. However, it was independent of studies of development.

After Waddington, there was spasmodic discussion of epigenetics by several scientists; much of this was reviewed by Nanney[10] and much more recently by Haig.[11] Some of the examples related to cytoplasmic inheritance, the phenotypes of cultured mammalian cells, or cancer cells. In general, observations that were not easily interpreted in genetic terms but had a heritable component, were liable to be labeled epigenetic. However, each author had his own idea of the meaning or definition of epigenetics. and no specific mechanisms were proposed. This was also true of the earlier work of Waddington, although he did introduce important new concepts such as canalization.[12]

THE NEED FOR EPIGENETIC MECHANISMS

The importance of the work of Waddington and Hadorn was to relate genes and gene action to development, in an environment in which most geneticists and most developmental biologists were not communicating with each other. As time went on, it became apparent that there were certain fundamental features of development that demanded explanation. One was the fact that differentiated cells, such as fibroblasts or lymphocytes, stably maintain their phenotypes through cell division. This means that some specialized genes which determine the phenotype of differentiated cells are permanently turned on, and other genes—active in some other cell type—are permanently turned off. These controls are heritable, just as the determined state of *Drososphila* disc cells are heritable. Traditionally, inheritance refers to the transmission of genes from generation to generation, but it was now realized that there is also mitotic inheritance in somatic cells of higher organisms. Of course, such inheritance had long been studied in yeasts and fungi, and then in cultured mammalian cells, but it had rarely been spelled out that it also regularly occurred in vivo, that is, in the normal somatic cells of higher organisms with specialized phenotypes. Another feature of higher organisms is the stem cell. Here an undifferentiated cell divides to produce a differentiated cell, and another undifferentiated stem cell. In the case of bone marrow stem cells, a variety of blood cell types are produced. In this situation there are clearly switches in gene activity associated with cell division. A third example is the X chromosome of female eutherian mammals. Early in development one X chromosome is randomly inactivated in every cell, whilst the other remains active. These two chromosome have almost identical DNA sequences, and they reside in a common nucleoplasm and cytoplasm, so the differences in gene activity are intrinsic to the chromosomes themselves. It is evident that there is a switch mechanism early in development, the result of which is the inactivity of one chromosome and the activity of the other. The switch is random and once made it is permanent. This example therefore embodies both a switch in gene activities and also its subsequent heritability.

The first suggestion that DNA methylation (or demethylation) might have an important biological role was made by Griffith and Mahler, who proposed in 1969 that it could provide a basis for long term memory in the brain.[13] In 1975 two papers were published which outlined a molecular model for the switching of gene activities, and also the heritability of gene activity or inactivity. It was based on the enzymic methylation of cytosine in DNA, which can also be referred to as DNA modification. The proposals by Riggs[14] and Holliday and Pugh[15] were very similar, but were made completely independently of each other. The suggestion was that DNA methylation could have strong effects on gene expression, and that changes in DNA methylation might therefore explain the switching on and off of genes during development. The enzyme(s) methylating a particular region of DNA would be sequence specific, or interact with another protein that was sequence specific. It was also proposed that the pattern of methylation could be heritable, if there was an enzyme called a maintenance methylase that recognized hemimethylated DNA soon after replication, but did not act on unmethylated DNA. This provides a mechanism for the heritability of the methylated and non-methylated state of DNA, and therefore for the heritability of a given pattern of gene activities. The issue of X chromosome inactivation was addressed particularly by Riggs. There might be an initial methylation that was immediately shut off, so that only one chromosome is marked. There would also have to be a spreading mechanism which inactivated the whole chromosome. Since it was much easier to envisage a processive methylating enzyme than the reverse, this implies that methylation of DNA is associated with gene inactivity. This can also explain the inactivation or silencing of autosomal DNA in several cases of X-autosome translocations.

There was also the possibility that developmental clocks might be important in unfolding the genetic program for development. This would be a mechanism that counts a specific number of cell divisions before a given gene or genes is activated or inactivated, and several molecular models were discussed.[15] Although there is scattered evidence for developmental clocks, it is not a commonly discussed topic, and only time will tell whether they are a significant component of development. As well as DNA methylation, there was also the possibility that specific base changes might occur, for example, the enzymic deamination of 5-methyl cytidine to form thymidine, and thus the substitution of an G-C base pair by a A-T base pair, a mechanism

that had previously been proposed by Scarano.[16] The existence of the enzyme cytidine deaminase which converts cytosine to uracil in DNA is now very well documented in the immune system and also in pluripotent cells.[17,18]

A third paper on DNA methylation by Sager and Kitchin also appeared in 1975, which proposed that there are enzymes in eukaryotic organisms that restrict unmodified DNA.[19] They explored the possibility that the many known examples of chromosome elimination or silencing might involve such a mechanism. It also became apparent that changes in DNA methylation might be important in tumor progression.[20,21] There was much accumulating evidence that changes in gene expression in cancer cells was due to mutation, but if the methylation model was correct, then aberrant changes in the distribution of 5-methyl cytosine in cancer cells could also result in changes in gene expression. The word epigenetics was not used in any of the 1975 papers on DNA methylation and gene expression, possibly because it had previously been used in several quite different contexts and remained undefined.[10,11]

EVIDENCE RELATING DNA METHYLATION TO GENE EXPRESSION

In 1975 when the DNA methylation models were proposed, there was no experimental evidence to support them. Nor did the models predict that specific DNA methylation would be associated with the activity or inactivity of genes. However, the spreading model for X chromosome inactivation did propose that methylation was the basis for such inactivation. With the cloning and sequencing of DNA, means were discovered for screening DNA methylation in specific DNA sequences. There were restriction enzymes which recognize and cut unmethylated sequences of DNA (usually four or six bases). In some cases there were two restriction enzymes which recognized the same base sequences, but only one of them would cut this sequence when it was methylated. This pair of enzymes were called isoschizomers, and examples were Hpa II and Msp I. Both cut DNA at GCGC sites, but only Msp cuts this sequence if the internal C is methylated. Using Southern blots it became possible to determine whether a given sequence containing a GCGC site was methylated or not. It was soon discovered that many genes with methylated promoter regions were inactive, and also that the corresponding active gene was unmethylated. This early work was reviewed by Doerfler.[22,23] A limitation of the method is that it detects only a subset of possible methylation sites, usually about 10%. Later on a more powerful method was introduced which can detect all methylated and non-methylated cytosine sites in a given stretch of DNA (see below).

Other evidence for the significance of DNA methylation came from the use of the nucleoside analogue 5-azacytidine. This is incorporated into DNA, inactivates DNA methyl transferase and thereby demethylates DNA. It was shown in many contexts that azacytidine reactivates silent genes, often at very high frequency (reviewed in ref. 24). This included the reactivation of genes on the inactive X chromosome. It had been shown that strains could be isolated in cultured mammalian cells which had biochemical deficiencies. Originally it was thought that these were mutations, but it now became apparent that they were often genes silenced by methylation, reactivable by 5-azacytidine.[25]

DEFINITIONS OF EPIGENETICS

Waddington did not use a specific definition for epigenetics. What he had in mind was: "All those events which lead to the unfolding of the genetic program for development." There is nothing wrong with that, except that it is not very specific. By the mid-1980s it was clear that there was a new type of inheritance, not based on changes in DNA sequence. In 1987 I wrote a paper "The inheritance of epigenetic defects."[26] In this I re-visited Waddington's use of the term, and I applied it to situations where changes in DNA methylation also changed gene activity. Possible epigenetic changes in cancer and also in ageing were discussed, and it was also suggested that some transgenerational effects that could not easily be explained by Mendelian genetics, might sometimes be due to the transmission of DNA methylation, or lack of it, through the germ line. It was also possible that some epigenetic defects might be recognized and repaired by genetic recombination at meiosis. At this time the word epimutation was introduced to describe heritable changes in genes which were not due to changes in DNA sequence. It has been suggested that this 1987 publication "was the critical paper that lit the fuse for the explosion in use of 'epigenetic' in the 1990s".[11]

Genomic imprinting in mammals had by now been discovered, and it was apparent that this was due to information superimposed on DNA that could be reversed at meiosis or during gametogenesis. New definitions of epigenetics were needed, and two were suggested in 1994: 1) The study of the changes in gene expression which occur in organisms with differentiated cells, and the mitotic inheritance of given patterns of gene expression, and 2) Nuclear inheritance which is not based on changes in DNA sequence.[27] The first definition is quite broad, which can include DNA methylation, but also a number of other mechanisms. The second definition includes imprinting and many other documented cases of epigenetic inheritance. It excludes cytoplasmic events, but they can be included in the first definition. Both definitions are in fact incomplete, but they seem to cover most known epigenetic processes. They do not include development itself, and for that we can use Waddington's general definition, which will become more specific as new information accumulates in the future.

DIFFERENCES BETWEEN GENETIC AND EPIGENETIC SYSTEMS

Much less is known about the epigenetic inheritance system than traditional genetics. Genetics is based on cell lineages and clonal inheritance. Gametogenesis produces single haploid cells that fuse to form a diploid zygote. The organism thus starts as a single cell, and ends up as a clone of cells. If a mutation or chromosomal change occurs in a somatic cell, then all its descendents would be expected to have the same genotype. In contrast, epigenetic changes often occur in groups of cells, for example, the induction of muscle tissue in mesoderm cells. This is due to a specific signal which impinges on a group of cells with the same receptor. Some epigenetic events are clonal, and X chromosome inactivation is an excellent example. Genetic changes are stable and rarely reversed, whereas epigenetic changes are often reversed. A good example of that is genomic imprinting, where the changes imposed on DNA sequences may be lost during development, or if they persist, are erased and re-set during gametogenesis. Environmental influences do not change the genotype (leaving aside mutagens), and there is no inheritance of acquired characteristics. Epigenetics is quite different, because normal development depends on communication between cells. Thus, a hormone, morphogen or growth factor may induce an epigenetic change that

may be heritable. This means that the environment of a cell may be all important in determining its properties or its fate in the developing organism. In this sense, epigenetics encompasses Lamarckian inheritance.

Maynard Smith[28] introduced the term dual inheritance, by which he meant that there is classical inheritance based on changes in DNA sequence, and also epigenetic inheritance which is not based on changes in DNA sequence. He was responding to the proposals by Jablonka and Lamb[29,30] that epigenetic inheritance in the germ line might introduce the possibility that environmental influences which induce phenotypic changes could become heritable. There are now many well documented examples of transgenerational effects, presumed to have an epigenetic basis.[31-38] Dual inheritance has also been demonstrated in experiments with cultured mammalian cells.[39] In some cases, what had long been thought to be a classical mutation has been shown to be due to a heritable change in DNA methylation, and a good example of that is a well known change in floral symmetry,[40] which can now be labeled as an epimutation. Transgenerational epigenetic inheritance and related topics have been recently reviewed by Jablonka and Lamb.[41]

It is well established that DNA methylation is involved in genomic imprinting, but the biological reasons for the existence of imprinting remain a matter for debate. (reviewed in ref. 42). One interesting possibility arises from the fact that imprinting results in haploid gene activity, because one of the gametes has an inactive gene. It may be important in early development to have single copies of single genes, particularly if a switch in gene activity takes place prior to or during division. Switching two copies has more than one consequence, but switching one simply leads to a plus and minus situation.[43] A challenge for the future is the unravelling the specificity of genomic re-programming when the germ cells and fertilized egg are formed. Little is yet known about this, although it is established that there are massive changes in DNA methylation at this time and also in early development. These are global changes, whereas information is needed about specific changes, as have been established in the case of imprinting.

OTHER EPIGENETIC MECHANISMS

Chromatin structure and gene expression has become an intensively active field of research. Chromatin can be in the open form that allows access of the machinery for transcription, and a closed form which does not allow transcription. The modification of histones, particularly acetylation and methylation, play a crucial role in this change, and many believe that it is this switch, rather than DNA methylation, which is the more important (reviewed in ref. 44). However, it is not at all obvious how chromatin configurations can be stably inherited. The evidence that DNA methylation can provide a primary switch is very strong,[45] and one likely possibility is that the presence of such methylation triggers the changes that lead to the closed chromatin configuration.

The role of RNA in epigenetic events has become increasingly important. The alternative splicing of gene transcripts can be regarded as an epigenetic mechanism This can produce many isoforms of a given protein that have subtly different properties, and distinct cell types are likely to have specific isoforms. The specificity of splicing events remains a problem, which might be solved if there were small RNA molecules that hybridized across splice junctions.[46] Another prediction is that there are large RNA molecules in the egg or early embryo that have an essential spatial, positional or structural role.[47] This could be essential for the correct 3-dimensional distribution of proteins. If substantiated, this can also be regarded as an epigenetic mechanism. It is now evident that there are a huge number of small regulatory RNA molecules in cells (reviewed in refs. 48–50), and their activities comprise new epigenetic controls. An exciting possibility is that some of these molecules can transmit signals by moving from one cell to another.

The DNA sequence remains constant in most somatic cells, but there is a special epigenetic mechanism in cells of the immune system that can join one constant and one variable sequence, from a pool of such sequences in the whole region, to form a particular genotype that is clonally inherited. Another mechanism to generate antibody variability depends on enzymes that can deaminate cytosine to uracil, or 5methyl cytosine to thymine.[17] This is in effect a mutation, but induced by an enzyme. It could be argued that such a mutation is not an epigenetic change, but it is certainly the result of a protein-DNA interaction and in this respect is epigenetic.[18]

THE GENOME, THE EPIGENOME AND EPIGENOTYPES

In the sequence of the human genome there are just four bases, yet with cytosine in methylated or non-methylated form, there are five, and there is the possibility of six.[51] The epigenome project sets out to determine the pattern of cytosine methylation in a variety of cell types.[52] This depends on the bisulphite sequencing technique introduced in 1992.[53] Since then the technique has been greatly improved, but the underlying chemistry remains the same. It relies on the fact that bisulphite can deaminate cytosine to uracil under conditions in which 5-methyl cytosine is not deaminated. Thus when bisulphite-treated DNA is amplified and sequenced, all the 5-methyl cytosine residues remain as cytosine, but the non-methylated cytosines have become thymines. This technique has been applied in a large number of contexts, and particularly to demonstrate the methylation of many inactive tumor suppressor genes in cancer cells.[54]

The epigenome project will take a long time to complete; nevertheless along the way, we can expect that interesting information will be continually uncovered. We might expect that some regions of the DNA will have the same, or a very similar pattern of methylation in all cell types. These sequences will include many repetitive or transposable elements which have entered the genome at some time and have been silenced by DNA methylation. Much more interesting information will come from specialized genes that are active in one cell type and inactive in another. The importance of DNA methylation in determining the cell phenotype will then be revealed. In the epigenome project, a new terminology will be necessary to classify differences in DNA methylation between cell types.

This introduces the concept of the epigenotype. It has been suggested that the epigenotype is the actual pattern of gene activity in a specialized cell type.[55] These cells are said to have household enzymes and proteins, necessary for normal metabolism in all cell types, and also luxury proteins which have specialized functions. The epigenotype includes all those genes necessary for both household and luxury functions, and also those that are silent or repressed in a given cell type. Thus, fibroblasts and lymphocytes have the same genotype, inherited from the fertilized egg, but they have very different epigenotypes. Of course, as in the case of genotypes, any terminology may apply just to one gene or a subset of genes.

CONCLUSIONS

This overview began with a brief historical account of genetics and developmental biology, and how they diverged for a major part of the twentieth century. Epigenetics is the field that attempted to unite them, and provide new insights into the mechanisms for unfolding the genetic program for development. In the last two decades of the twentieth century much progress has been made on the relationship between DNA methylation and gene expression in a variety of biological contexts, and the experimental study of epigenetics was established. The field has now widened to include another of other mechanisms, especially those involving RNA. Many new insights into the mechanisms for development will be gained in this century.

The sequencing of the human genome is being followed by the epigenome project, which will eventually unravel the significance of DNA methylation in the control of specialized gene functions. It will become apparent whether the primary controls are at the DNA or at the chromatin level. In either case, the nature of the continual interactions between proteins and DNA will further advance the field of epigenetics, and illuminate current problems, such as the re-programming of the genome which initiates the normal processes of development.

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Spring 2021 – Epigenetics and Systems Biology
Lecture Outline (Systems Biology)
Michael K. Skinner – Biol 476/576
Weeks 5, 6 and 7 (February 2021)

Epigenetics (History / Molecular Processes/ Genomics)

- Definitions and History
- Molecular Factors (DNA Methylation, Histone Modification, Chromatin Structure, ncRNA)
- Epigenetics Technology and Genomics

Required Reading

Holliday R. Epigenetics: a historical overview. Epigenetics. 2006 Apr-Jun;1(2):76-80.

Books (Reserve in Library)

Kevin V. Morris (2012) Non-coding RNAs and Epigenetic Regulation of Gene Expression: Drivers of Natural Selection. Caister Academic Press.

Russo, V.E.A., Martienssen, A.and Riggs, A.D. (eds.). 1996. Epigenetic Mechanisms of Gene Regulation. Cold Spring Harbor Press. Cold Spring Harbor.

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Spring 2021 – Epigenetics and Systems Biology
Discussion Session (Epigenetics)
Michael K. Skinner – Biol 476/576
Week 5 (February 18)

Epigenetics (History / Molecular Processes/ Genomics)

Primary Papers

1. Singer J, et al. (1977) J Biol Chem. 10;252(15):5509-13. (Classic) (PMID: 195953)
2. Holliday and Pugh (1975) Science 24;187(4173):226-32. (PMID: 1111098)
3. Wen, et al. (2012) BMC Genomics 13:566. (PMID: 23102236)
4. Kempfer and Pombo (2020) Nat Rev Genet. Apr;21(4):207-226. (PMID: 31848476)

Discussion

Student 10 – Ref #1 & 2 above

- How did BRdU effect DNA methylation?
- What new assay for DNA methylation was developed?
- What observations were used to document DNA methylation?

Student 11 – Ref #3 above

- How does euchromatin correlate to DNA methylation?
- What is CTCF and its function?
- What integration in epigenetics is observed?

Student 12 – Ref #4 above

- What are chromatin remodeling proteins?
- What are the different methods for chromatin structure mapping?
- What are the functions of the epigenetic modifications?

Spring 2021 – Epigenetics and Systems Biology
Discussion Session (Epigenetics)
Michael K. Skinner – Biol 476/576
Week 6 (February 25)

Epigenetics (History / Molecular Processes/ Genomics)

Primary Papers

1. Cuerda-Gil and Slotkin (2016) Nat Plants 2(11):16163. (PMID: 27808230)
2. Rao et al. (2014) Cell 159:1665. (PMID: 25497547)
3. Morris and Mattick (2014) Nat Rev Genet. 15(6):423-37. (PMID: 24776770)

Discussion

Student 13 – Ref #1 above

- What is RdDM and its structure?
- What are the effects of RdDM and different mechanisms?
- How are histone modifications linked to DNA methylation?

Student 14 – Ref #2 above

- What is mitotic technology used to map genome structure?
- What are the different loops identified and role CTCF?
- What is the hypothesis on the role of chromatin looping?

Student 15 – Ref #3 above

- What are non-coding RNAs?
- How does ncRNA influence gene expression?
- How do lncRNA influence chromatin structure?

Spring 2021 – Epigenetics and Systems Biology
Discussion Session (Epigenetics)
Michael K. Skinner – Biol 476/576
Week 7 (March 4, 2021)

Epigenetics (History / Molecular Processes / Genomics)

Primary Papers

1. Yao B, et al, (2018) Mol Cell. 2018 Sep 6;71(5):848-857.e6. (PMID: 30078725)
2. Booth, et al. (2012) Science 336:934. (PMID: 22539555)
3. Kelsey, et al. (2017) Science 358:69. (PMID: 28983045)

Discussion

Student 16 – Ref #1 above

- What epigenetic mark was identified?
- What was the technology used?
- What function does the epigenetic mark have?

Student 17 – Ref #2 above

- What is hydroxymethylcytosine and how distinct from 5mC?
- What technology was used?
- What is the function of 5hmC and where expressed?

Student 18 – Ref #3 above

- What epigenetic marks were identified?
- What technology was used?
- How did the genomic profiling correlate with cellular differentiation?

Epigenetic Definition

Conrad Waddington in the 1940s introduced the term epigenetic, but he had a very broad definition, namely:

"The total developmental system consisting of interrelated developmental pathways through which the adult form of an organism is realized".

This is too general and all-embracing to be useful, hence the more specific definition introduced here.

Huxley (1957) used epigenetics "to denote the analytic study of individual development (ontogeny) with its central problem of differentiation."

For Huxley, "The method by which tissues and organs differentiate in the course of normal development is at the moment the main blank space in biology's map. . .

In 1990, Holliday defined epigenetics as 'the study of the mechanisms of temporal and spatial control of gene activity during the development of complex organisms'.

His definition rescues Waddington's original meaning of developmental biology, although it does not differentiate between the action of what we currently know as epigenetic mechanisms and the action of genetic regulators of gene expression such as transcription factors.

Riggs and colleagues in 1996 states that epigenetics is

'the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence'.

However, the term heritable is generally used in reference to generational inheritance and is not associated with growth of cells or tissues.

Bird (1990s) defines epigenetics as the 'structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states'.

Because there are several epigenetic elements that do not fit into this definition such as non-coding RNA and minor modifications of histones and DNA methylation of promoters, this definition appears insufficiently global to encompass all of epigenetics.

Epigenetics

Molecular factors/processes around the DNA that regulate genome activity, independent of DNA sequence, and are mitotically stable

Opposition

Epigenetics is an attempt to oppose the reductionist, piecemeal approach of genetics: epigenetics—and what belongs to epigenetics—can be defined only as a reaction against the current, dominant, reductionist approach of genetics. (Michel Morange 2002)

Epigenetic History

An Epigenetic Perspective on the Midwife Toad Experiments of Paul Kammerer (1880-1926).
J Exp Zool B Mol Dev Evol. 2017 Jan;328(1-2):179-192.
Vargas AO, Krabichler Q, Guerrero-Bosagna C.

Kammerer's hybrid cross using a modified male "water-breeding" toad and an unmodified (normal) female "land-breeding" toad. Kammerer reported obtaining a result like that of a Mendelian cross, with dominance of "water-breeding" toads, which were 100% of all toads in the F1 generation, and roughly three-fourths of all toads in the F2 generation. Note that the F1 are water-breeding toads despite coming from eggs that grew on land, exposed to dry conditions. Likewise, the F2 generation includes one-fourth of land-breeding toads from eggs that grew submerged in water. Drawings of the toads are taken from Kammerer (1910). N is for "Normal" and V for "Verändert" (meaning "changed").

History of Epigenetics

1940s Conrad Waddington defined epigenetics as environment-gene interactions induce phenotype.

1975 Holliday and Pugh, and Riggs identify DNA methylation

1988 X- chromosome inactivation and DNA methylation

1990s Imprinted genes, allelic expression and DNA methylation

1995s Histone modifications and chromatin structure

2000s Small micro RNAs

2005s Epigenome mapping

Waddington argued the development of differences within a single organism, for example:

the difference between an eye and a nose…is clearly *neither* genotypic nor phenotypic. It is due…to the different sets of developmental processes which have occurred in the two masses of tissue; and these again can be traced back to local interactions between the various genes of the genotype and the already differentiated regions of the cytoplasm in the egg. One might say that the set of organizers and organizing relations to which a certain piece of tissue will be subject during development make up its *epigenetic constitution or epigenotype*; then the appearance of a particular organ is the product of the genotype and the epigenotype, reacting with the external environment." [italics added]

development as an *epigenetic process*

FROM

genotype + environment = phenotype

TO

genotype + *epigenotype* + environment = particular phenotype

⇓

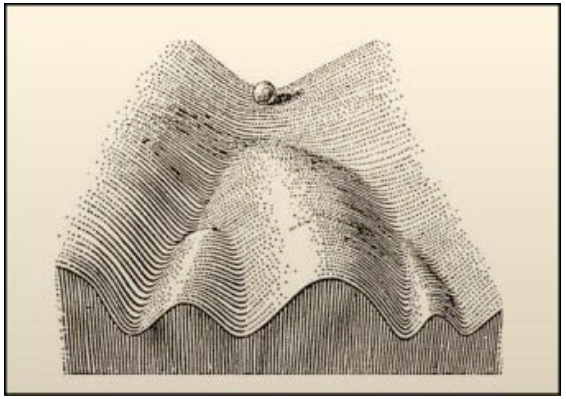
= epigenetic constitution of tissue/cell

= set of organizers and organizing relations to which tissue is subject during development

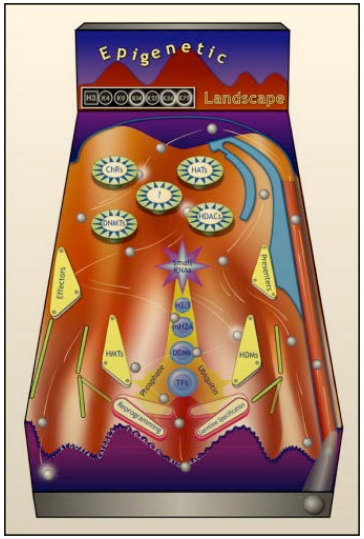
Figure 2. Scheme of Waddington's expansion of the classical model on the phenotype-genotype distinction.

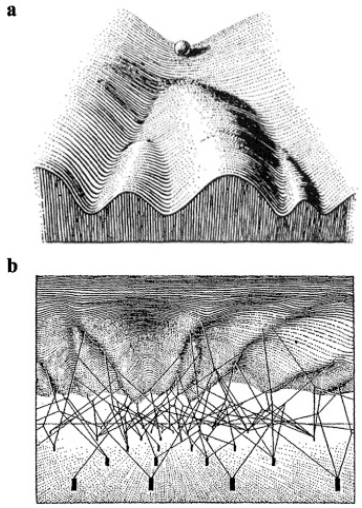
Waddington's Evo-Devo Program

Stressing the developing phenotype in evolutionary theory expands the classical focus on the transmission of genetic information with a second focus on gene regulation or instructions on how to use the genetic information. To bring evolution and development to full synthesis, however, a developmental theory is needed. Therefore, Waddington's epigenetics mainly situates itself on the developmental plane, as a model to link the genotype and the phenotype during development in a specific environmental context.



Waddington's Classical Epigenetic Landscape
In 1957, Conrad Waddington proposed the concept of an epigenetic landscape to represent the process of cellular decision-making during development. At various points in this dynamic visual metaphor, the cell (represented by a ball) can take specific permitted trajectories, leading to different outcomes or cell fates. Figure reprinted from Waddington, 1957.





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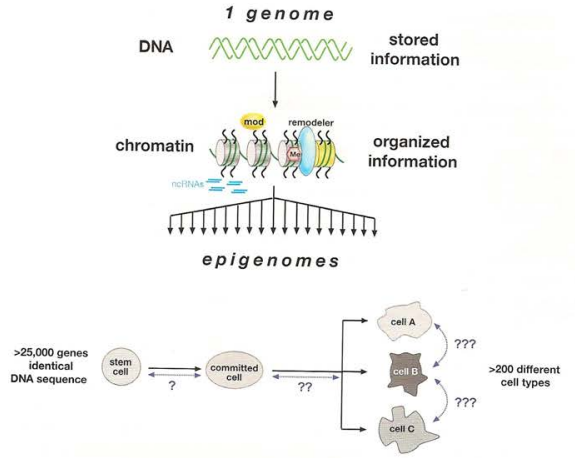
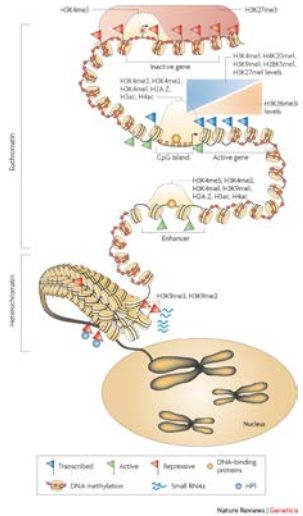


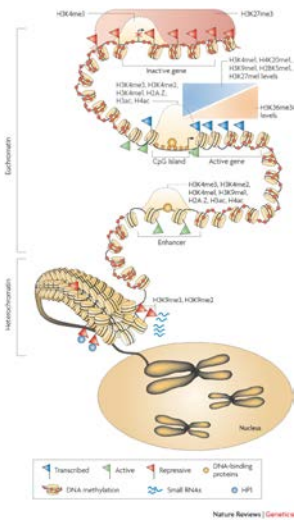
Epigenetic Mechanisms of Gene Regulation

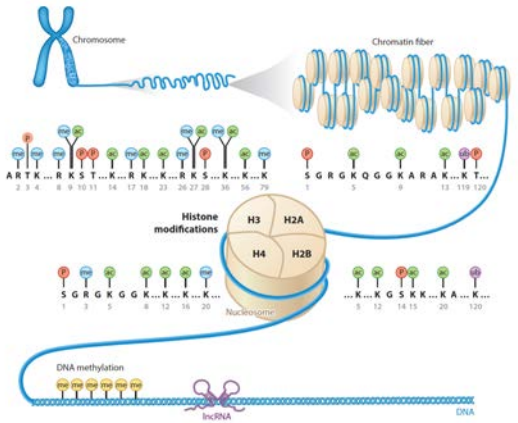
-DNA Methylation
-Histone Modification
-Chromatin Structure
-DNA Organization into Domains (eg Loops)
-Nuclear Compartmentalization (eg nuclear matrix)
-Noncoding functional RNAs



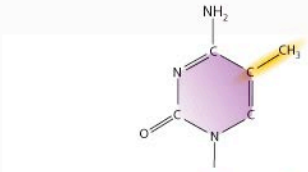
Epigenetic Mechanisms of Gene Regulation

- -DNA Methylation
- -Histone Modification
- -Chromatin Structure
- -DNA Organization into Domains (eg Loops)
- -Nuclear Compartmentalization (eg nuclear matrix)
- -Noncoding functional RNAs
- -RNA Methylation
- - Adenine DNA Methylation

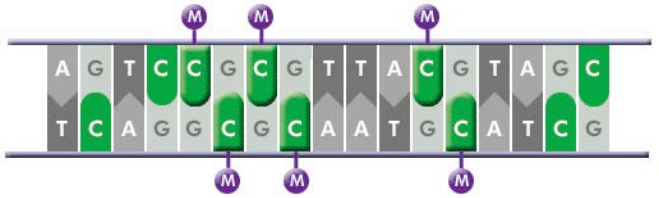


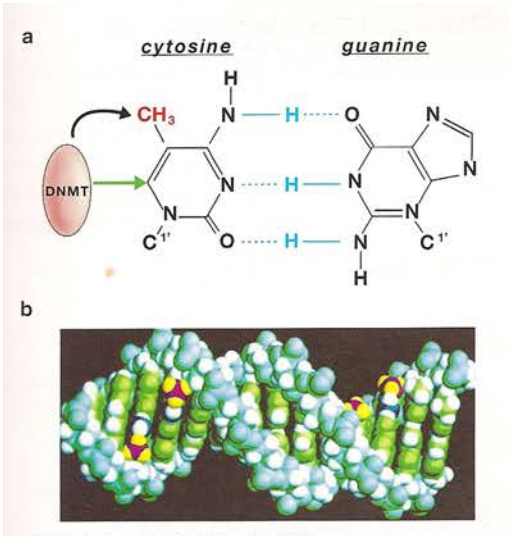


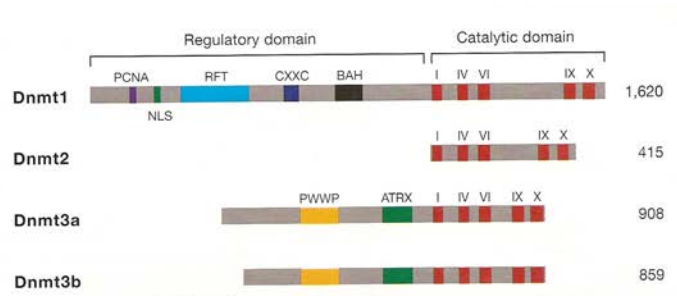
Epigenetics
DNA Methylation

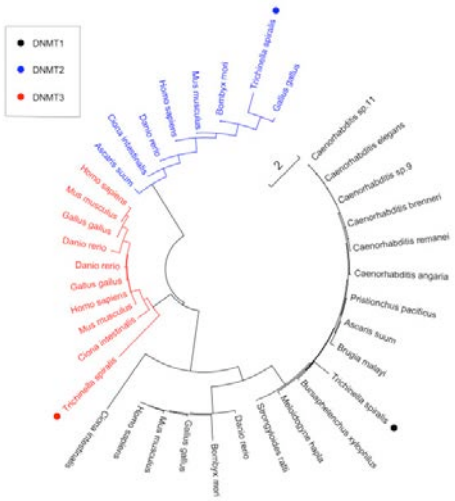


DNA Methylation is the addition of a methyl group (M) to the DNA base cytosine (C) in a CpG sequence

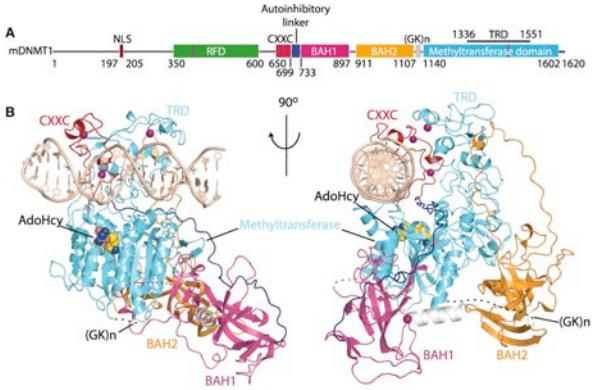








Structure of DNMT1-DNA complex reveals a role for autoinhibition in maintenance DNA methylation.
Song J, et al. Science. 2011 Feb 25;331(6020):1036-40



Structural overview of mDNMT1(650–1602)–DNA 19-nucleotide oligomer complex with bound AdoHcy. (A) Color-coded domain architecture and numbering of mDNMT1 sequence. The thin vertical light blue bars indicate binding positions of zinc ions. (B) Ribbon representation of the complex in two orthogonal views. The CXXC, BAH1, BAH2, and methyltransferase domain are colored in red, light purple, orange and light blue, and DNA and zinc ions are colored in light brown and dark purple, respectively; CXXC-BAH1 linker in dark blue, BAH1-BAH2 linker in silver, (GK)n-containing BAH2-methyltransferase linker in black, and bound AdoHcy as in space-filling representation.

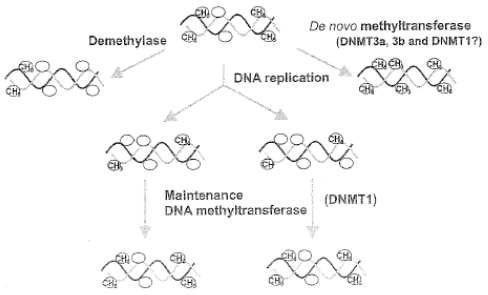
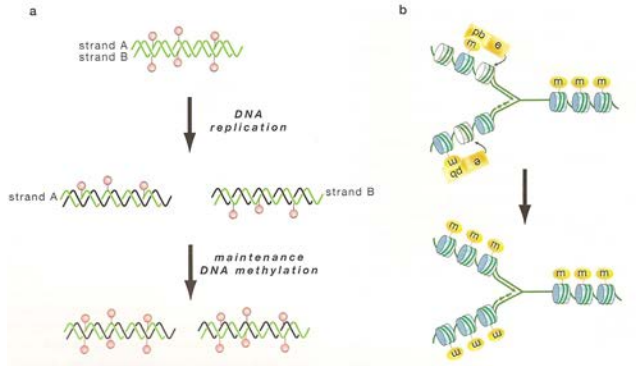
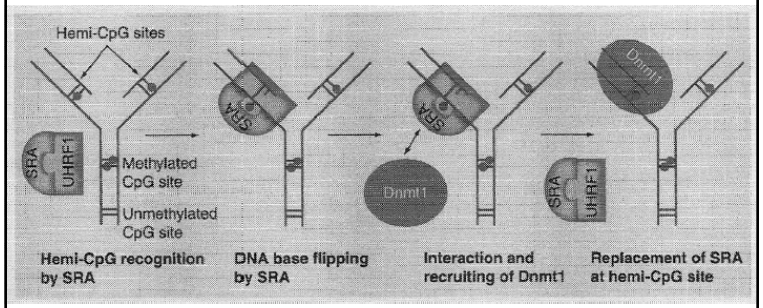
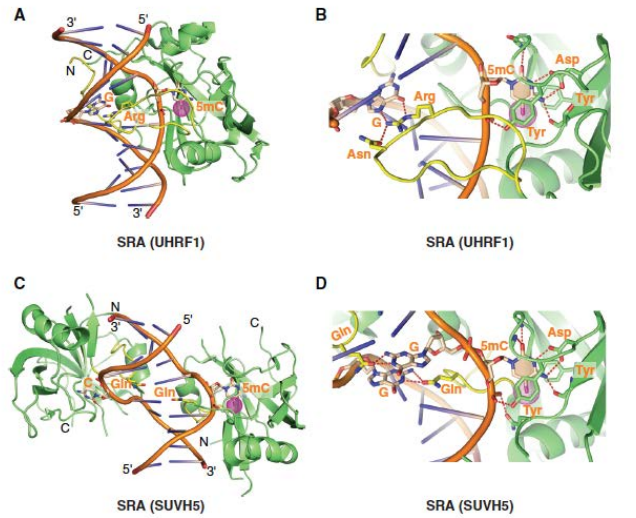
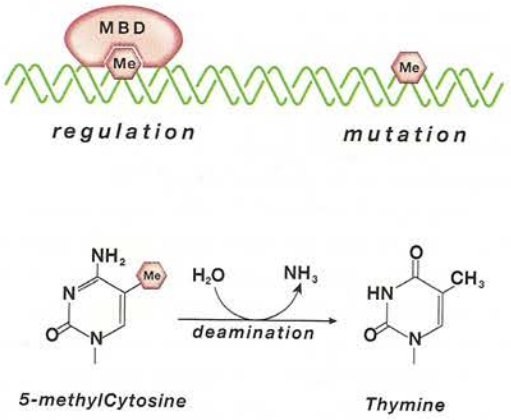


Figure 1.2 DNA methylation reactions. During embryogenesis the pattern of methylation is sculpted by a combination of DNA demethylation by demethylases and *de novo* methylation by DNA methyltransferases (DNMT). The pattern is then maintained in a tissue specific manner by maintenance methyltransferases (DNMT1) that copy the methylation pattern from parental to daughter strands.









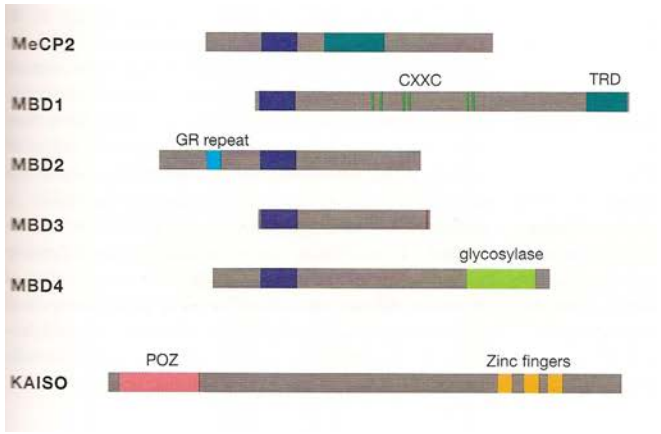
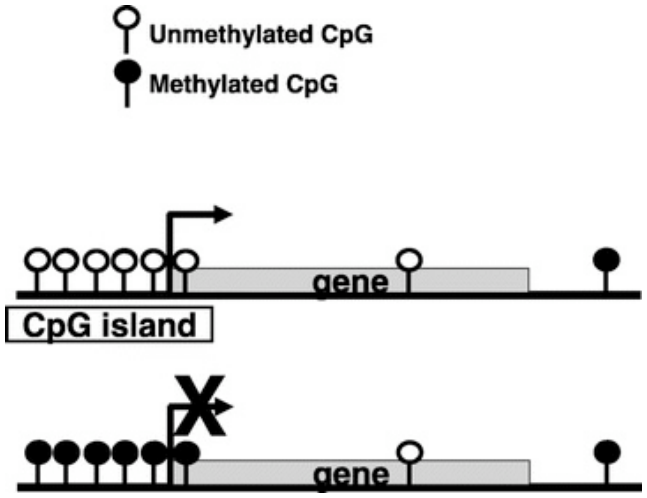
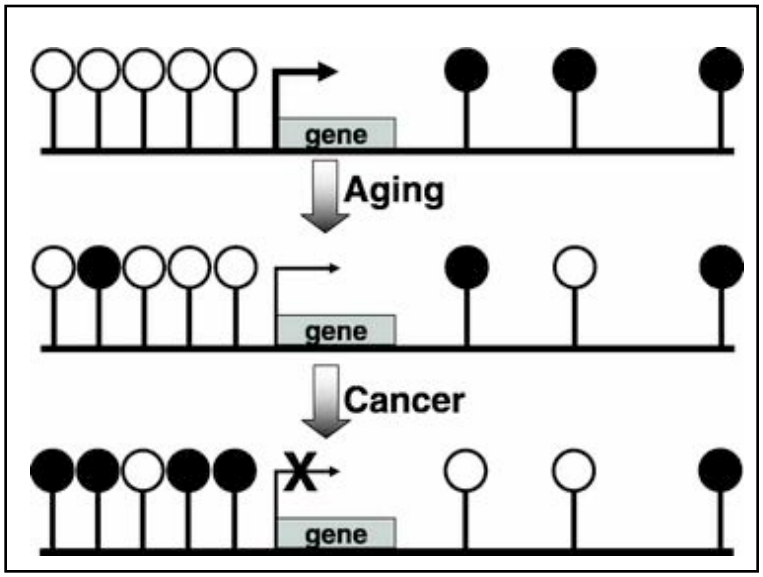


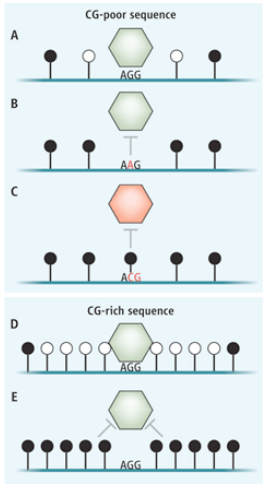
Table 2. Functions of methyl-CpG-binding proteins

MBP	Major activity	Species	Major phenotypes of loss-of-function mutations
MeCP2	binds mCpG with adjacent A/T run; transcriptional repressor	mouse	delayed onset neurological defects including inertia, hindlimb clasping, nonrhythmic breathing, and abnormal gait; postnatal survival ~10 weeks
MECP2	binds mCpG with adjacent AT run; transcriptional repressor	human	heterozygotes suffer from Rett syndrome, a profound neurological disorder characterized by apraxia, loss of purposeful hand use, breathing irregularities, and microcephaly
Mbd1	binds mCpG via MBD; a major splice form is also able to bind CpG via a CxxC domain	mouse	no overt phenotype, but subtle defects in neurogenesis detected
Mbd2	binds mCpG; transcriptional repressor	mouse	viable and fertile, but show reduced maternal nurturing behavior; defective gene regulation in T-helper-cell differentiation leading to altered response to infection; highly resistant to intestinal tumorigenesis
Mbd3	core component of NuRD co-repressor complex; does not show strong binding to mCpG	mouse	early embryonic lethal
Mbd4	DNA repair protein that binds mCpG and T:G mismatches at mCpG sites; thymine DNA glycosylase that excises T from T:G mismatches	mouse	viable and fertile; three- to fourfold increase in mutations at CpG sites; increased susceptibility to intestinal cancer correlates with C-to-T transitions within the Apc gene; Mbd4 functions to minimize the mutability of 5-methylcytosine
Kaiso	binds mCGmCG and CTGCNA; transcriptional repressor	mouse	no overt phenotype; small but significant delay in tumorigenesis on Min background





Molecular biology. Epigenetic islands in a genetic ocean.
Schübeler D.
Science. 2012 Nov 9;338(6108):756-7. doi: 10.1126/science.1227243. No abstract available.



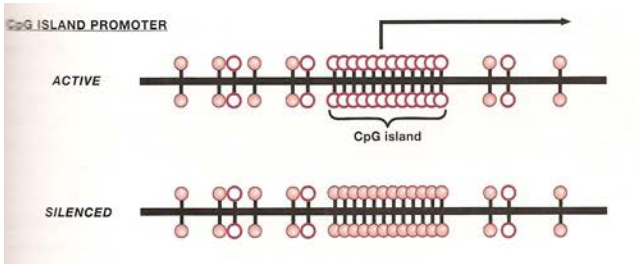
Genetics and epigenetics of transcription factor binding.

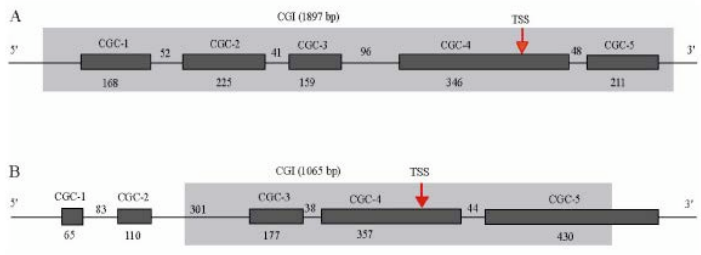
(A) Binding of a transcription factor in CG-poor regions leads to a local unmethylated state. (B) Mutations in the binding site prevent binding and result in increased methylation. (C) Some transcription factors could be sensitive to methylation even in CG-poor regions. (D) Transcription factor binding in a CG-rich area (CG island) requires the region to be unmethylated, and (E) can be blocked if methylated. (Hexagon) Transcription factors, (black circles) methylated CG, (white circles) unmethylated CG.

CpG Island

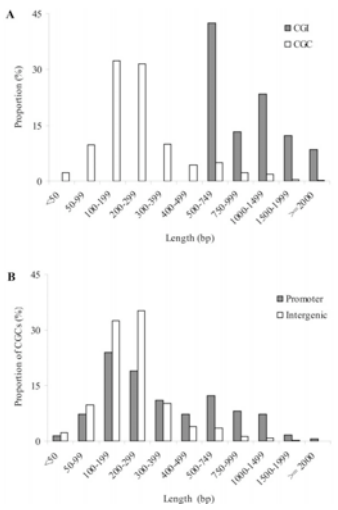
- 70- 80% of all CpG dinucleotides are heavily methylated in human cells.
- CpG islands (CGI) are 0.2 to 1-kb long DNA sequence stretches of GC-rich (G+C content: >50-60%) DNA that appear to be protected from the modification in somatic cells.
- CpG islands are frequently located in the promoters and first exon regions of 40 to 50% of all genes, but can be in introns and exons or between genes.
- Methylation typically results in loss of expression of adjacent genes.

WO 2007050706 20070503: DNA METHYLATION BIOMARKERS IN LYMPHOID AND HEMATOPOIETIC



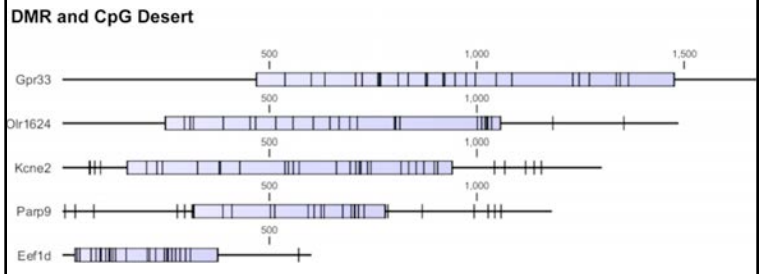


Multiple short CGCs embedded in one CGI in the promoter region. Dark box: CGCs identified by CpGcluster. Grey box: CGI identified by Takai and Jones' algorithm. The length of each CGC is labeled below the dark box and the distance between two neighboring CGCs is above the line. The transcription start site (TSS) is marked by an arrow. **(A)** CAP1. **(B)** ADAM33.

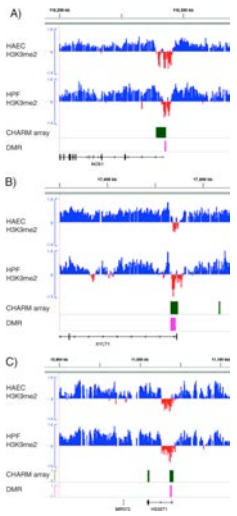


Length distribution of CGIs or CGCs in the human genome. (A) CGIs versus CGCs. **(B)** For CGCs, promoter regions versus intergenic regions.

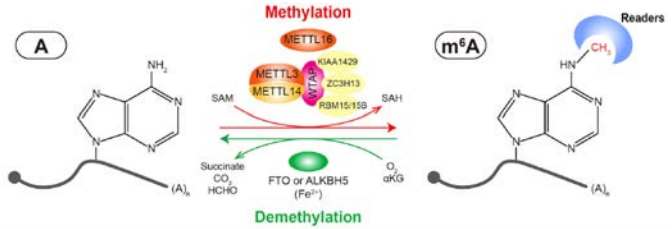
BMC Genomics. 2014 Aug 20;15:692. doi: 10.1186/1471-2164-15-692.
Role of CpG deserts in the epigenetic transgenerational inheritance of differential DNA methylation regions.
Skinner MK[1] and Guerrero-Bosagna C
[1]Center for Reproductive Biology, School of Biological Sciences, Washington State University, Pullman, WA 99164-4236, USA. skinner@wsu.edu.



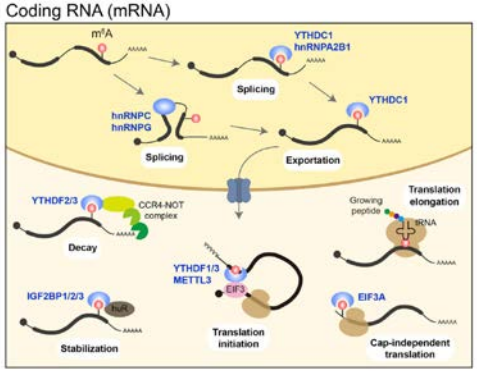
Euchromatin islands in large heterochromatin domains are enriched for CTCF binding and differentially DNA-methylated regions.
Wen B, et al. BMC Genomics. 2012 Oct 26;13:566.



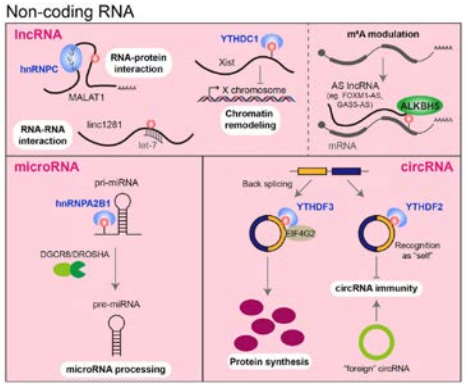
m⁶A Modification in Coding and Non-coding RNAs: Roles and Therapeutic Implications in Cancer.
Huang H, Weng H, Chen J.
Cancer Cell. 2020 Mar 16;37(3):270-288.



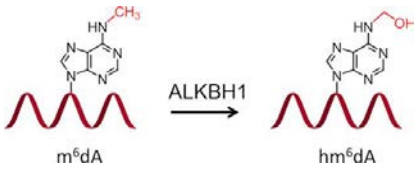
Coding RNA (mRNA)



Non-coding RNA

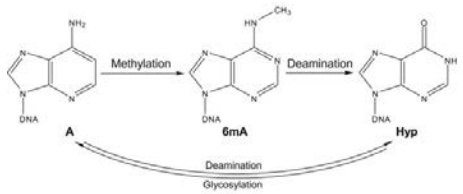


N 6-Hydroxymethyladenine: a hydroxylation derivative of N6-methyladenine in genomic DNA of mammals
Xiong J, Ye T-T, Ma C-J, et al.
Nucleic Acids Res. 2019 Feb 20;47(3):1268-1277.

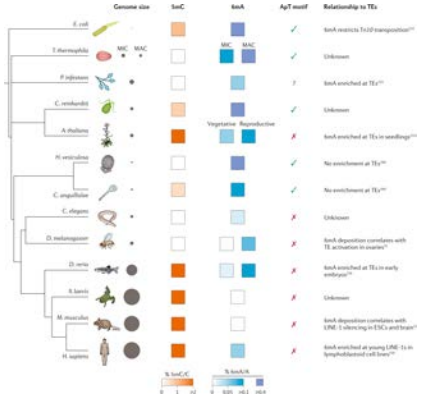


Schematic illustration of the formation of hm^6dA in DNA from the hydroxylation of m^6dA by the Fe^{2+}- and 2-oxoglutarate-dependent ALKBH1 protein.

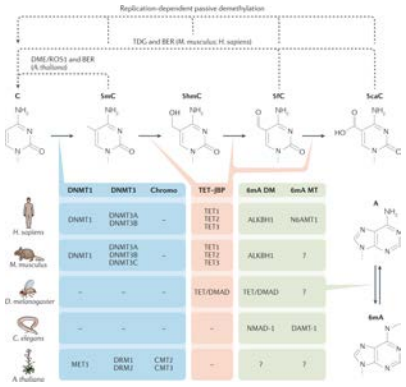
Epigenetically modified N 6-methyladenine inhibits DNA replication by human DNA polymerase iota
Zhang S, Li B, Du K, et al.
Biochimie. 2020 Jan;168:134-143.



Structure illustration of adenine (A), N^6-methyladenosine (6 mA), and hypoxanthine (Hyp), and the scheme of the conversions among A, 6 mA, and Hyp.

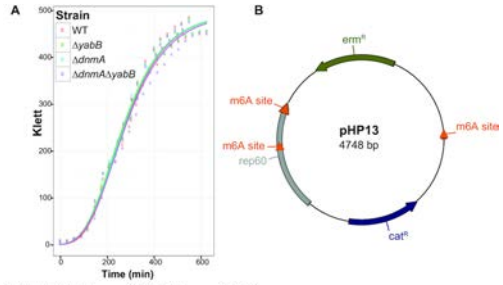


Variation in 6mA abundance and relationship with TEs across species.

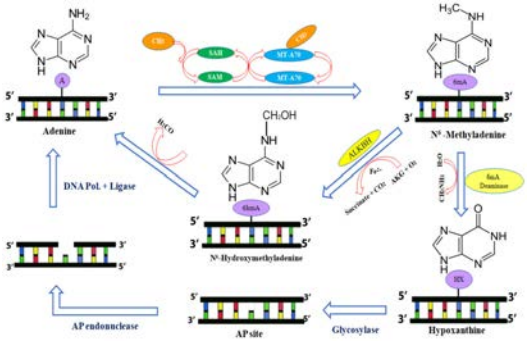


DNA modifications and DNA-modifying enzymes.

Methyltransferase DnmA is responsible for genome-wide N6-methyladenosine modifications at non-palindromic recognition sites in Bacillus subtilis
Taylor M Nye TM, van Gijtenbeek LA, Stevens AG.
Nucleic Acids Res. 2020 Jun 4;48(10):5332-5348.

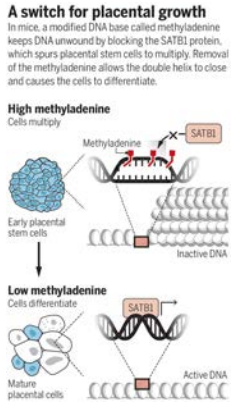


Relevant genotype	Growth rate	95% CI
Wild type	1.52	[1.43, 1.61]
Δ*dnmA*	1.51	[1.43, 1.59]
Δ*yabB*	1.51	[1.43-1.58]
Δ*dnmA*Δ*yabB*	1.46	[1.35-1.57]

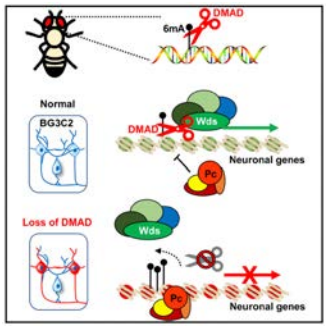


Mechanism of reversible DNA Adenine methylation (6mA)—Using S-adenosyl L-methionine as a methyl group donor, the writer MT-A70 protein family adds methyl group in the 6th position of purine ring of adenine. It has been shown that 6mA can be converted to unmethylated adenine by the two mechanisms. In the first pathway, 6mA reverts back to adenine by ALKBH family proteins (eraser) by oxidative demethylation via intermediary hydroxymethyladenine (HO-6mA). In yet another mechanism, 6mA is converted back to adenine by 6mA deaminase (eraser) releasing the intermediate product of hypoxanthine, which is recognized and cleaved by glycosylase leaving an apurinic site (AP site). This AP site is further rectified by base excision repair involving AP endonuclease, DNA polymerase and DNA ligase. The 6mA reader proteins, which are efficient enough to recognize methylated adenine motifs, remain unknown.

Altered DNA base could play key role in pregnancy
Elizabeth Pennisi
Science. 2020 Jul 31;369(6503):495.



Active N 6-Methyladenine Demethylation by DMAD Regulates Gene Expression by Coordinating with Polycomb Protein in Neurons
Yao B, Li Y, Wang Z, et al.
Mol Cell. 2018 Sep 6;71(5):848-857.e6.

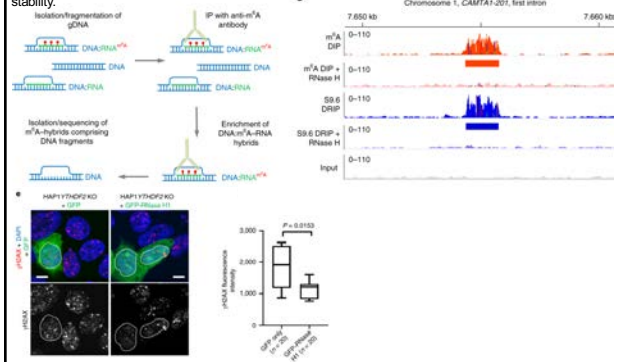


Highlights

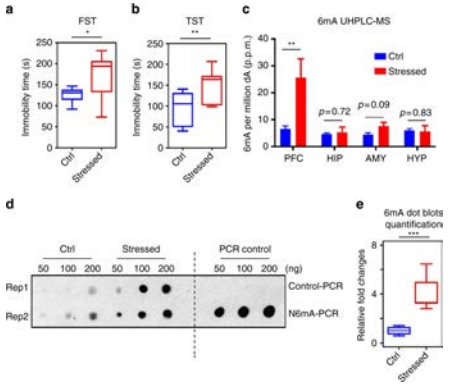
- DMAD is critical in *Drosophila* brain by actively demethylating 6mA
- DMAD interacts with Trithorax-related protein Wds to regulate gene expression
- 6mA correlates with the binding of Polycomb proteins in *Drosophila* genome
- DMAD and 6mA coordinate with Trithorax and Polycomb proteins for gene regulation

N 6-methyladenosine regulates the stability of RNA:DNA hybrids in human cells
Abakir A, Giles TC, Cristini A, et al.
Nat Genet. 2020 Jan;52(1):48-55.

Our results suggest that m⁶A regulates accumulation of R-loops, implying a role for this modification in safeguarding genomic stability.



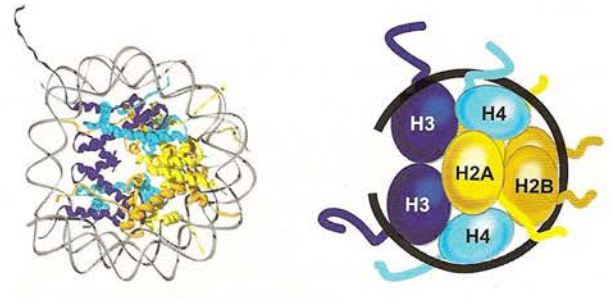
DNA N6-methyladenine is dynamically regulated in the mouse brain following environmental stress
Yao B, Cheng Y, Wang Z, Li Y, et al.
Nat Commun. 2017 Oct 24;8(1):1122.

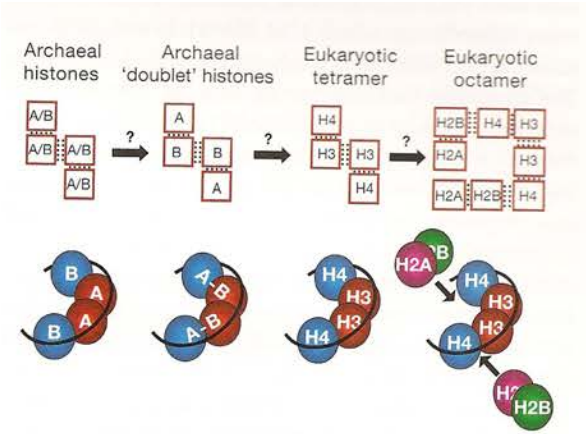


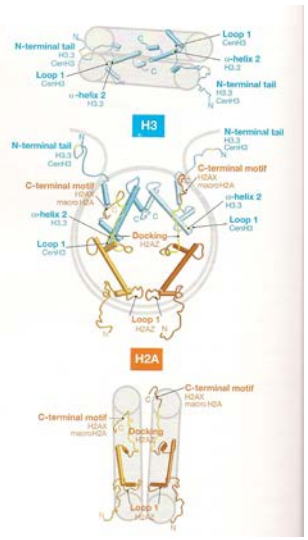
DNA N ^{6}-Methyladenine Modification in Plant Genomes-A Glimpse into Emerging Epigenetic Code
Karanthamalai J, Chodon A, Chauhan S, Pandi G.
Plants (Basel). 2020 Feb 14;9(2):247.

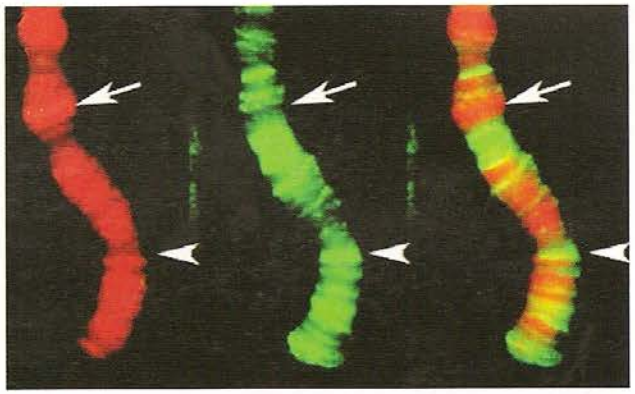
N^6-methyladenine (6mA) is a DNA base modification at the 6th nitrogen position; recently, it has been resurfaced as a potential reversible epigenetic mark in eukaryotes. Despite its existence, 6mA was considered to be absent due to its undetectable level. However, with the new advancements in methods, considerable 6mA distribution is identified across the plant genome. Unlike 5-methylcytosine (5mC) in the gene promoter, 6mA does not have a definitive role in repression but is exposed to have divergent regulation in gene expression. Though 6mA information is less known, the available evidences suggest its function in plant development, tissue differentiation, and regulations in gene expression. The current review article emphasizes the research advances in DNA 6mA modifications, identification, available databases, analysis tools and its significance in plant development, cellular functions and future perspectives of research.

Epigenetics
Histone Modification

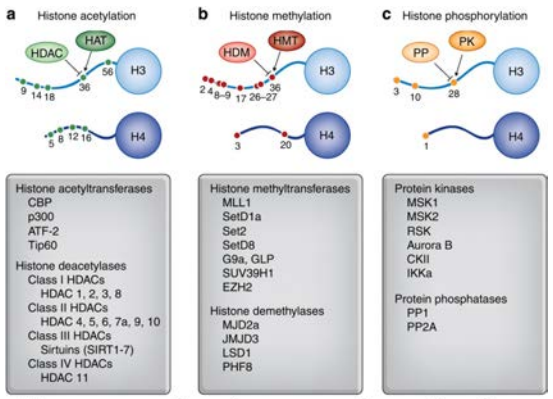




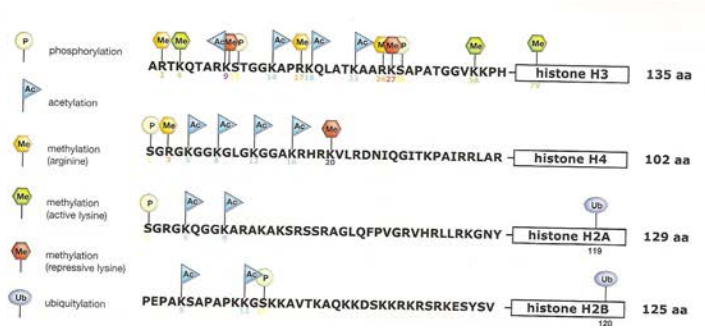


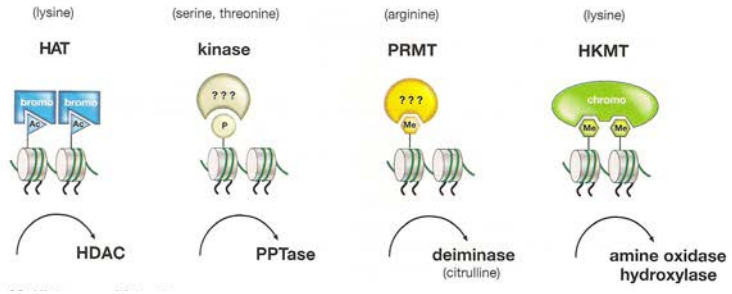


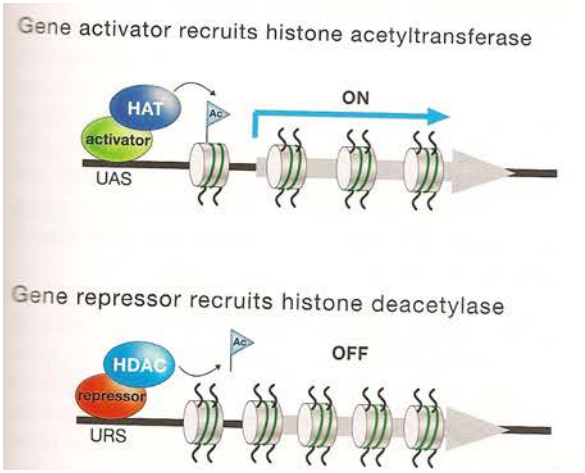
histone H3		
H3	HFD	135
H3.3	HFD	135
CenpA	HFD	133
histone H4		
H4	HFD	102
histone H2A		
H2A	HFD	129
H2A.Z	HFD	127
H2A.X	HFD	142
macroH2A	HFD MACRO	355
histone H2B		
H2B	HFD	125
histone H1		
(7 isoforms)	H15	179-223

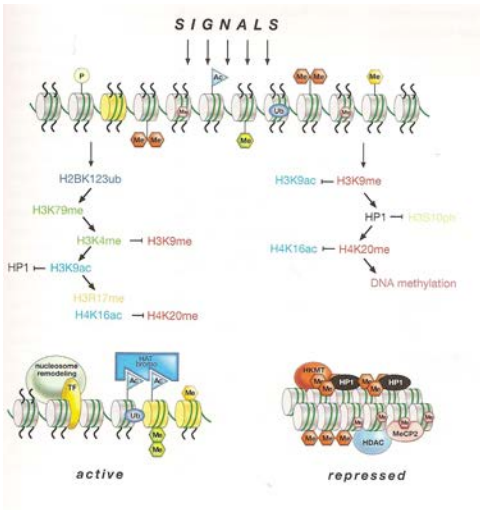


Summary of well-understood histone modifications and histone-modifying enzymes. (a) Histone acetylation at numerous lysine residues on histone tails is catalyzed by histone acetyltransferases (HATs) and removed by histone deacetlyases (HDACs). Histone acetylation is generally a transcriptionally permissive mark. Different HAT and HDAC enzymes are listed below. Importantly, specific HDACs isoforms are differentially expressed across brain structures and appear to uniquely regulate different aspects of cognition. (b) Histone methylation at lysine and arginine residues on histone tails is catalyzed by histone methyltransferases (HMTs) and removed by histone demethylases (HDMs). Histone methylation at different amino acid residues has been linked to both transcriptional activation and transcriptional repression. Methylation can occur in mono-, di-, or even tri-methylated states. Many HDMs and HMTs are specific for modifications at individual amino acids on histone tails or even a specific number of methyl groups. (c) Histone phosphorylation at serine residues is catalyzed by protein kinases (PKs) such as mitogen- and stress-activated protein kinase 1 (MSK1), whereas phosphorylation marks are removed by protein phosphatases such as protein phosphatase 1 (PP1). Histone phosphorylation is generally linked to transcriptional activation.









Histone deacetylase inhibitor (HDACI) mechanisms of action: emerging insights.
Bose P, Dai Y, Grant S.
Pharmacol Ther. 2014 Sep;143(3):323-36.

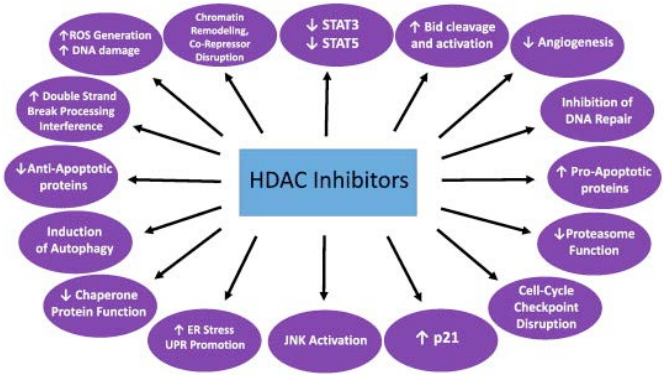
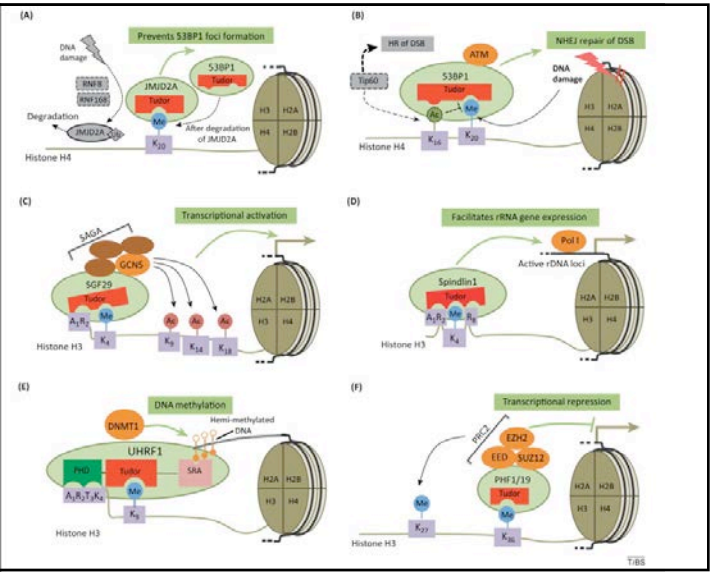
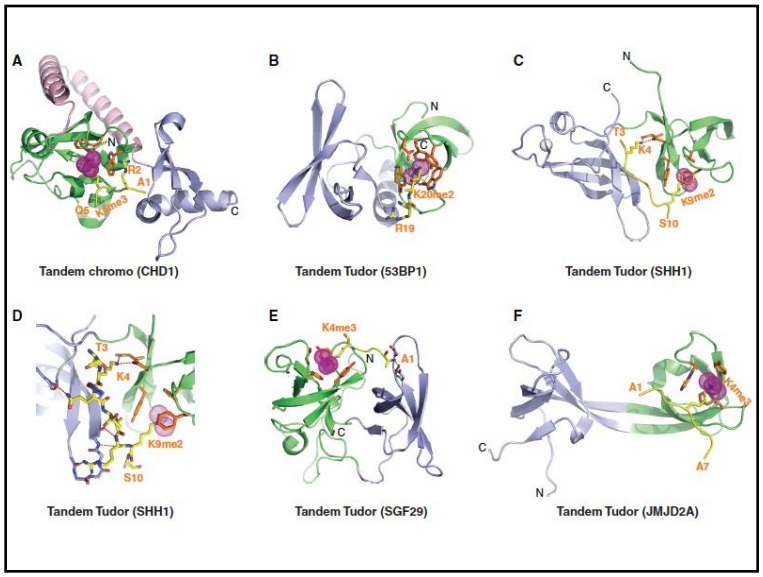


Table 1. Mammalian Tudor-domain-containing proteins as histone methylation readers, and their biological functions

Protein	Domain architecture	Tudor ligand	K_d (μM)	Biological functions	Refs
JMJD2A	100 aa; JmjN JmjC PHD PHD Tudor Tudor	H3K4me3	~0.5	H3K9me3 and H3K36me3-specific demethylase; transcriptional regulation and regulator of DNA damage response	[28,45,48]
		H4K20me3	~0.4		
		H4K20me2	~2		
53BP1	Tudor Tudor BRCT BRCT	H4K20me2	20~50	Substrate of ATM; promote non-homologous end joining DNA repair	[29,44]
SGF29	Tudor Tudor	H3K4me3	1~4	Component of SAGA complex; mediate transcriptional activation	[30,31]
Spindlin1	Tudor Tudor Tudor	H3K4me3	~0.8	Nucleolar protein; promote rRNA transcription	[32,33]
UHRF1	UBL Tudor Tudor PHD SRA RING	H3K9me3 by Tudor	1~3	Partner of DNMT1; maintain the level of DNA methylation during DNA replication	[35,37,66, 88,89]
		H3 N terminus and K9me3 by Tudor–PHD	~0.4		
PHF1	Tudor PHD PHD	H3K36me3	5–50a	Accessory component of PRC2 complex; promote transcriptional repression	[38,39,40]
PHF19	Tudor PHD PHD	H3K36me3	6–35a	Accessory component of PRC2 complex; promote transcriptional repression	[38,40,41,42]
LBR	Tudor; Transmembrane regions	H4K20me2	N.D.	Inner nuclear membrane protein; promote formation of nuclear peripheral heterochromatin	[84]
TDRD3	UBA Tudor	H4R3me2a; H3R17me2a; H3R2me2a	>500	Transcriptional coactivator and interacts with CARM1 and PRMT1	[105,109]

Abbreviations: ATM, ataxia telangiectasia mutated; K_d, dissociation constant; N.D., not defined; PRC2, polycomb repressive complex 2.
Modifications: me1, monomethylation; me2, dimethylation; me3, trimethylation; me2a, asymmetric dimethylation.
Protein domains: BRCT, BRCA1 C terminus domain; JmjC, jumonji C domain; JmjN, jumonji N domain; PHD, plant homeodomain; RING, really interesting new gene finger domain; SRA, SET and RING finger associated domain; UBA, ubiquitin-associated domain; UBL, ubiquitin-like domain.
a, for PHF1 or PHF19 binding to H3K36me3 peptides, a K_d of 5–6 μM was obtained at 4 °C in a buffer of 100 mM NaCl and 20 mM Tris-HCl pH 7.5, and a higher K_d of 35–50 μM obtained at 25° in a buffer of 150 mM NaCl and 20 mM Tris, pH 6.8–7.5.





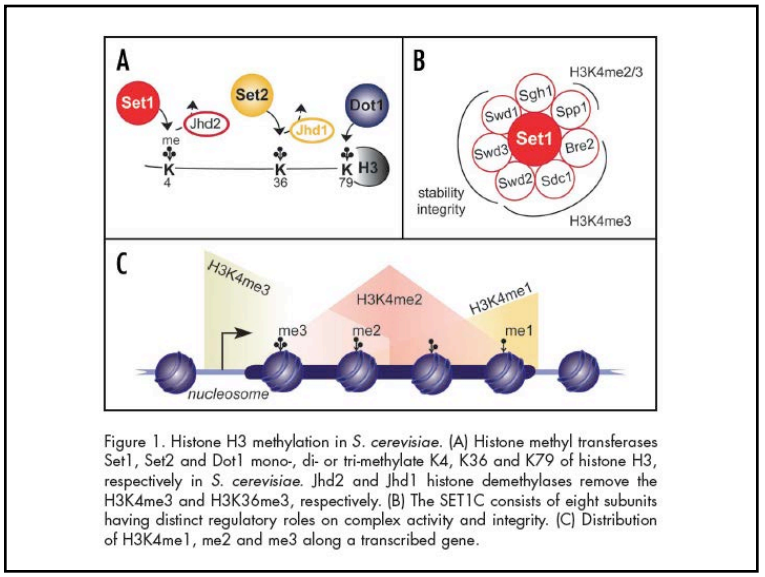
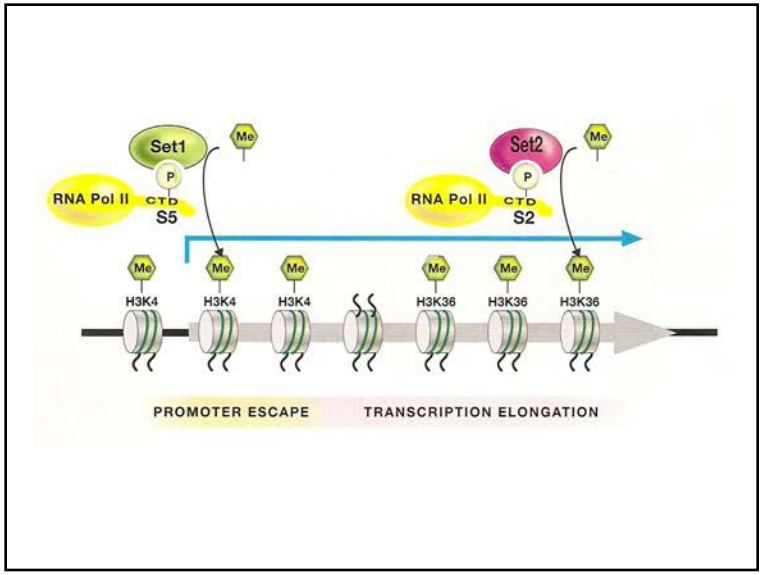
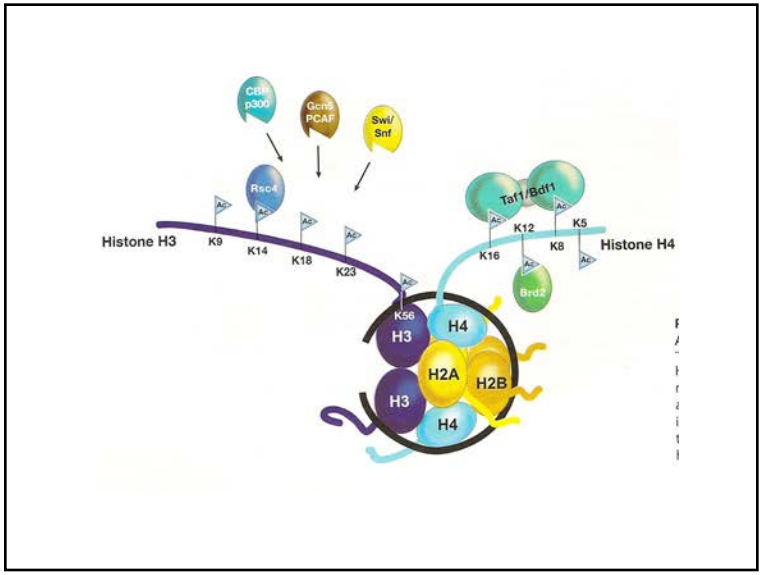


Figure 1. Histone H3 methylation in *S. cerevisiae*. (A) Histone methyl transferases Set1, Set2 and Dot1 mono-, di- or tri-methylate K4, K36 and K79 of histone H3, respectively in *S. cerevisiae*. Jhd2 and Jhd1 histone demethylases remove the H3K4me3 and H3K36me3, respectively. (B) The SET1C consists of eight subunits having distinct regulatory roles on complex activity and integrity. (C) Distribution of H3K4me1, me2 and me3 along a transcribed gene.





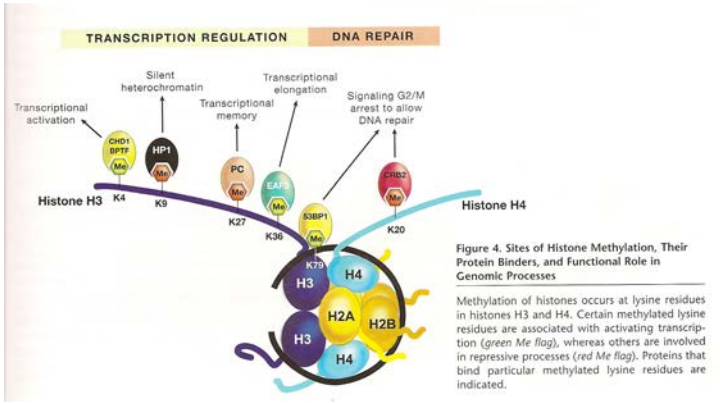


Figure 4. Sites of Histone Methylation, Their Protein Binders, and Functional Role in Genomic Processes

Methylation of histones occurs at lysine residues in histones H3 and H4. Certain methylated lysine residues are associated with activating transcription (*green Me flag*), whereas others are involved in repressive processes (*red Me flag*). Proteins that bind particular methylated lysine residues are indicated.

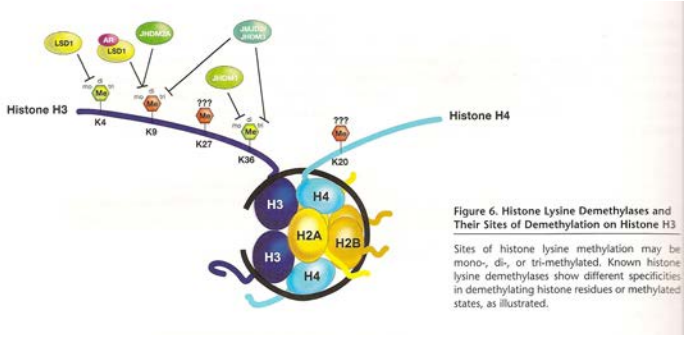


Figure 6. Histone Lysine Demethylases and Their Sites of Demethylation on Histone H3

Sites of histone lysine methylation may be mono-, di-, or tri-methylated. Known histone lysine demethylases show different specificities in demethylating histone residues or methylated states, as illustrated.

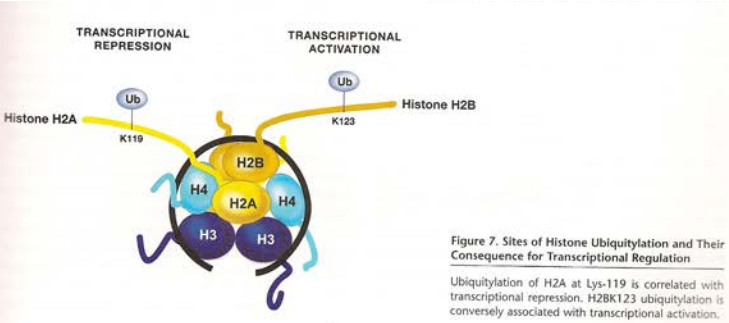
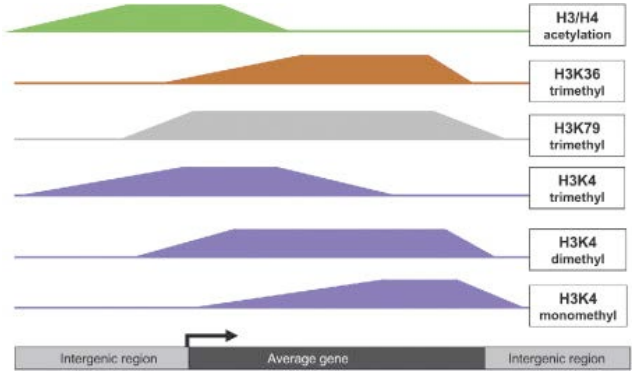
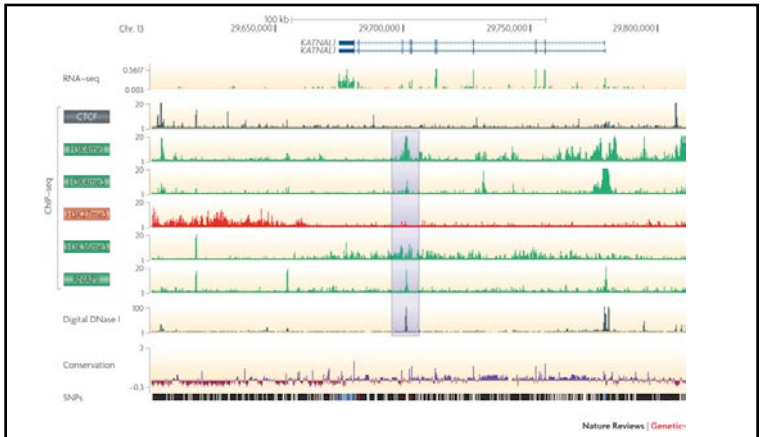


Figure 7. Sites of Histone Ubiquitylation and Their Consequence for Transcriptional Regulation

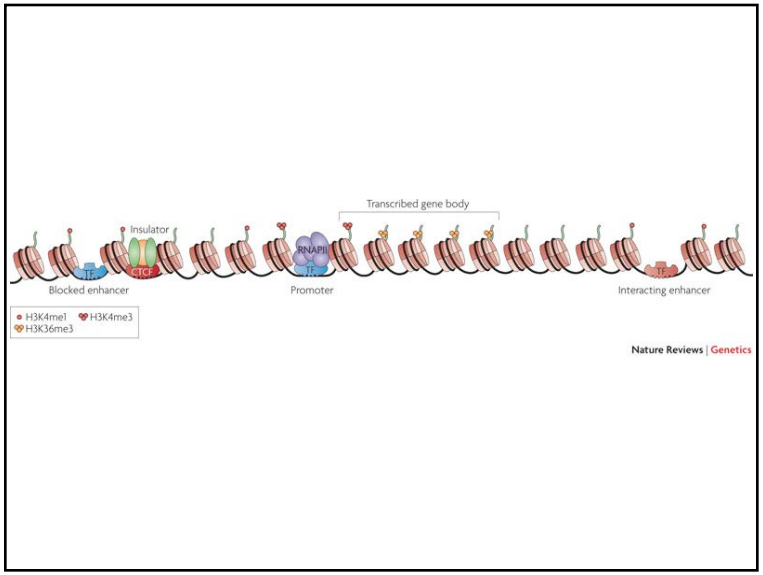
Ubiquitylation of H2A at Lys-119 is correlated with transcriptional repression. H2BK123 ubiquitylation is conversely associated with transcriptional activation.

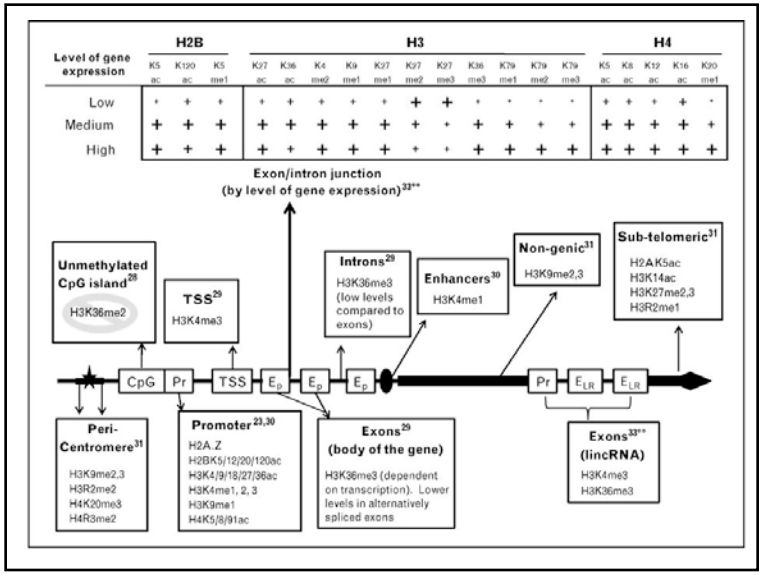


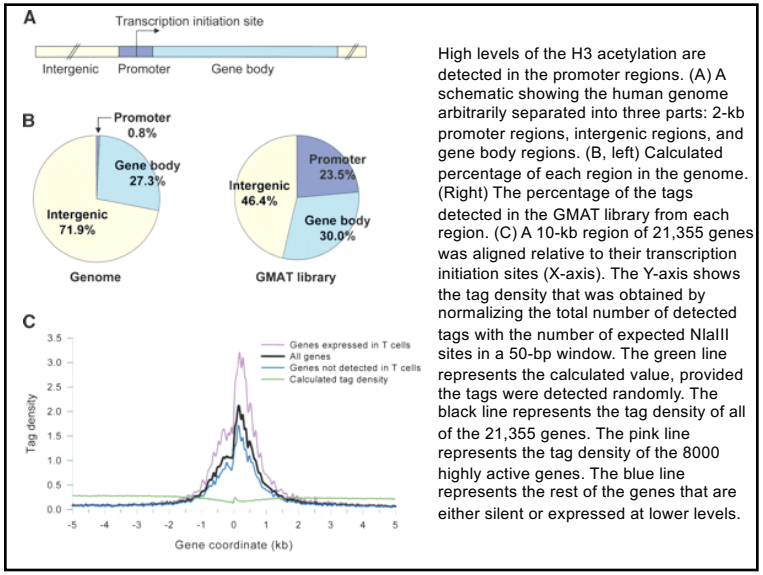
Distribution of Histone Modifications across a Typical Yeast GeneIn their ChIP-chip assay, Pokholok et al. (2005) immunoprecipitated formaldehyde-crosslinked yeast chromatin with antibodies to acetylated histones H3 and H4, and to H3 histone mono-, di-, or trimethylated at lysine 36 (H3K36), lysine 79 (H3K79), and lysine 4 (H3K4). The authors amplified immunoprecipitated and input DNA by ligation-mediated PCR, labeled them with different fluorescent dyes, and mixed and hybridized them to a single DNA microarray. The ratio of both fluorescent dyes on each spot provides a measure of the relative enrichment of the histone modification under investigation. Collectively, this provides information with which to construct a genome-wide map of the consensus distribution of histone modifications in yeast. Each of the distributions shown represents a composite profile derived from several hundred genes.



The University of California-Santa Cruz (UCSC) Genome Browser is a tool for viewing genomic data sets. A vast amount of data is available for viewing through this browser. This example from the browser shows numerous data types in K562 cells from the ENCODE Consortium. A random gene was selected — katanin p60 subunit A-like 1(KATNAL1) — that shows several points that can be identified by using this tool. The promoter has a typical chromatin structure (a peak of histone 3 lysine 4 trimethylation (H3K4me3) between the bimodal peaks of H3K4me1), is bound by RNA polymerase II (RNAPII) and is DNase hypersensitive. The gene is transcribed, as indicated by RNA sequencing (RNA–seq) data, as well as H3K36me3 localization. The gene lies between two CCCTC-binding factor (CTCF)-bound sites that could be tested for insulator activity. An intronic H3K4me1 peak (highlighted) predicts an enhancer element, corroborated by the DNase I hypersensitivity site peak. There is a broad repressive domain of H3K27me3 downstream, which could have an open chromatin structure in another cell type.





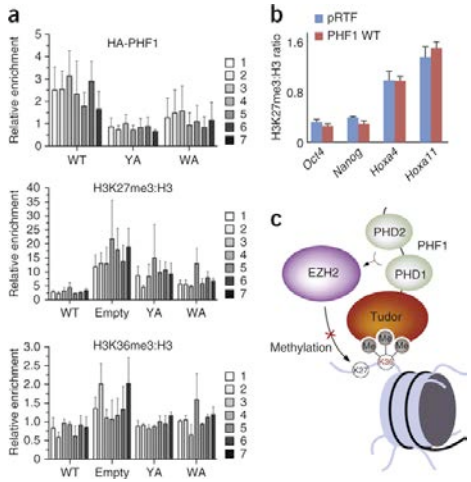


High levels of the H3 acetylation are detected in the promoter regions. (A) A schematic showing the human genome arbitrarily separated into three parts: 2-kb promoter regions, intergenic regions, and gene body regions. (B, left) Calculated percentage of each region in the genome. (Right) The percentage of the tags detected in the GMAT library from each region. (C) A 10-kb region of 21,355 genes was aligned relative to their transcription initiation sites (X-axis). The Y-axis shows the tag density that was obtained by normalizing the total number of detected tags with the number of expected NlaIII sites in a 50-bp window. The green line represents the calculated value, provided the tags were detected randomly. The black line represents the tag density of all of the 21,355 genes. The pink line represents the tag density of the 8000 highly active genes. The blue line represents the rest of the genes that are either silent or expressed at lower levels.

Molecular basis for H3K36me3 recognition by the Tudor domain of PHF1.
Musselman CA, et al. Nat Struct Mol Biol. 2012; 19(12):1266-72.

Abstract

The PHD finger protein 1 (PHF1) is essential in epigenetic regulation and genome maintenance. Here we show that the Tudor domain of human PHF1 binds to histone H3 trimethylated at Lys36 (H3K36me3). We report a 1.9-Å resolution crystal structure of the Tudor domain in complex with H3K36me3 and describe the molecular mechanism of H3K36me3 recognition using NMR. Binding of PHF1 to H3K36me3 inhibits the ability of the Polycomb PRC2 complex to methylate Lys27 of histone H3 in vitro and in vivo. Laser microirradiation data show that PHF1 is transiently recruited to DNA double-strand breaks, and PHF1 mutants impaired in the H3K36me3 interaction exhibit reduced retention at double-strand break sites. Together, our findings suggest that PHF1 can mediate deposition of the repressive H3K27me3 mark and acts as a cofactor in early DNA-damage response.

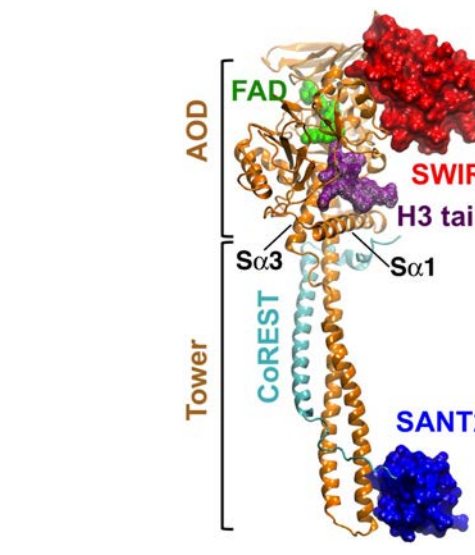


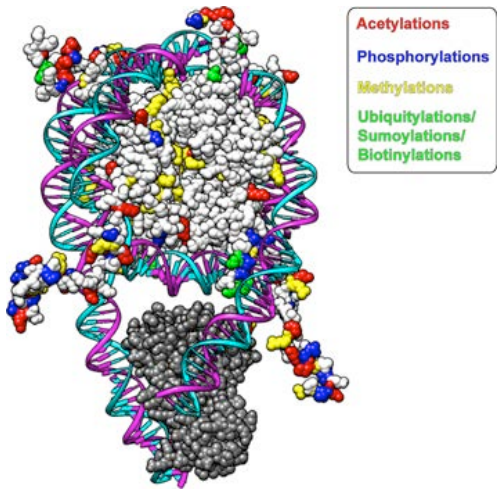
Binding of PHF1 to H3K36me3 decreases PRC2-mediated deposition of H3K27me3. (a) ChIP assays on chromatin from HEK293T cells fixed and harvested 48 h after transfection with either empty vector, wild-type HA-PHF1, or W41A or Y47A mutants. The levels of HA-PHF1, H3K27me3, H3K36me3 and histone H3 were probed across the MYT1 promoter using seven primer sets. Above, occupancy of wild-type and mutant HA-PHF1. Middle, H3K27me3 level normalized to H3 (H3K27me3:H3). Below, H3K36me3 level normalized to H3 (H3K36me3:H3). All data are relative to the PCLB4 nontarget control gene. Error bars represent s.d. based on three experiments. (b) ChIP assays on chromatin harvested from mES cells transduced with empty vector (pRTF, blue) or a vector containing Flag-PHF1 (PHF1, red). Using quantitative PCR (qPCR), levels of H3K27me3 and histone H3 were probed at the Oct4, Nanog, Hoxa4 and Hoxa11 promoters. Data are presented as the ratio of H3K27me3 to total H3 signal to correct for possible differences in nucleosome density at the different loci examined. Error bars represent s.d. based on two experiments. (c) A model for inhibition of EZH2–PRC2 activity through recognition of H3K36me3 by the Tudor domain of PHF1.

LSD1/CoREST is an allosteric nanoscale clamp regulated by H3-histone-tail molecular recognition.
Baron R, Vellore NA. Proc Natl Acad Sci U S A. 2012 Jul 31;109(31):12509-14.

Abstract

The complex of lysine-specific demethylase-1 (LSD1/KDM1A) with its corepressor protein CoREST is an exceptionally relevant target for epigenetic drugs. Here, we provide insight into the local and global changes of LSD1/CoREST conformational dynamics that occur upon H3 binding on the basis of a total cumulative time of one microsecond molecular dynamics simulation. The LSD1/CoREST complex functions as an allosteric nanoscale-binding clamp, which is regulated by substrate binding. In the unbound state, LSD1/CoREST reversibly visits clamp states that are more open or significantly more closed compared with the available X-ray crystal structures. The Lys triad of residues Lys355, Lys357, and Lys359 gates the entrance of the H3 pocket. H3 binding shifts the pocket breathing dynamics toward open, higher-volume states while reducing the overall flexibility of the LSD1/CoREST nanoscale clamp. We show that the H3 pocket is an allosteric site for the regulation of the rotation of the amino oxidase domain with respect to the Tower domain. The allosteric mechanism relies on the specific reduction of nanoscale domain rotation upon local H3-tail binding. Instead, clamp opening/closing motions that do not involve domain rotation only reduce in amplitude yet are dominant in the bound state. Overall, our data suggest that the H3 binding pocket is a central target site to (i) switch off LSD1 amino oxidase activity, thus H3-tail demethylation; (ii) block the competitive binding of transcription factors; and (iii) prevent chromatin anchoring to LSD1/CoREST. This study underscores the importance of receptor flexibility for future epigenetic drug discovery.

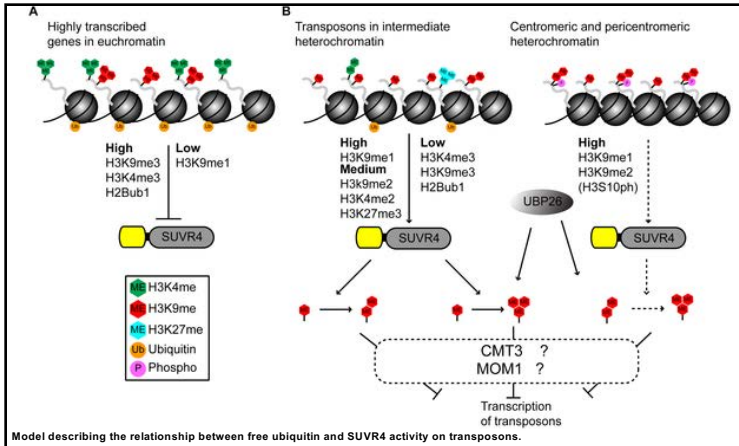




The SUVR4 histone lysine methyltransferase binds ubiquitin and converts H3K9me1 to H3K9me3 on transposon chromatin in Arabidopsis.
Veiseth SV, et al. PLoS Genet. 2011 Mar;7(3):e1001325.

Abstract

Chromatin structure and gene expression are regulated by posttranslational modifications (PTMs) on the N-terminal tails of histones. Mono-, di-, or trimethylation of lysine residues by histone lysine methyltransferases (HKMTases) can have activating or repressive functions depending on the position and context of the modified lysine. In Arabidopsis, trimethylation of lysine 9 on histone H3 (H3K9me3) is mainly associated with euchromatin and transcribed genes, although low levels of this mark are also detected at transposons and repeat sequences. Besides the evolutionarily conserved SET domain which is responsible for enzyme activity, most HKMTases also contain additional domains which enable them to respond to other PTMs or cellular signals. Here we show that the N-terminal WIYLD domain of the Arabidopsis SUVR4 HKMTase binds ubiquitin and that the SUVR4 product specificity shifts from di- to trimethylation in the presence of free ubiquitin, enabling conversion of H3K9me1 to H3K9me3 in vitro. Chromatin immunoprecipitation and immunocytological analysis showed that SUVR4 in vivo specifically converts H3K9me1 to H3K9me3 at transposons and pseudogenes and has a locus-specific repressive effect on the expression of such elements. Bisulfite sequencing indicates that this repression involves both DNA methylation-dependent and -independent mechanisms. Transcribed genes with high endogenous levels of H3K4me3, H3K9me3, and H2Bub1, but low H3K9me1, are generally unaffected by SUVR4 activity. Our results imply that SUVR4 is involved in the epigenetic defense mechanism by trimethylating H3K9 to suppress potentially harmful transposon activity.



Model describing the relationship between free ubiquitin and SUVR4 activity on transposons.
(A) SUVR4 is repressed by H3K4me3 in vitro, and has no activity on genes with high H3K4me3, H3K9me3, H2Bub1 and a low level of H3K9me1. (B) SUVR4's preference for heterochromatic transposons intercalated within euchromatin is maintained by its specificity for H3K9me1 which is highly enriched at transposons, and its repression by activating marks like H3K4me3. The deubiquitinase UBP26 regulates H3K9me2/me3 at the same targets as SUVR4, and might produce free ubiquitin that stimulates the H3K9me2/me3 activity of SUVR4 at target transposons. Although SUVR4 normally is repressed by H3K9me2 and H3S10ph which is high in pericentric heterochromatin, these regions may be targets for SUVR4 activity when ubiquitin levels are high. Since the transposons also contain a medium level of H3K27me3 in addition to H3K9me3, this could possibly create a binding site for CMT3 in order to repress transcription in a DNA methylation-dependent manner at some transposons. At other transposons, transcription may be repressed in a DNA methylation- independent manner by the MOM transcriptional repressor (See text for details).

Structural Paradigms in the Recognition of the Nucleosome Core Particle by Histone Lysine Methyltransferases
Janna A, Davarinejad H, Joshi M, Couture J-F.
Front Cell Dev Biol. 2020 Jul 31;8:600.

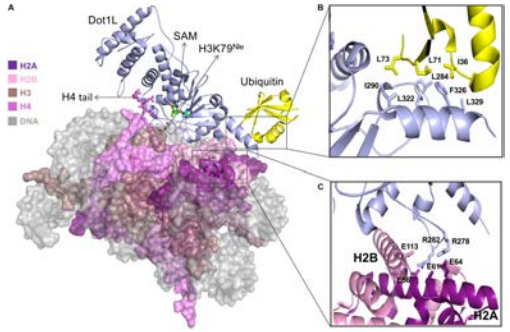
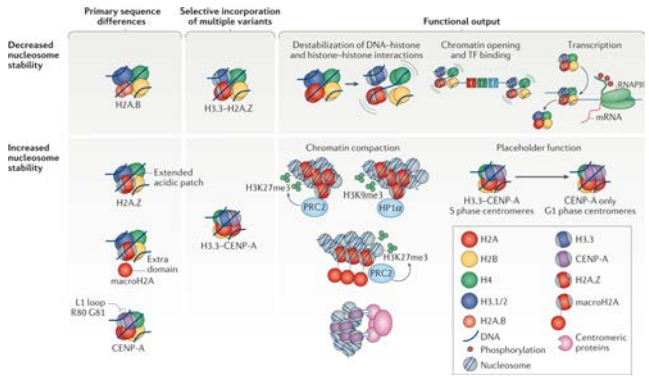
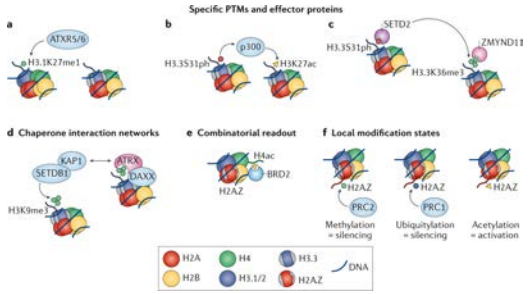


Figure 1. (A) Cryo-EM structure of Dot1L bound to H2B ubiquitinated nucleosome in active state. SAM cofactor, H4 tail, H3K79^{Me} and H2A-H2B acidic patch residues are depicted in stick model and nucleosome core particle (NCP) is depicted in surface representation **(B)** Detailed view of interaction between Dot1L and ubiquitin. Important residues at the Dot1L-ubiquitin interface are shown as sticks **(C)** Close-up of residues interactions between Dot1L and H2A-H2B acidic patch. Figures are generated using the cryo-EM structure of the Dot1L bound to H2B-Ubiquitin Nucleosome complex in active state (PDB accession number 6NJ9; Worden et al., 2019).

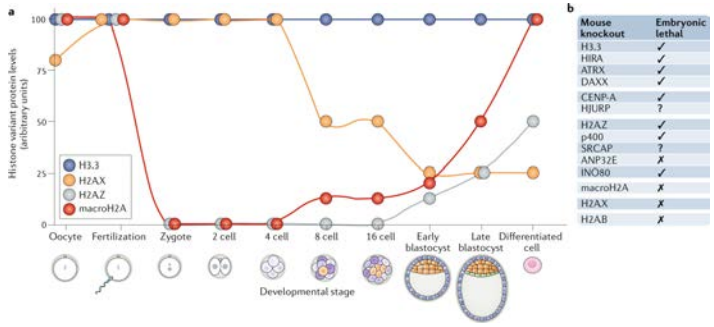


Variant-containing nucleosomes influence nucleosome stability and chromatin properties.



Variant-containing nucleosomes influence chromatin through various mechanisms.

a–c | Histone variants can harbour post-translational modifications (PTMs) that have direct effects on chromatin structure and function through the recruitment of specific readers. *Arabidopsis thaliana* trithorax-related protein 5/6 (ATXR5/6) methylates only histone H3.1 and not H3.3 owing to steric hindrance by H3.3 Ser31 (part **a**). Variant-specific modification can promote chromatin-associated enzyme activity, specifically H3.3 Ser31 phosphorylation (ph) increases activity of the histone acetyltransferase p300 (part **b**). Additionally, H3.3S31ph promotes the histone methyltransferase SET domain-containing 2 (SETD2), resulting in H3.3K36me3 deposition — a mark of transcriptionally active chromatin. In turn, H3.3K36me3 is specifically recognized by zinc-finger MYND-type-containing 11 (ZMYND11), a reported repressor of transcription elongation, hence the role of H3.3S31ph in transcription regulation is likely multifaceted (part **c**). **d** | Histone variants can also influence chromatin state through chaperone interaction with other chromatin-associated proteins to promote distinct chromatin states at regions of deposition. In the example shown, H3.3 incorporation leads to death domain-associated protein (DAXX)-dependent recruitment of Krüppel-associated box (KRAB)-associated protein 1 (KAP1) co-repressor and histone methyltransferase SET domain bifurcated 1 (SETDB1), all of which appear stabilized at chromatin in the presence of H3.3. **e** | Another mechanism is combinatorial readout, such as the presence of acetylated histone H4 (H4ac) coupled with histone H2A.Z.1 which promotes bromodomain-containing 2 (BRD2) binding and transcription activation. **f** | Histone variants can be modified in accordance with their site of deposition and thus modulate transcription. For example, H2A.Z can be acetylated at euchromatin or methylated and ubiquitylated at heterochromatin, contributing to gene activation or silencing, respectively. See Table 1 for additional details and references. PRC, Polycomb repressive complex.



Expression of histone variants during embryonic development.

a | The expression levels of histone variants change during the early stages of embryonic development. Each dot represents the level of the histone variant at the specific developmental stage. Histones H3.3 and H2A.X are expressed already in the zygote and importantly contribute to all stages of development. H2A.Z is expressed only later in the blastocyst. MacroH2A is present in the oocyte but is excluded after fertilization, and its levels do not increase until the eight-cell stage. **b** | Requirement of histone variants and associated chaperones[169,170,243,244,245] for embryonic development in knockout mouse models. See the text for additional details and references. ANP32E, acidic leucine-rich nuclear phosphoprotein 32 family member E; ATRX, α-thalassaemia mental retardation syndrome X-linked; CENP-A, centromeric protein A; DAXX, death domain-associated protein; HIRA, histone regulator A; HJURP, Holliday junction recognition protein; SRCAP, Snf2-related CREBBP activator protein.

"Epigenetics and Systems Biology"

Spring 2021 (Odd Years)

Biol 476/576

Schedule/Lecture Outline –

Week 1	(Lesson 1)	Systems Biology (History/ Definitions/ Theory)
Week 2	(Lesson 2)	Systems Biology (Networks & Emergence)
Week 3	(Lesson 3)	Systems Biology (Components: DNA to Phenotype)
Week 4	(Lesson 4)	Systems Biology (Genomics / Technology)
Week 5	(Lesson 5)	Epigenetics (History / Molecular Processes)
Week 6	(Lesson 6)	Epigenetics (Molecular Processes & Integration)
Week 7	(Lesson 7)	Epigenetics (Genomics and Technology)
Week 8	(Lesson 8)	Cell & Developmental Biology
Week 9	(Lesson 9)	Epigenetics of Cell & Developmental Biology
Week 10	(Lesson 10)	Environmental Impact on Biology
Week 11	(Lesson 11)	Environmental Epigenetics
Week 12	(Lesson 12)	Disease Etiology
Week 13	(Lesson 13)	Epigenetics & Disease Etiology
Week 14	(Lesson 14)	Evolutionary Biology & Genetics
Week 15	(Lesson 15)	Epigenetics & Evolutionary Biology
Week 16	(Lesson 16)	Grant Review/ Study Section Meeting

Spring 2019 – Epigenetics and Systems Biology
Lecture Outline (Systems Biology)
Michael K. Skinner – Biol 476/576
Weeks 5, 6 and 7 (February 5, 12 and 19)

Epigenetics (History / Molecular Processes/ Genomics)

- Definitions and History
- Molecular Factors (DNA Methylation, Histone Modification, Chromatin Structure, ncRNA)
- Epigenetics Technology and Genomics

Required Reading

Holliday R. Epigenetics: a historical overview. Epigenetics. 2006 Apr-Jun;1(2):76-80.

Books (Reserve in Library)

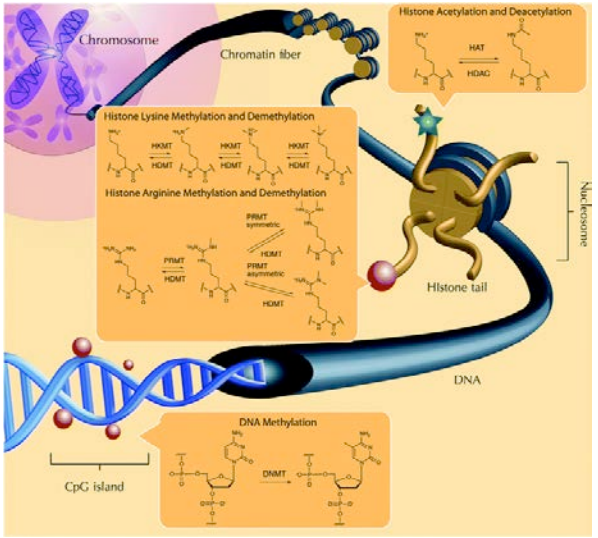
Kevin V. Morris (2012) Non-coding RNAs and Epigenetic Regulation of Gene Expression: Drivers of Natural Selection. Caister Academic Press.

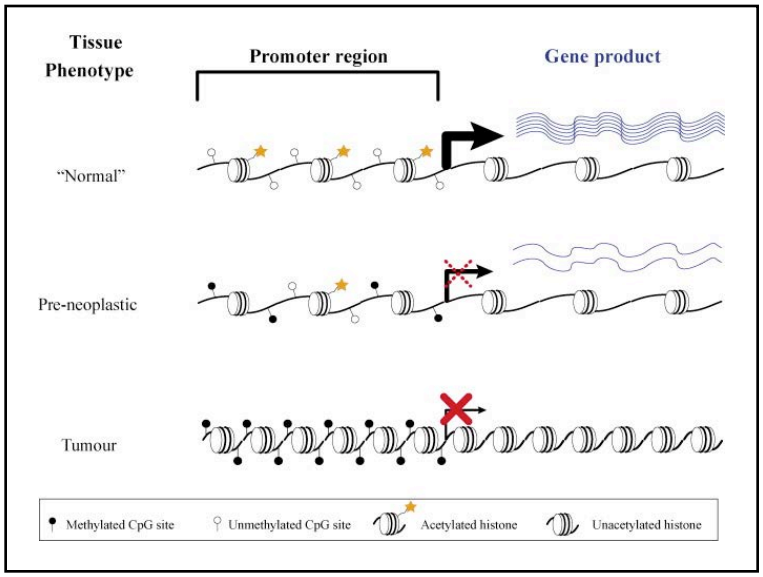
Russo, V.E.A., Martienssen, A.and Riggs, A.D. (eds.). 1996. Epigenetic Mechanisms of Gene Regulation. Cold Spring Harbor Press. Cold Spring Harbor.

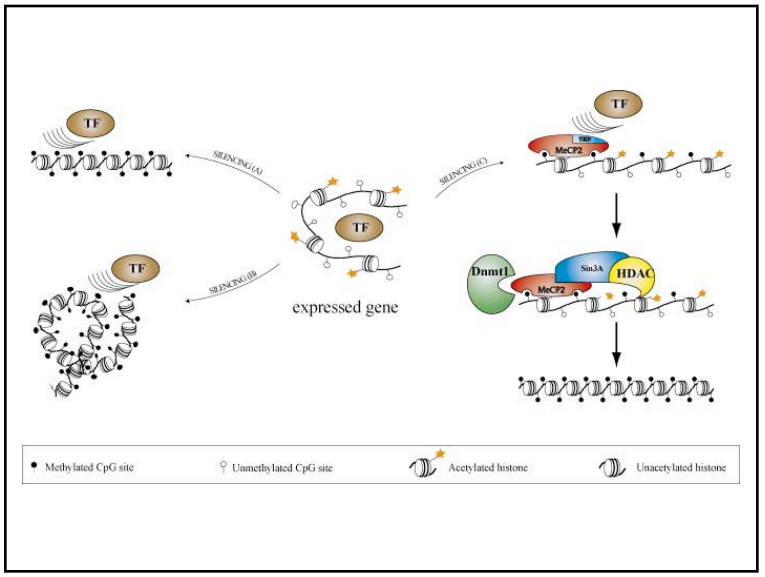
Allis, C.D., Jenuwein, T. & Reinberg, D., Eds. (2007). Epigenetics. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

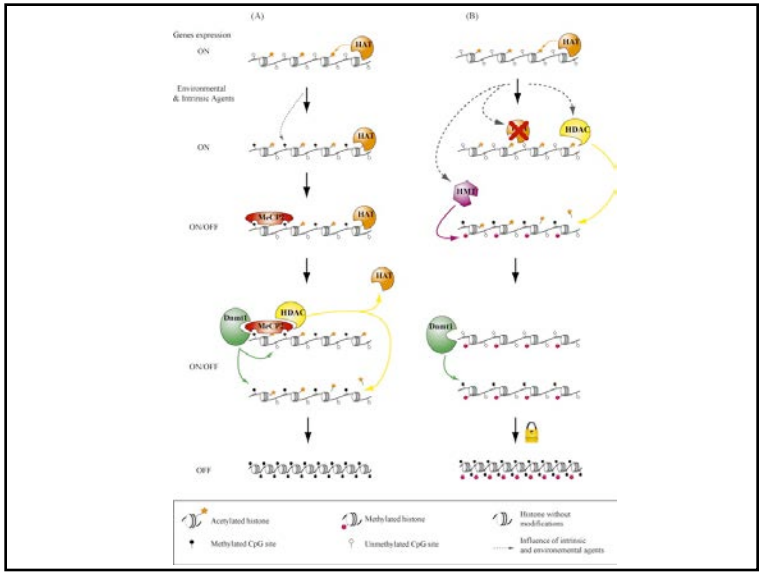
Jeanteur, P. EPIGENETICS AND CHROMATIN. Progress in Molecular and Subcellular Biology, 2005, Volume 38, 151-167, DOI: 10.1007/3-540-27310-7_6

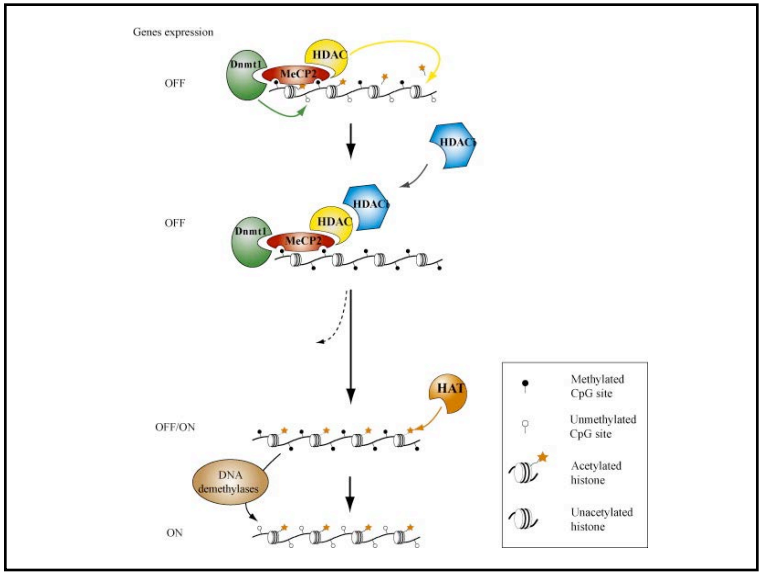
Epigenetics
DNA Methylation & Histone Modification Integration

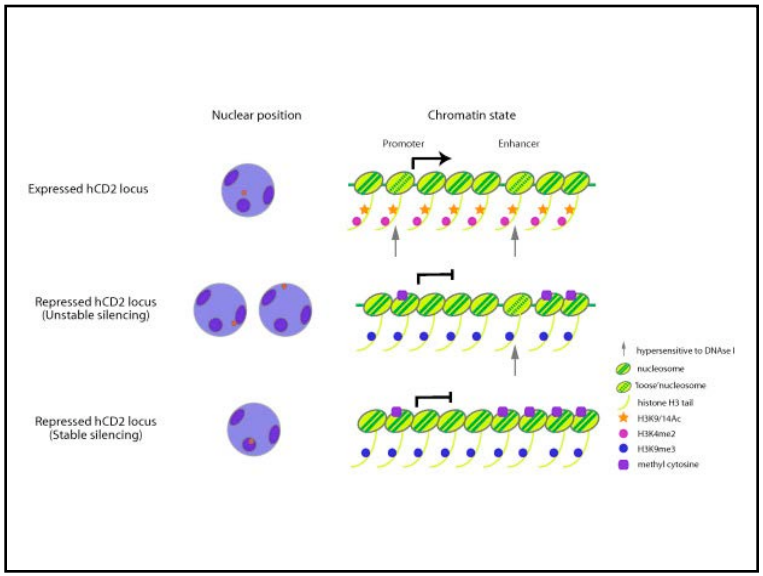



Tissue Phenotype
Promoter region
Gene product
"Normal"
Pre-neoplastic
Tumour
Methylated CpG site
Unmethylated CpG site
Acetylated histone
Unacetylated histone

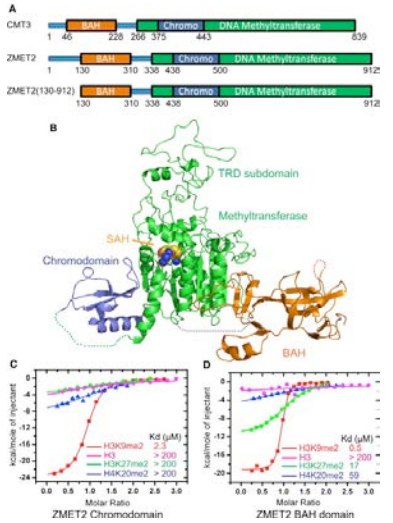

TF
SILENCING (A)
SILENCING (B)
SILENCING (C)
expressed gene
MeCP2
Sin3A
HDAC
Dnmt1
Methylated CpG site
Unmethylated CpG site
Acetylated histone
Unacetylated histone

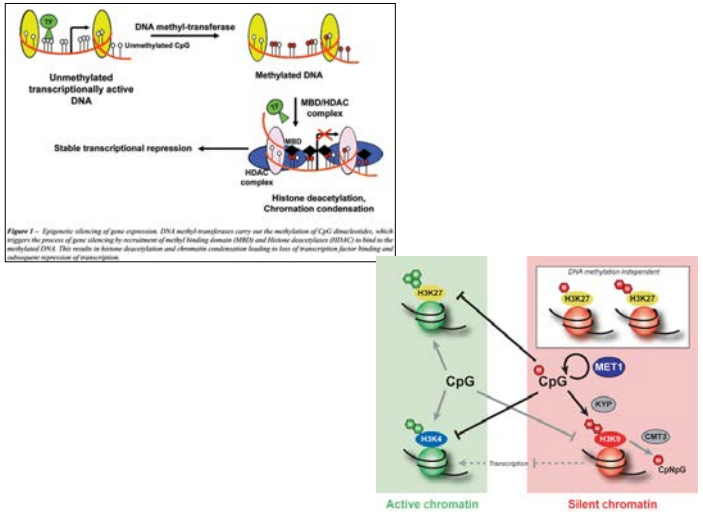

(A)
(B)
Genes expression
ON
Environmental & Intrinsic Agents
ON/OFF
OFF
HAT
HDAC
MeCP2
Dnmt1
HMT
Acetylated histone
Methylated CpG site
Methylated histone
Unmethylated CpG site
Histone without modifications
Influence of intrinsic and environmental agents

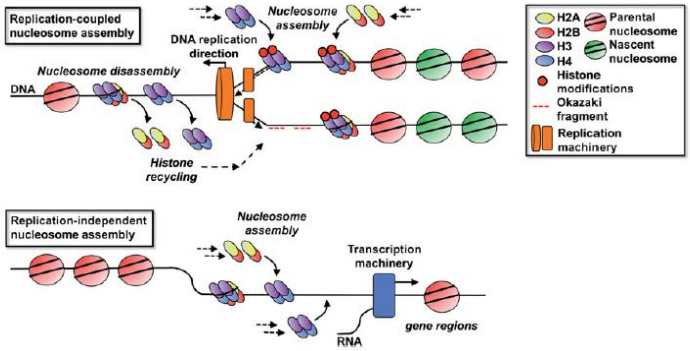

Genes expression
OFF
OFF
OFF/ON
ON
HDAC
MeCP2
Dnmt1
HAT
DNA demethylases
Methylated CpG site
Unmethylated CpG site
Acetylated histone
Unacetylated histone



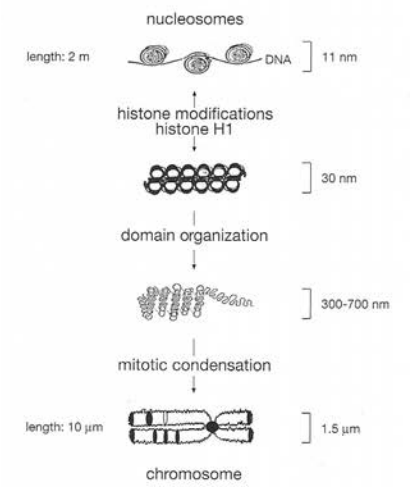
Dual binding of chromomethylase domains to H3K9me2-containing nucleosomes directs DNA methylation in plants.
Du J, et al. Cell. 2012 Sep 28;151(1):167-80.

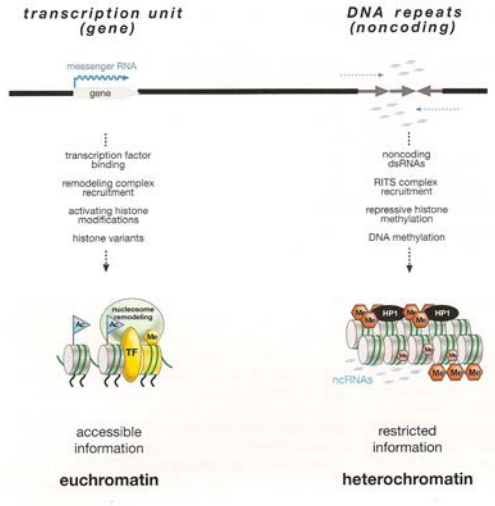


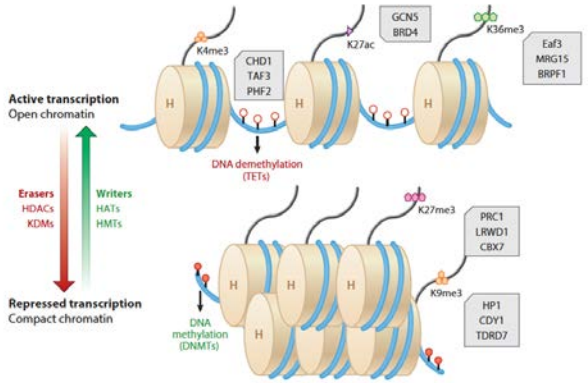


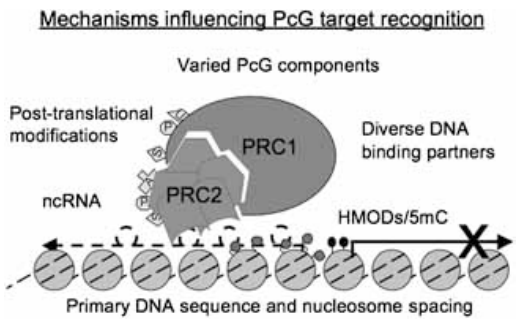


Epigenetics
Chromatin Structure



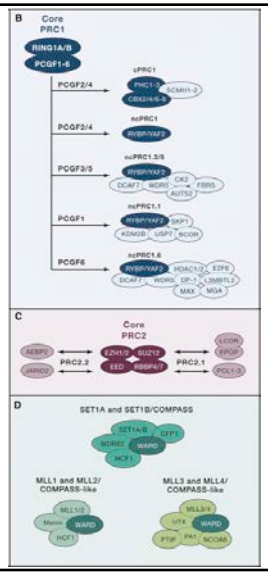


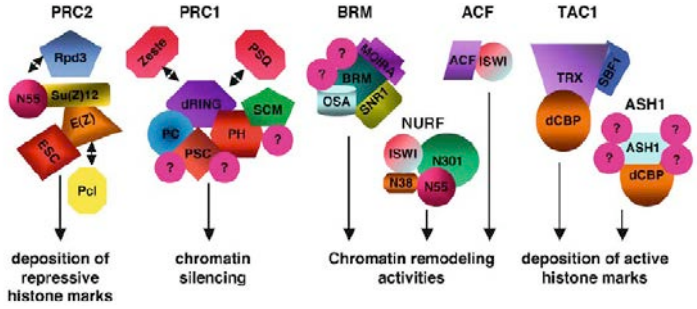


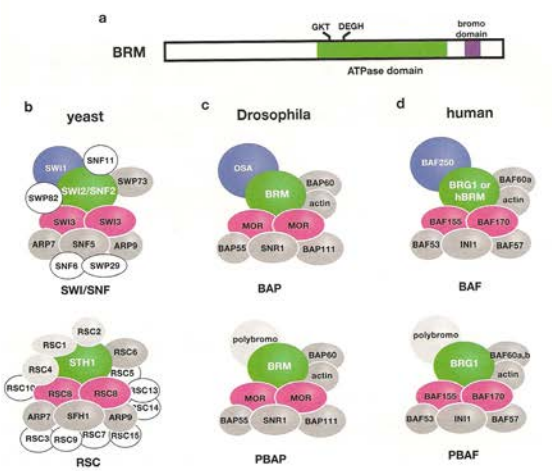


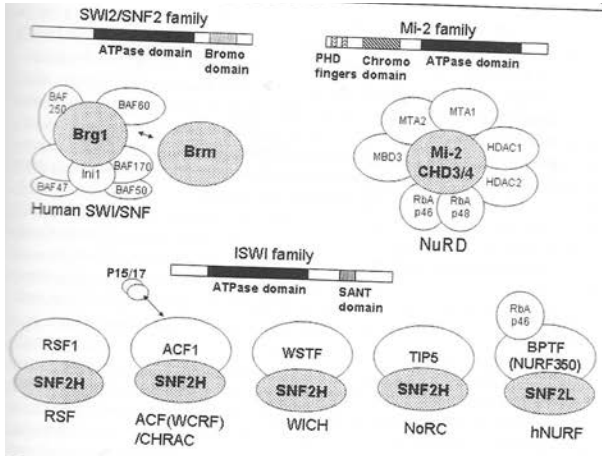
Summary of diverse mechanisms that may alter PcG binding to target DNA sequences, augment protein-protein interactions and/or modify enzymatic activity.

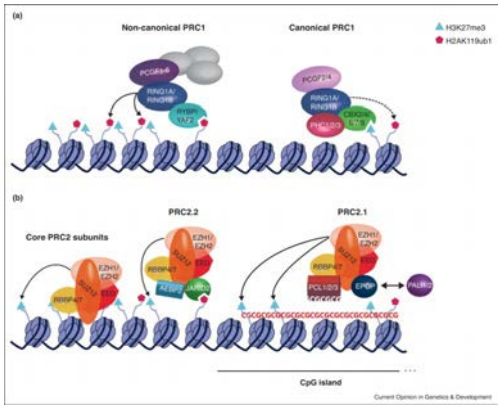
PcG complexes		trxG complexes	
PRC1	PC PH PSC dRING SCM	TAC1	TRX dCBP SBF1
PRC2	E(Z) ESC Su(Z)12 NURF-55	ASH1	ASH1 dCBP ...
		ASH2	ASH2 ...
PHO/PHOL Pipsqueak Grainyhead	DNA-binding PcG/trxG recruiters		Zeste GAF
	PcG/trxG cofactors		
Asx E(Pc) Su(Z)2 Corto Lola/Batman PCL Domino dMi2	ACF ISWI ACF	BRM BRM MOIRA OSA SNR1	Kismet Tonalli Skuld Kohtalo NURF NURF-301 ISWI NURF-55 NURF-38



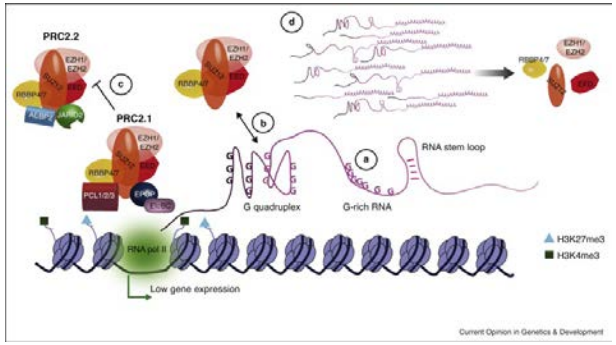






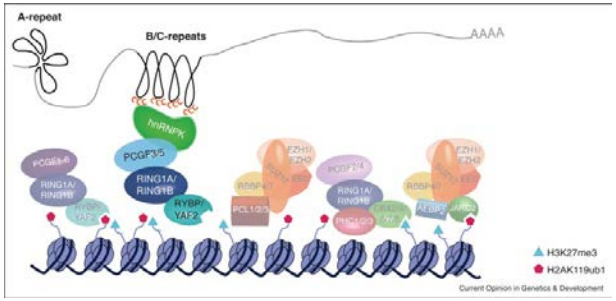


Polycomb repressive complexes subunits, recruitment mechanisms and catalytic activity in mammals.



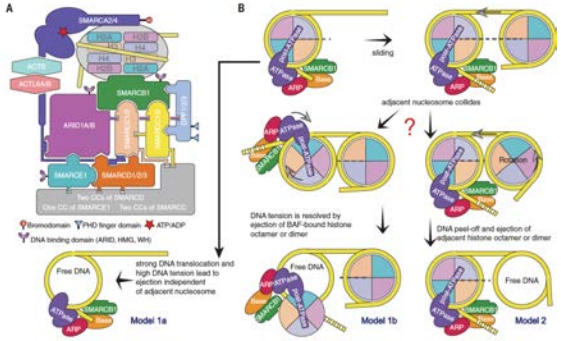
PRC2-RNA interaction depends on RNA structure and composition modulating PRC2 function.

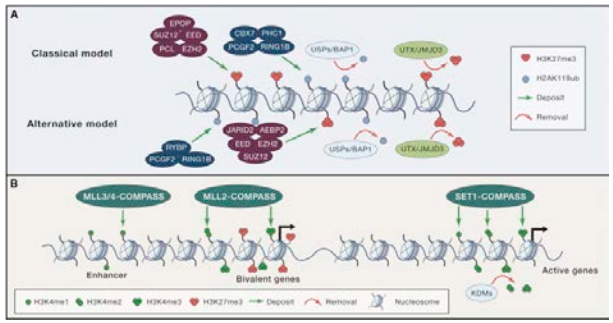
The many faces of Polycomb regulation by RNA
Almeida M, Bowness JS, Brockdorff N.
Curr Opin Genet Dev. 2020 Apr;61:53-61.



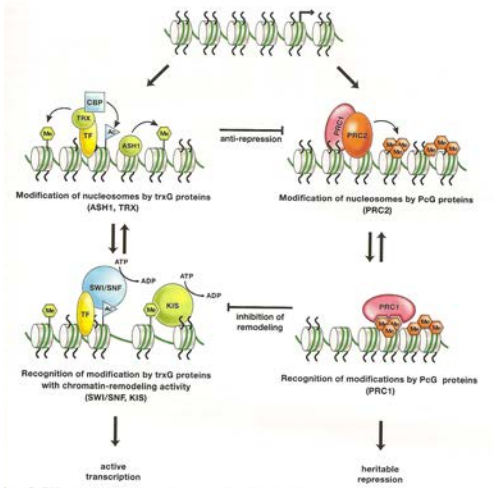
Model illustrating Polycomb recruitment by Xist lncRNA.

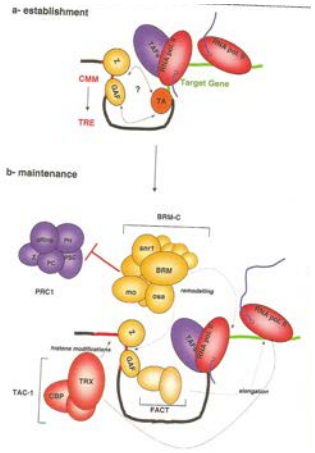
Structure of nucleosome-bound human BAF complex
He S, Wu Z, Tian Y, et al.
Science. 2020 Feb 21;367(6480):875-881.

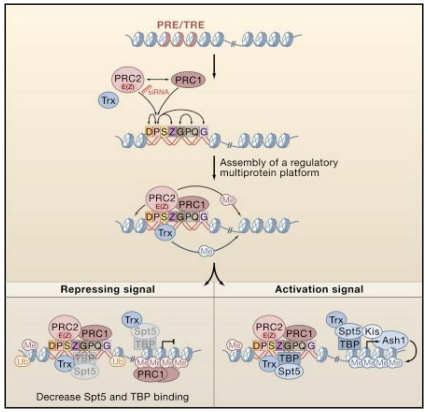




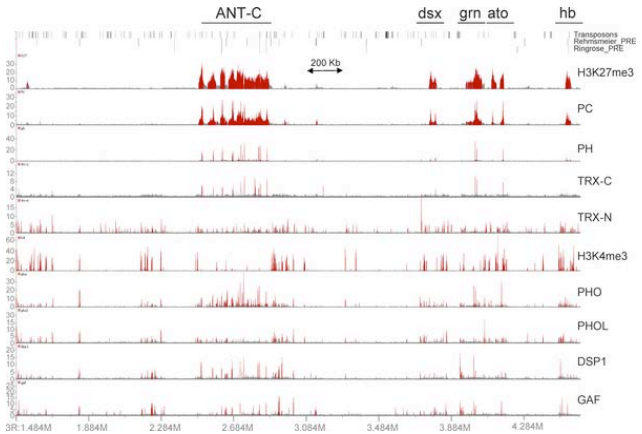
Deposition and Removal of PcG and TrxG-Mediated Histone Marks Contributes to Their Balanced Action on Chromatin. (A) According to the "classical model," targeting of the PRC2 complex leads to the methylation of H3K27. This modification is recognized by the Cbx subunit of the canonical PRC1 complex, which in turn catalyzes monoubiquitination of histone H2A at lysine 119 (H2AK119ub). An alternative recruitment mechanism posits that ncPRC1 is targeted to unmethylated CpG islands through the FBXL10/KDM2B subunit. ncPRC1 seems to be responsible for the majority of the H2AK119ub modifications present at PcG-target sites, which might facilitate the recruitment of PRC2. H3K27 methyl marks can be erased by histone demethylases, such as UTX/KDM6A and JMJD3/KDM6B, while several deubiquitinases (including BAP1, USP16 and USP21) can remove the monoubiquitin moiety from histone H2A. **(B)** Set1A/B COMPASS complexes catalyze mono-, di-, and trimethylation on H3K4 at active promoters. The activity of the partially redundant COMPASS complexes containing MLL3/KMT2C and MLL4/KMT2D leads to the deposition of H3K4me1 at enhancers, facilitating the recruitment of other activators such as CBP/p300. The deposition of methyl marks on H3K4 at bivalent regions is performed by MLL2/COMPASS. Multiple histone demethylases, including members of the KDM1/LSD, KDM2A/FBXL11, and KDM5/JARID families, are implicated in the removal of methyl groups on H3K4.

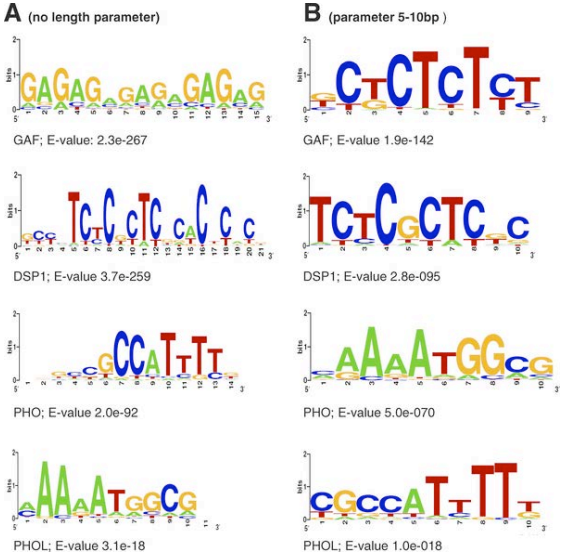


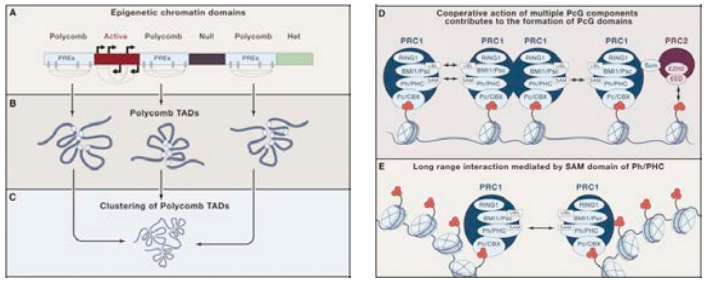


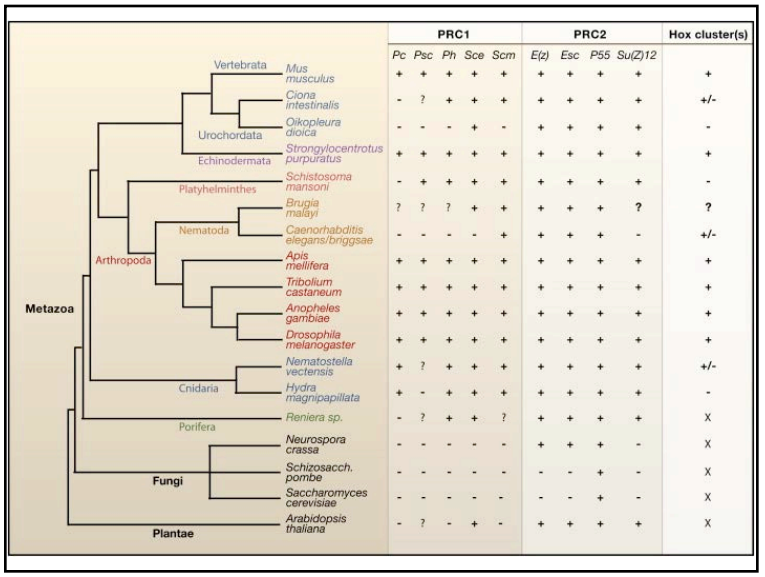


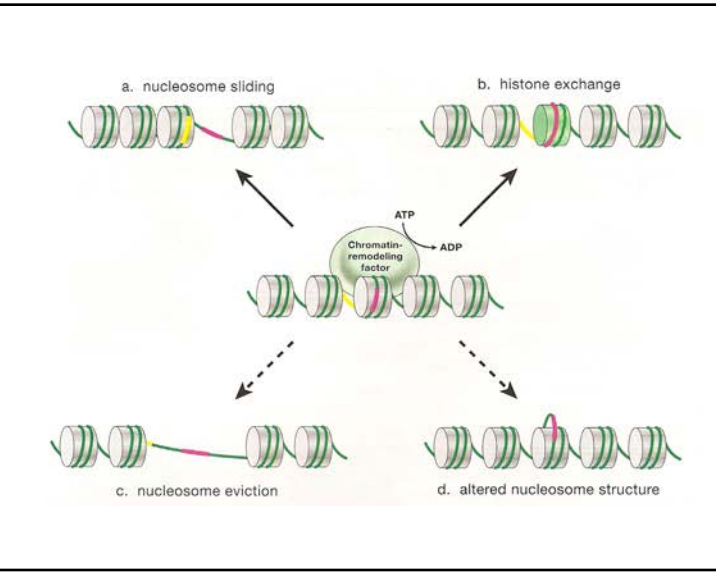
PREs and TREs as Molecular Binding PlatformsMultiple DNA binding proteins like Pho (P), Dsp1 (D), SP1/KLF (S), Zeste (Z), GAGA factor (G), Pipsqueak (PQ), and Grainyhead (G) recruit PcG complexes to PREs. The recruitment of either PcG or TrxG proteins to the PRE sequence does not depend on the activation status of the gene. Moreover, general transcription and elongation factors, such as TBP and Spt5, are constitutively bound to the PRE. A developmental signal then determines whether the PRE mediates gene activation or gene repression, which are accompanied by trimethylation (Me) of histone H3 on lysine 4 or lysine 27, respectively. Upon activation, Kismet (Kis), a protein facilitating elongation, and Ash1, leading to local H3K4 methylation, are recruited to the promoter region. Small RNAs (red ladder) may also contribute to PcG protein recruitment.

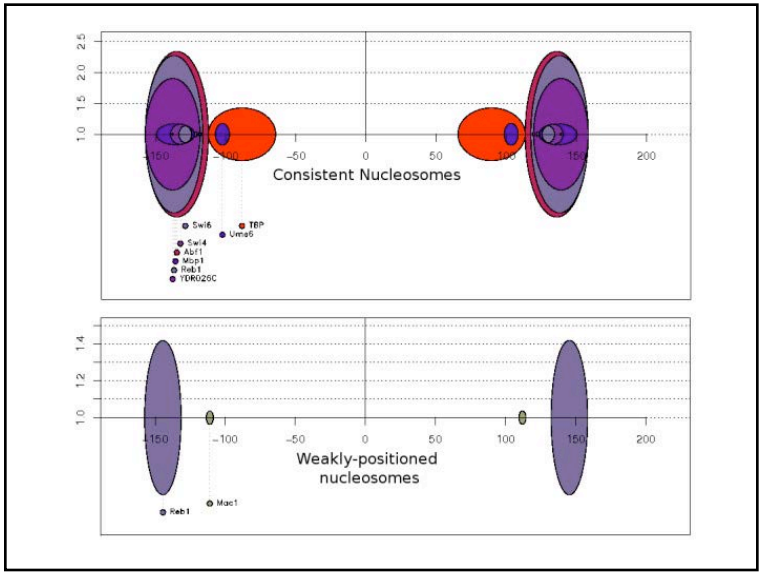


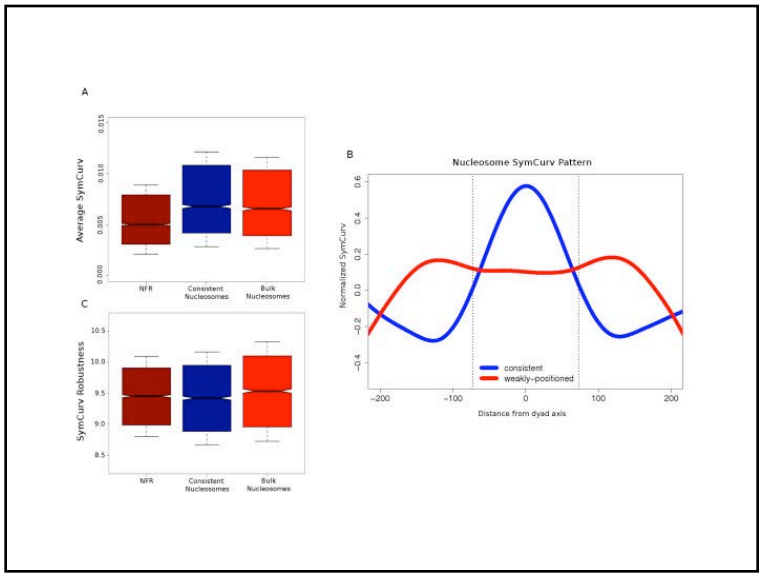


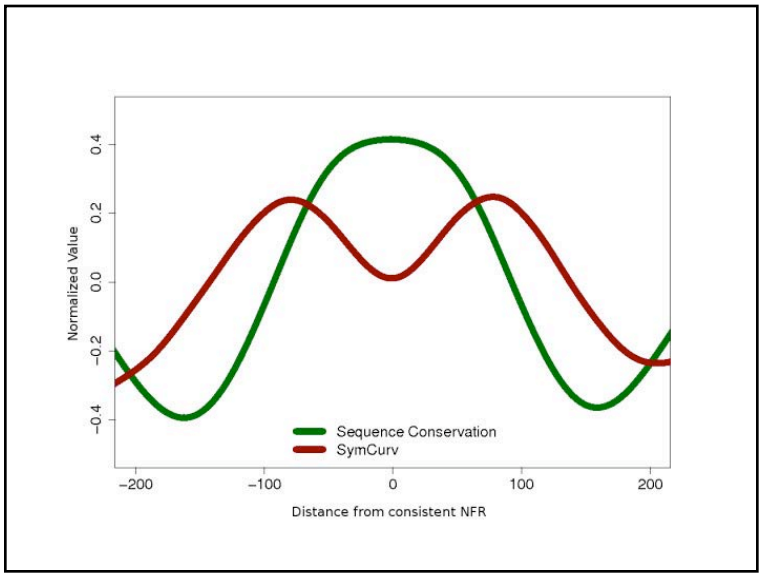

A
Epigenetic chromatin domains
Polycomb
Active
Polycomb
Null
Polycomb
Het
B
Polycomb TADs
C
Clustering of Polycomb TADs
D
Cooperative action of multiple PcG components contributes to the formation of PcG domains
PRC1
PRC1
PRC1
PRC1
PRC2
E
Long range interaction mediated by SAM domain of Ph/PHC
PRC1
PRC1

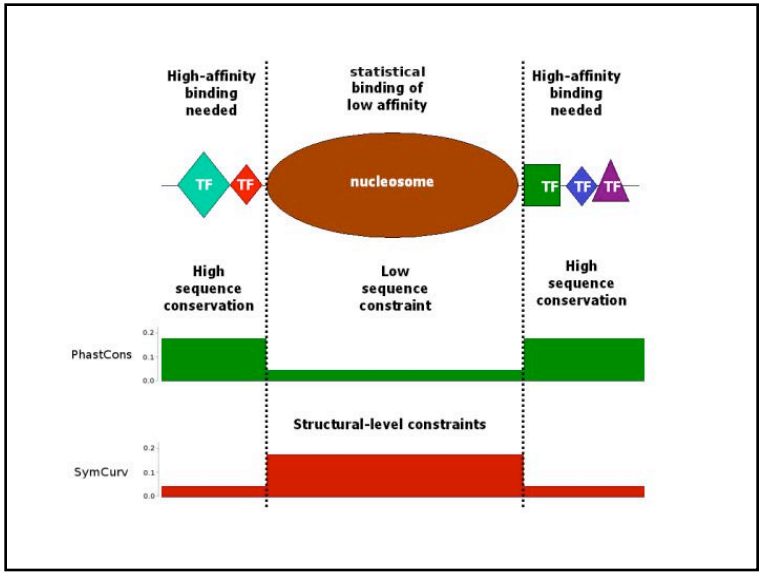

PRC1
PRC2
Hox cluster(s)
Pc Psc Ph Sce Scm
E(z) Esc P55 Su(Z)12
Vertebrata
Mus musculus + + + + + + + + + +
Ciona intestinalis - ? + + + + + + + +/-
Urochordata
Oikopleura dioica - - - + - + + + + -
Echinodermata
Strongylocentrotus purpuratus + + + + + + + + + +
Platyhelminthes
Schistosoma mansoni - + + + + + + + + -
Nematoda
Brugia malayi ? ? ? + + + + + ? ?
Caenorhabditis elegans/briggsae - - - - + + + + - +/-
Arthropoda
Apis mellifera + + + + + + + + + +
Tribolium castaneum + + + + + + + + + +
Anopheles gambiae + + + + + + + + + +
Drosophila melanogaster + + + + + + + + + +
Metazoa
Nematostella vectensis + ? + + + + + + + +/-
Cnidaria
Hydra magnipapillata + - + + + + + + + -
Porifera
Reniera sp. - ? + + ? + + + + X
Fungi
Neurospora crassa - - - - - + + + - X
Schizosacch. pombe - - - - - - - + - X
Saccharomyces cerevisiae - - - - - - - + - X
Plantae
Arabidopsis thaliana - ? - + - + + + + X

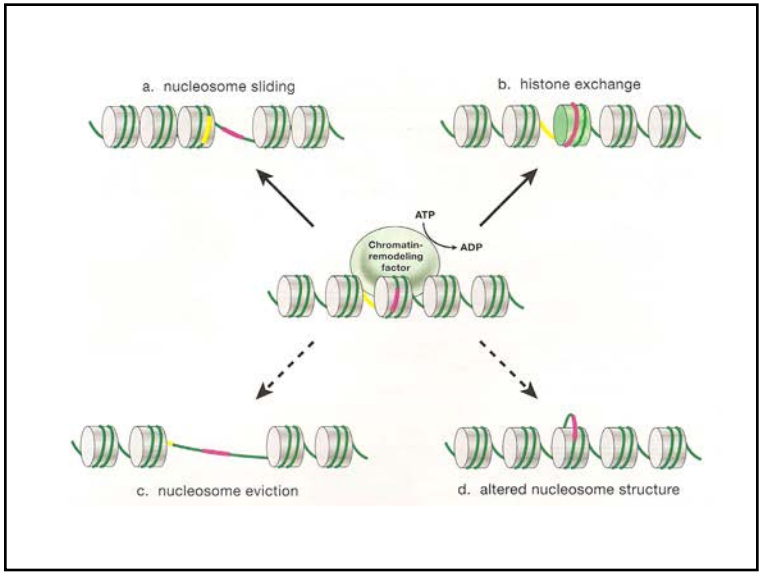

a. nucleosome sliding
b. histone exchange
ATP
ADP
Chromatin-remodeling factor
c. nucleosome eviction
d. altered nucleosome structure

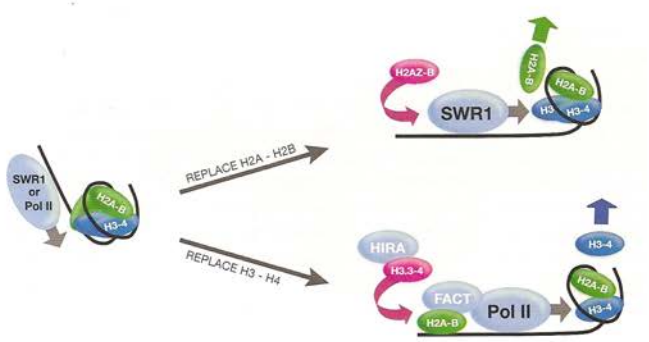

Consistent Nucleosomes
Swi6
Swi4
Abf1
Mbp1
Reb1
YDR026C
TBP
Ume6
Weakly-positioned nucleosomes
Reb1
Mac1

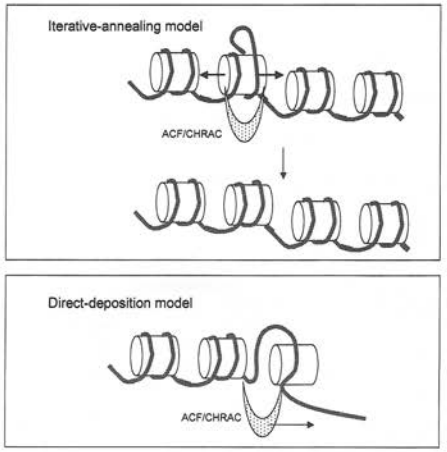

A
Average SymCurv
NFR
Consistent Nucleosomes
Bulk Nucleosomes
C
SymCurv Robustness
B
Nucleosome SymCurv Pattern
Normalized SymCurv
consistent
weakly-positioned
Distance from dyad axis


Normalized Value
Sequence Conservation
SymCurv
Distance from consistent NFR


High-affinity binding needed
statistical binding of low affinity
High-affinity binding needed
TF
nucleosome
High sequence conservation
Low sequence constraint
High sequence conservation
PhastCons
Structural-level constraints
SymCurv


a. nucleosome sliding
b. histone exchange
ATP
ADP
Chromatin-remodeling factor
c. nucleosome eviction
d. altered nucleosome structure

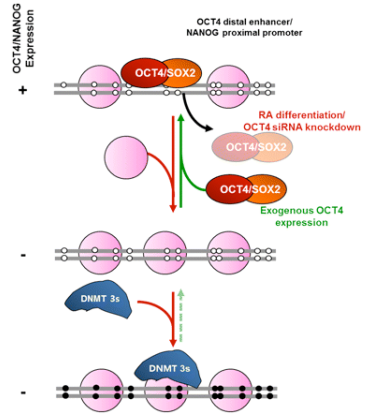




OCT4 establishes and maintains nucleosome-depleted regions that provide additional layers of epigenetic regulation of its target genes

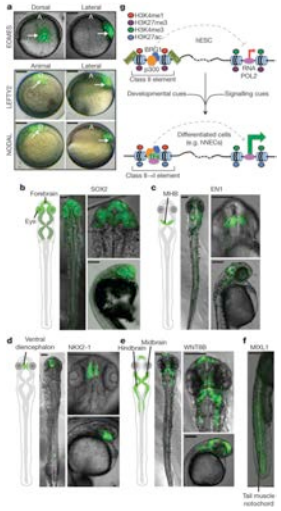
Jueng Soo You, Theresa K. Kelly, Daniel D. De Carvalho, Phillippa C. Taberlay, Gangning Liang, and Peter A. Jones[1]

PNAS | August 30, 2011 | vol. 108 | no. 35 | 14497–14502



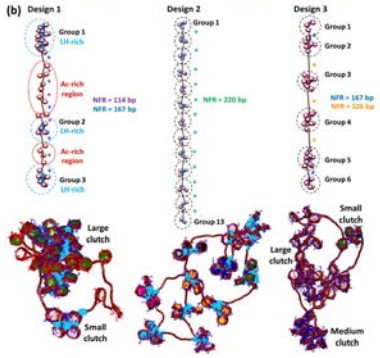
A proposed model of the mechanism of repression and de novo DNA methylation of OCT4 and NANOG during cell differentiation. (Refer to Discussion for a detailed explanation.)

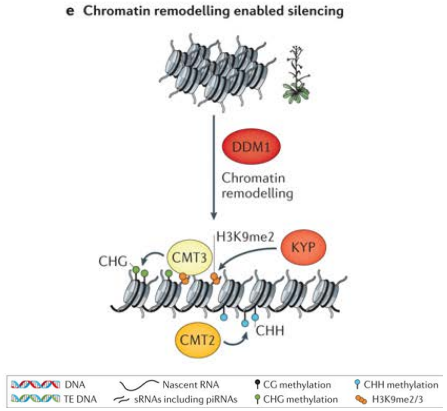
A unique chromatin signature uncovers early developmental enhancers in humans.
Rada-Iglesias A, et al. Nature. 2011 Feb 10;470(7333):279-83

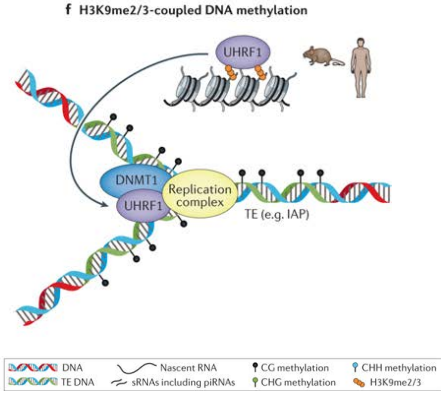


Class II elements have developmental enhancer activity *in vivo*. a, Merged bright-field and GFP images are shown for representative shield stage zebrafish embryos injected with class II elements proximal to human EOMES, LEFTY2 and NODAL. For the EOMES enhancer, dorsal (anterior to top) and lateral (shield to right) views are presented in the left and right panels, respectively. For LEFTY2 and NODAL, animal pole (shield to top) and lateral (shield to right) views are presented in the left and right panels, respectively. White arrows indicate the location of the shield in each image. A, anterior; D, dorsal. Scale bar, 150 μm. b–f, Merged bright-field and GFP images are shown for representative 24–28 h.p.f. zebrafish embryos injected with class II elements proximal to SOX2 (b), EN1 (c), NKX2-1 (d), WNT8B (e) and MIXL1 (f) genes. In b–e, schematics highlighting the relevant anatomical structures where GFP expression was reproducibly observed are shown on the left, and three images correspond, from left to right and top to bottom, to whole-embryo flattened dorsal views, dorsal anterior views and lateral anterior views, respectively. In f, a lateral posterior view is shown. In b–f, scale bar = 150 μm. MHB, midbrain–hindbrain boundary. g, Proposed model for enhancer bookmarking during early embryonic development. Poised developmental enhancers (class II) are marked by a unique chromatin signature, involving occupancy of chromatin modifiers p300, BRG1 and PRC2 and nucleosomal regions marked by H3K4me1 and H3K27me3. During differentiation, appropriate developmental and signalling cues are able to rapidly transition these poised, pre-marked enhancers into an active state represented by the acquisition of H3K27ac, RNA POL2 binding, recruitment of tissue-specific transcription factors (TFs) and loss of H3K27me3, leading to the establishment of tissue-specific gene expression patterns.

Nucleosome Clutches are Regulated by Chromatin Internal Parameters
Portillo-Ledesma S, Tsao LH, Wagley M, et al.
J Mol Biol. 2020 Nov 9;166701.

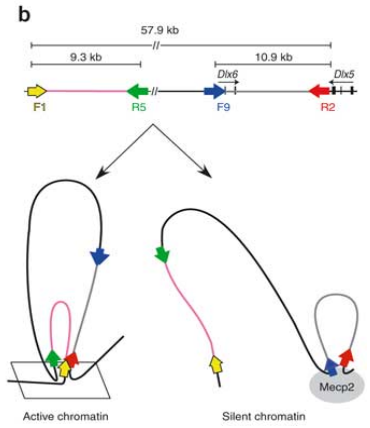






- Gene expression and transcription is regulated at many levels, including at the chromatin level
- Differences in methylation can result in different chromatin loops
- Thus, CpG methylation can regulate nearby genes or genes 10-100kb away

- Take home: It's unclear whether regulated methylation events are impacting proximal or distal genes



Nat Gen 37, 31 (2005)

CpG unmethylated – in open chromatin

CpG methylated – in closed chromatin

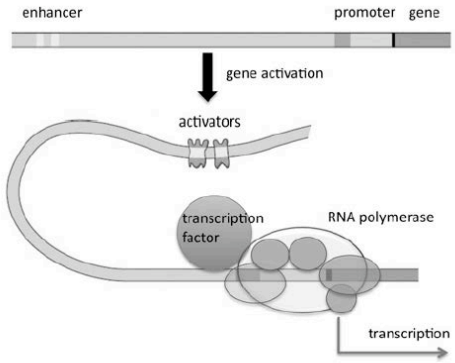


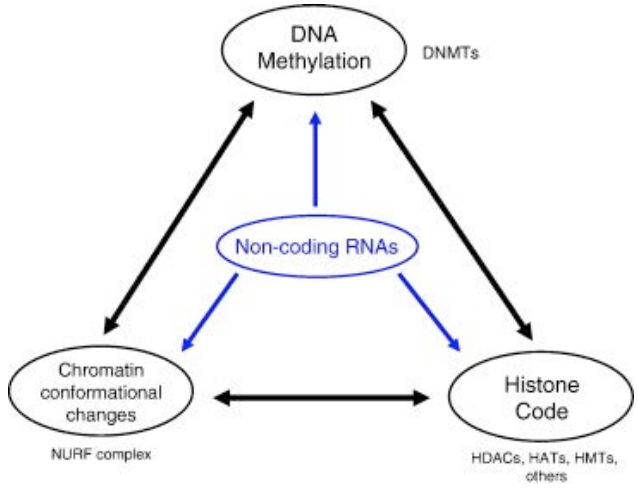
Figure 2 *Enhancers and long-range chromatin interactions*

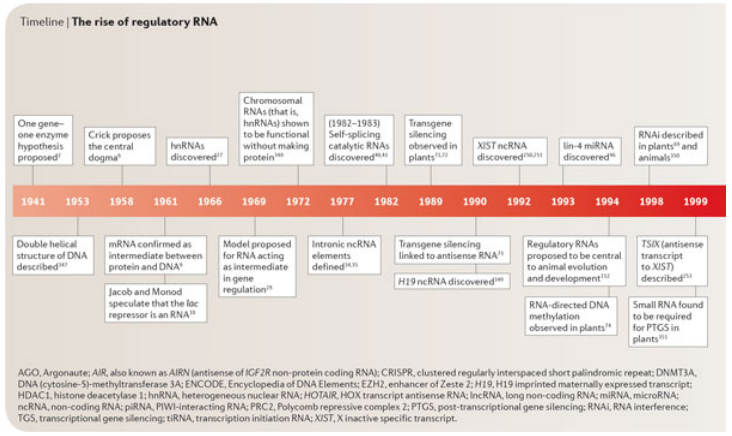
Enhancer is a short DNA region to which activators can bind. Activators bind to an enhancer and affect the transcription of a gene that is located away from the enhancer by recruiting transcription factors and the Pol II complex to the relevant promoter. This process requires the role of three-dimensional chromatin looping

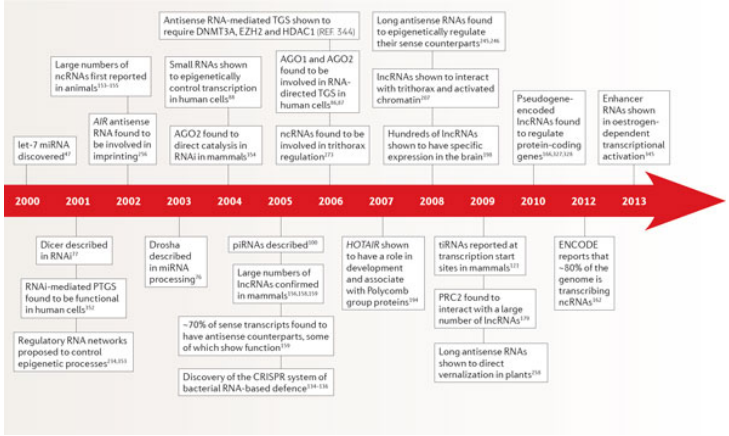
Table 2. Selected genetic disorders affecting chromatin structure in *trans*

Disorder	Gene	Comments
Rubinstein-Taybi syndrome	*CREBBP, EP300*	
Rett syndrome	*MECP2*	loss of function as well as duplication causes a broad spectrum of phenotypes
α-Thalassemia and X-linked mental retardation	*ATRX*	somatic mutations cause α-thalassemia and myelodysplastic syndrome
ICF Syndrome	*DNMT3B*	
Schimke immuno-osseous dysplasia	*SMARCAL1*	
Mental retardation	*MTHFR*	

Epigenetics
non-coding RNAs







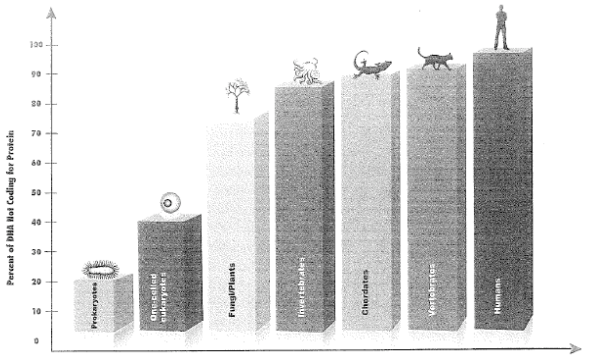
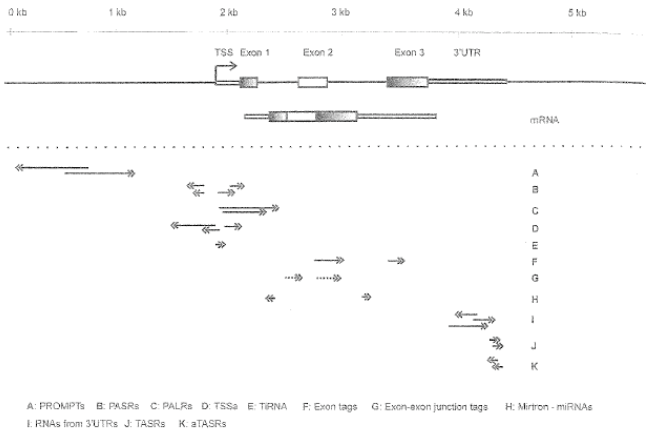


Figure 7.3 Non-coding RNAs as drivers of evolution and complexity? The histogram depicts the percentage of non-coding DNA in different organisms along the evolutionary scale. The Y axis represents the percentag of DNA not coding for protein and the X axis shows histograms for different organisms from prokaryote to humans. It is clear that in prokaryotes the percentage of DNA not coding for proteins is very sma (approximately 20% or less). On the other hand, organisms such as chordates, vertebrates and human have more than 85% of non-coding DNA in their genomes. Importantly, a big proportion of this non-codin DNA is transcribed into lncRNAs, most of which still have unknown functions. Histograms and chart adapte by Kathryn A. Bischof.

Table 16.2 RNA classes emanating from transcriptional units

RNA class	Size	Location	Orientation	Reference
PROMPTs	long	0.5–2.5 kb upstream of TSS	Sense or antisense	Preker (2008)
PALRs	200 nt to 1 kb	Within 0.5 kb of TSS, can extend into 1st exon or intron	sense	Kapranov (2007), Fejes-Toth (2009)
PASRs	22–200 nt	Start downstream or upstream of TSS, within 0.5 kb of TSS	Sense or antisense	Kapranov (2007), Fejes-Toth (2009)
aPASRs	<200 nt	PASRs with poly-U tail at 5′ end. Proposed to be generated by RDRP-like copying from poly adenylated transcripts emanating from promoter regions	antisense	Kapranov (2010)
TiRNA	18 nt	−60 to +120 of TSS	Sense	Taft (2009)
TSSa	20–90 nt	250 nt upstream or 50 nt downstream of TSS	Sense or antisense	Seila (2008)
TASRs	20–200 nt	Near termination sites	Sense or antisense	Kapranov (2007)
aTASRs	<200 nt	Contain a poly-U tail at 5′ end. Proposed to be generated by an RDRP-like copying mechanism from 3′ ends of polyadenylated RNA	Antisense	Kapranov (2010)

TSS, transcription start sites; PROMPTs, promoter upstream transcripts; PALRs, promoted-associated long RNA; PASR, promoter-associated short RNA; TASRs, termini-associated short RNA; aTASRs, antisense TASR; TSSs, TSS-associated RNAs; RDRP, RNA-dependent RNA polymerase.



A: PROMPTs B: PASRs C: PALRs D: TSSa E: TIRNA F: Exon tags G: Exon-exon junction tags H: Mirtron - miRNAs
I: RNAs from 3'UTRs J: TASRs K: aTASRs

Table 1. ncRNA classes and their function

ncRNA class	Size	Function	References
miRNAs	~23 nt	Regulation of hundreds to thousands of protein-coding and non-coding genes by mechanisms such as post-translational gene silencing in animals and plants	[31–44]
piRNAs	26–31 nt	Repeat silencing in the genome and regulation of DNA methylation affecting gene expression	[45–49]
Small RNAs	~20–300 nt	Diverse functions from RNA modification to genomic imprinting in eukaryotic cells	[50–55]
LncRNAs	>300 to thousands of nt	Diverse functions from structural mechanisms to gene regulation by epigenetic modifications	[9, 10, 56–61]

LncRNAs, long ncRNAs; miRNAs, microRNAs; nt, nucleotides; piRNAs, Piwi-interacting RNAs.
The classification of ncRNAs is mainly based in size and functionality and this categorization is very subjective. piRNAs and miRNAs are classified as small RNAs by several groups; however, they are described separately here since they differ in function.

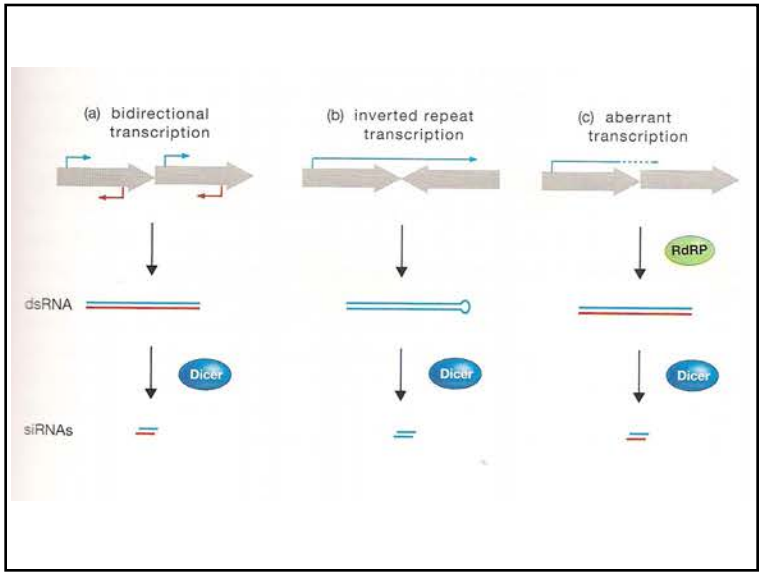
Table 3. ncRNAs produced by eukaryotic genomes that have been recently described

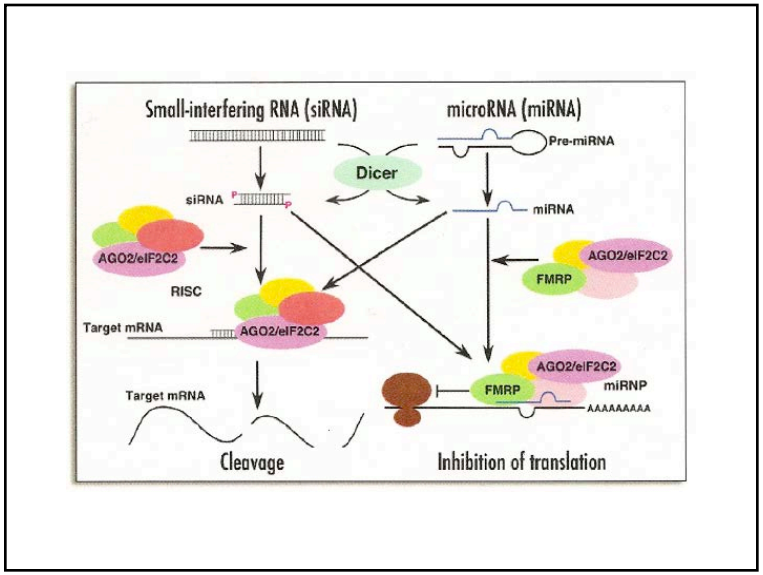
ncRNA class	Size	Organism	Function	References
CUTs and SUTs	>200 nt	Yeast	Broad regulators of gene transcription in yeast by epigenetic mechanisms in bidirectional promoters	[12, 13]
Repetitive RNAs	Diverse	Mouse and human	Epigenetic regulation? Other mechanisms?	[14]
LincRNAs	>200 nt	Mouse and human	Associated with diverse biological processes based on high conservation between different species	[9, 10]
tiRNAs	~18 nt	Human, chicken and fruit fly	Putative function in chromatin modifications and protein recruitment for transcription initiation	[11]
PASR RNAs	Produced as long ncRNAs (PALRs) and processed to small RNAs of ≤200 nt	Human	The majority has obscure function but it is speculated that they could serve as important components of regulatory circuitries	[23]
TSSa RNAs	20 to 90 nt	Human	Possible role in maintaining the structure of the chromatin and driving the transcription of nascent RNAs	[65]
NRO-RNAs	~100 nt	Human	They may play a role in gene transcription	[66]
PROMPTs and PALRs	An average of 500 nt	Human	Produced upstream of active genes and associated with chromatin changes	[23, 67]
ncmtRNAs	>200 nt	Human	Regulation of cell cycle and proliferation by unknown mechanisms	[16]
TUFs	>200 nt to several kb	Human	Important functions in stem cell differentiation	[70]

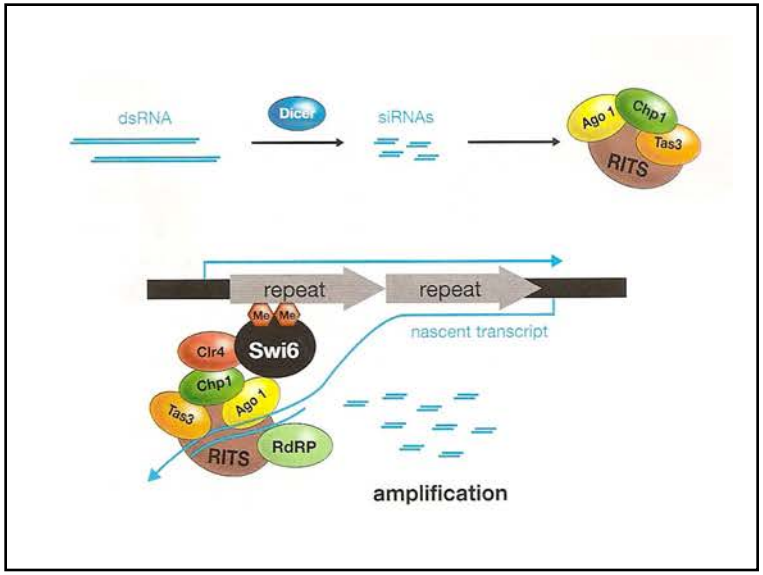
CUTs, cryptic unstable transcripts; kb, kilobases; LincRNAs, large intergenic ncRNAs; ncmtRNA, non-coding mitochondrial RNAs; NRO-RNA, nuclear run-on RNAs; nt, nucleotides; PALR, promoter-associated long RNAs; PASR, promoter-associated short RNAs; PROMPT, promoter upstream transcripts; tiRNAs, transcription initiation RNAs; TSSa, transcription start site-associated RNA; TUF, transcripts of unknown function.

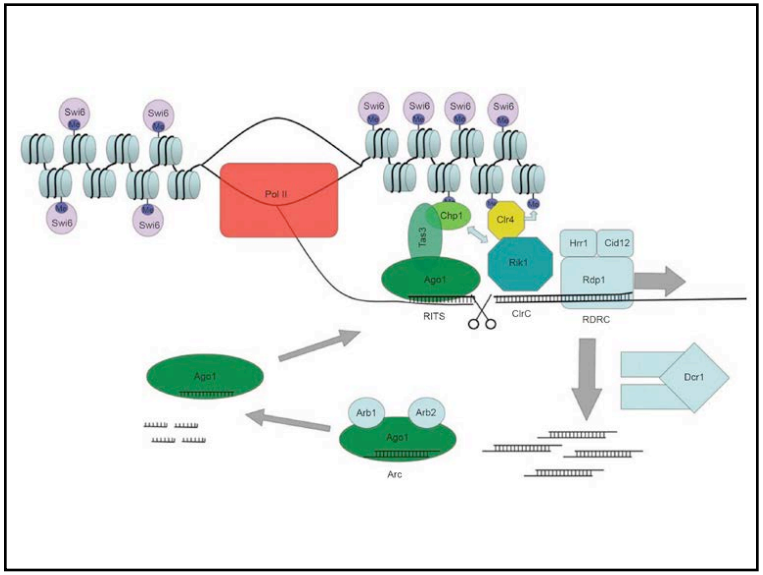
miRNA,siRNA and RNAi

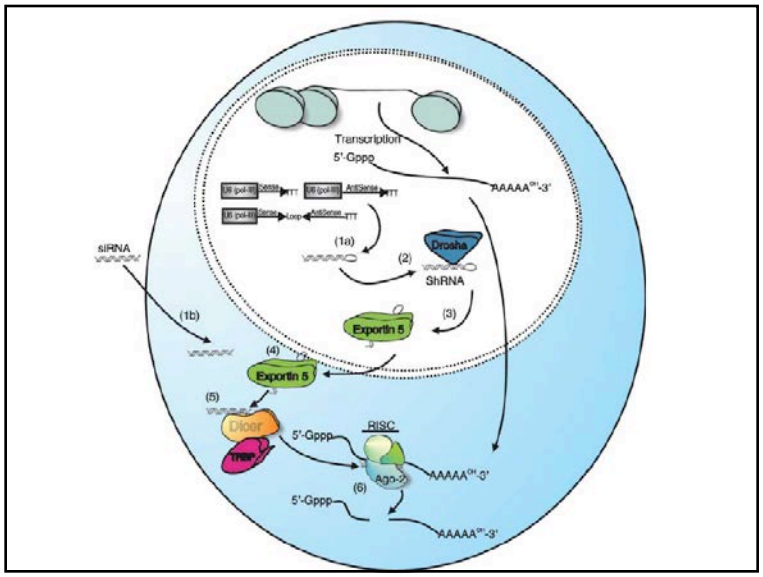
Sequence dependent mRNA stability and translation control

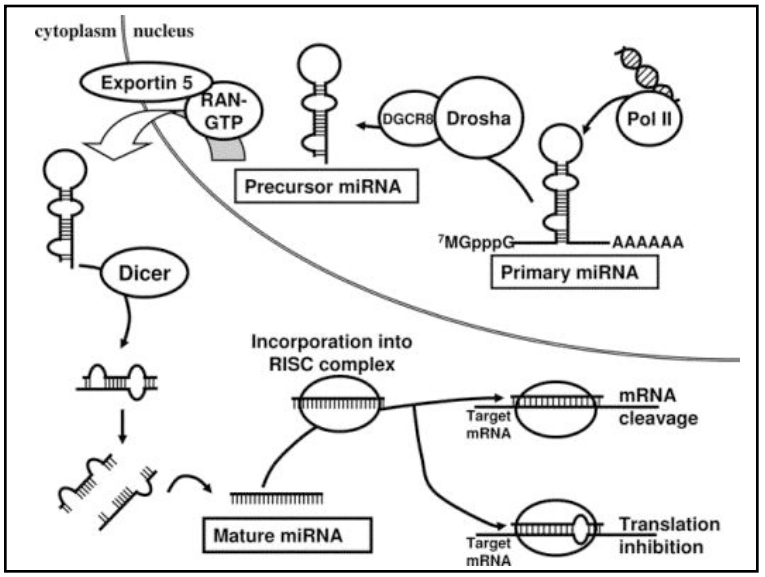

(a) bidirectional transcription
(b) inverted repeat transcription
(c) aberrant transcription
RdRP
dsRNA
Dicer
Dicer
Dicer
siRNAs


Small-interfering RNA (siRNA)
microRNA (miRNA)
Pre-miRNA
Dicer
siRNA
miRNA
AGO2/eIF2C2
RISC
AGO2/eIF2C2
FMRP
Target mRNA
AGO2/eIF2C2
AGO2/eIF2C2
FMRP
miRNP
Target mRNA
AAAAAAAAA
Cleavage
Inhibition of translation


dsRNA
Dicer
siRNAs
Ago 1
Chp1
Tas3
RITS
repeat
repeat
Me
Me
nascent transcript
Clr4
Swi6
Chp1
Ago 1
Tas3
RITS
RdRP
amplification


Swi6
Swi6
Swi6
Swi6
Swi6
Swi6
Swi6
Swi6
Pol II
Chp1
Clr4
Tas3
Hrr1
Cid12
Rik1
Ago1
Rdp1
RITS
ClrC
RDRC
Ago1
Dcr1
Arb1
Arb2
Ago1
Arc





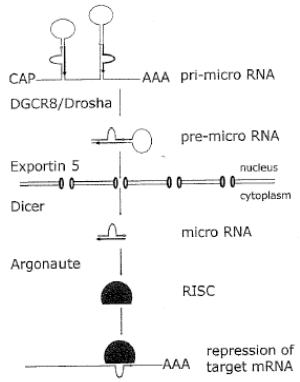
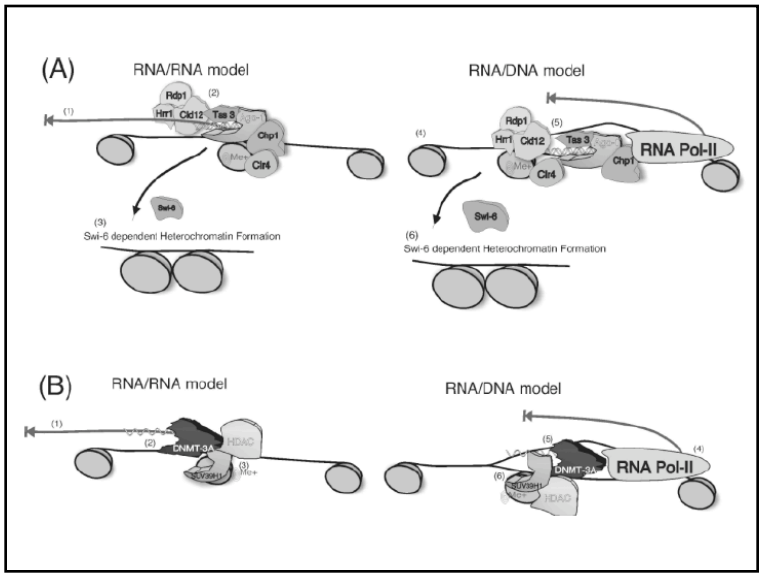


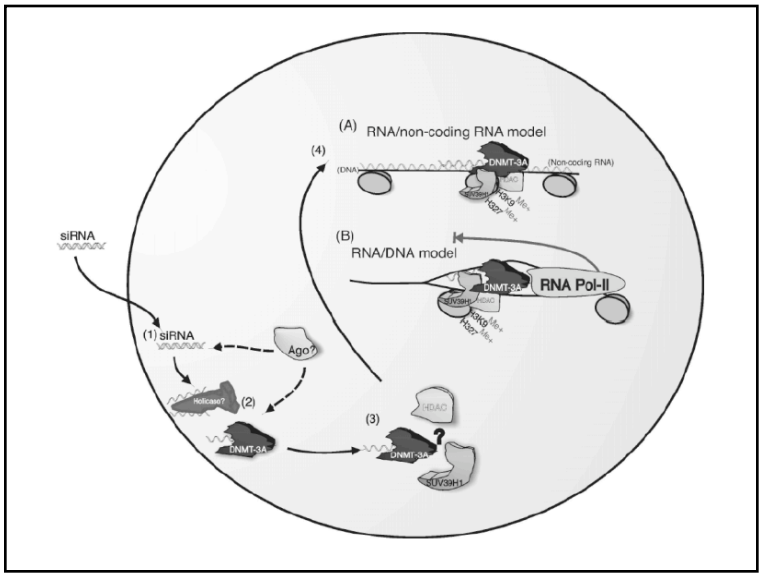
Figure 2.1 Schematic representation of miRNA processing. Stem loop structures in the pri-miRNA transcript are recognized and cleaved by DGCR8/Drosha to form the pre-miRNA structures. The stem loop is recognized by Exportin 5 and shuttled to the cytoplasm. Dicer removes the stem loop to generate microRNAs that can enter the pre-RISC complex. Strand displacement results in mature RISC formation and eventual repression of the target mRNA.

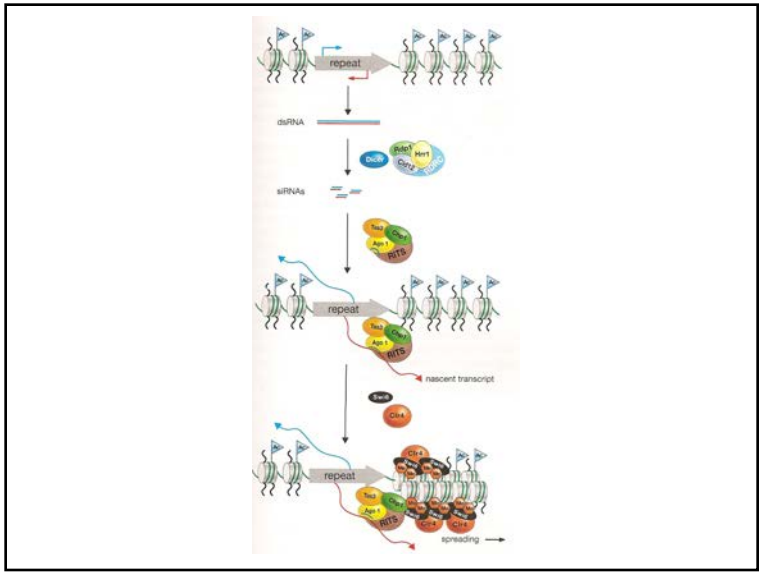
Table 1 Compilation of features of heterochromatin and RNAi from yeast, animals and plants

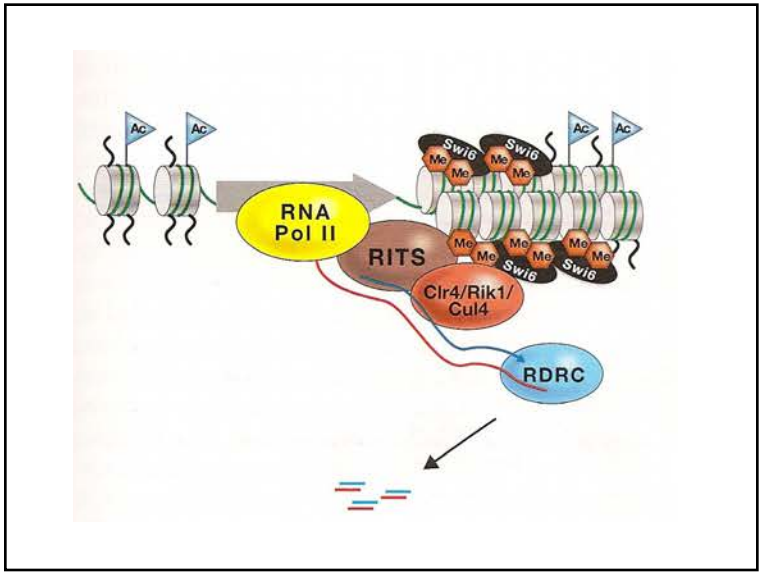
	Fungi		Ciliates	Animals			Plants
	S. pombe	*N. Crassa*	*T. thermophila*	*C. elegans*	*D. melanogaster*	*H. sapiens*	*A. thaliana*
H3K9me	Yes	Yes	Yes	Yes	Yes	Yes	Yes
HP1	Swi6, Chp1 & Chp2	HP1	Pdd1p &Pdd3p	HPL1 &HPL2	HP1, HP1b & HP1c	HP1α, β & γ	TFL2/LHP1
DNA methylation	–	Yes	Yes	–	Low levels	Yes	Yes
short RNA	siRNA	siRNA	scnRNA	miRNA & siRNA	miRNA, siRNA, piRNA	miRNA, siRNA & piRNA	miRNA & siRNA
Dicer	Dcr1	DCL1 & DCL2	DCL1	Dcr1	Dcr1 & Dcr2	DCR1	DCL1, DCL2, DCL3 & DCL4
Argonaute	Ago1	QDE2 & SMS2	TWI1	27 AGO genes	Ago1-3, Piwi1-4	AGO1-4, PIWI1-4	AGO1-10
RDP	Rdp1	QDE1, SAD1 & RRP3	–	Ego1, Rrf1-3	–	–	RDR1-6
ds siRNA ribonuclease	Eri1	n.d.	–	Eri1	CG6393	THEX1	–
pol IV	–	–	–	–	–	–	NRPD1a & b

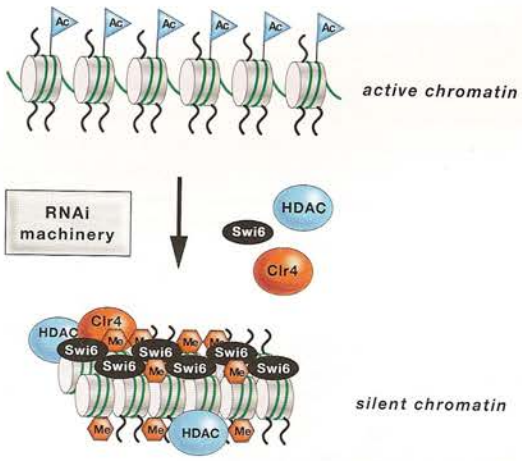
n.d.: Not determined

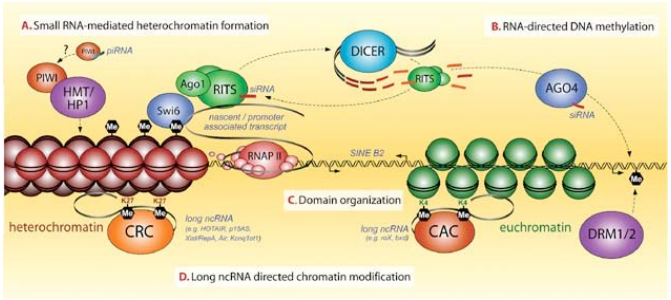

(A)
RNA/RNA model
RNA/DNA model
Rdp1
Hrr1
Cid12
Tas 3
Chp1
Clr4
Swi-6
RNA Pol-II
Swi-6 dependent Heterochromatin Formation
(B)
RNA/RNA model
RNA/DNA model
DNMT-3A
HDAC
SUV39H1
RNA Pol-II

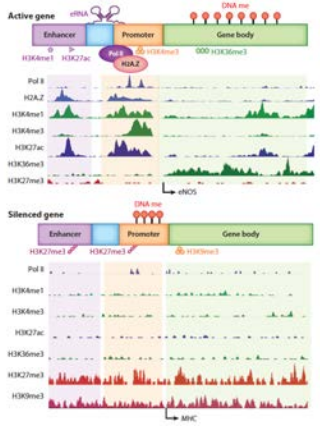

(A) RNA/non-coding RNA model
(B) RNA/DNA model
siRNA
(1) siRNA
Ago?
Helicase?
(2)
(3)
(4)
DNMT-3A
SUV39H1
HDAC
RNA Pol-II
(Non-coding RNA)

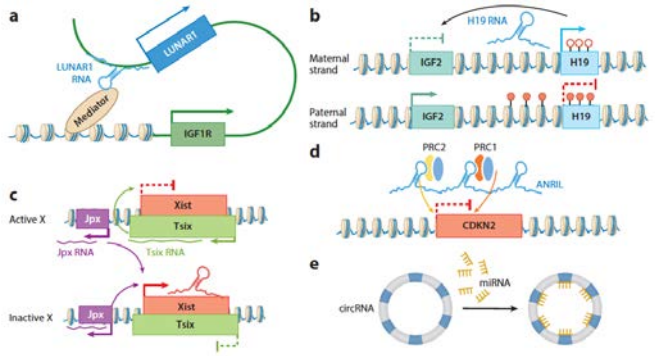

repeat
dsRNA
siRNAs
RITS
nascent transcript
spreading


Ac
Me
Swi6
RNA
Pol II
RITS
Clr4/Rik1/
Cul4
RDRC

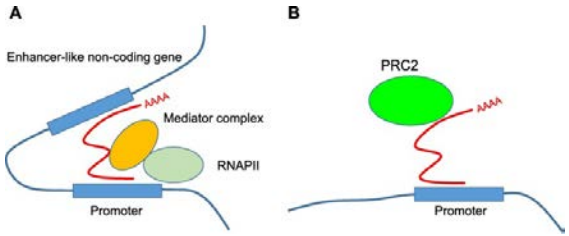

Ac
active chromatin
RNAi machinery
HDAC
Swi6
Clr4
Me
silent chromatin


A. Small RNA-mediated heterochromatin formation
B. RNA-directed DNA methylation
piRNA
PIWI
HMT/ HP1
Ago1
RITS
siRNA
DICER
AGO4
Swi6
nascent / promoter associated transcript
RNAP II
SINE B2
C. Domain organization
heterochromatin
CRC
long ncRNA
CAC
euchromatin
DRM1/2
D. Long ncRNA directed chromatin modification


Active gene
eRNA
DNA me
Enhancer
Promoter
Gene body
Pol II
H2A.Z
eNOS
Silenced gene
MHC


a
LUNAR1
LUNAR1 RNA
Mediator
IGF1R
b
H19 RNA
Maternal strand
Paternal strand
IGF2
H19
c
Active X
Xist
Tsix
Jpx
Jpx RNA
Tsix RNA
Inactive X
d
PRC2
PRC1
ANRIL
CDKN2
e
circRNA
miRNA

GenomicsTranscriptional and Post-transcriptional Gene Regulation by Long Non-coding RNA.
Proteomics Bioinformatics. 2017 Jun;15(3):177-186.
Dykes IM, Emanueli C.



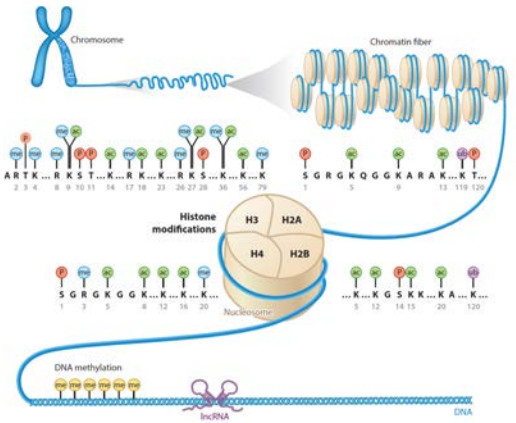
Models of transcriptional regulation.
In the bridging scaffold model **(A)**, activating RNAs (red line) are transcribed from enhancer-like non-coding genes and are required to recruit the mediator complex and to mediate chromatin conformational changes bridging the enhancer-like non-coding gene and the promoter of a coding gene. In the tethered scaffold model **(B)**, lncRNA (red line) recognises specific DNA motifs and recruits histone modifying enzymes such as PRC2 to the locus. lncRNA, long non-coding RNA; PRC2, polycomb repressive complex 2; RNAPII, RNA polymerase II.

Table 1. *Oncogenic MiRNAs*

miRNA	Regulation	Role in cancer	References
miR-17-92 cluster	c-MYC induces the expression	Overexpressed in B-cell lymphomas and can accelerate tumor progression in a mouse B-cell lymphoma model. Plays a role in tumor angiogenesis. Overexpressed in lung cancers.	(46,47,62,63)
miR-372, miR-373		Overexpressed in testicular germ cell tumors. Permit proliferation and tumorigenesis of primary human cells that have both oncogenic RAS and active wild-type p53.	(54)
miR-21		Expressed in glioblastomas and breast cancers. An anti-apoptotic factor.	(34,36,37)
miR-155		Overexpressed in breast, colon, and lung cancers, as well as in B-cell lymphomas. Overexpression correlates with poor survival in lung cancers and poor prognosis in DLCBL. High expression in children with Burkitt's lymphoma.	(34,55,56,60,64–66)
miR-146	NF-κB	Overexpressed in breast, pancreas, and prostate cancers.	(66,67)

Table 2. *Tumor-suppressor MiRNAs*

miRNA	Regulation	Role in cancer	References
miR-127	DNA methylation and histone modifications	Decreased expression in bladder and prostate cancers. Targets proto-oncogene *BCL6*.	(49)
miR-15a, miR-16-1		Often down-regulated in B-cell chronic lymphocytic leukemias. Targets *BCL2*.	(32,33)
let-7		Targets RAS. Reduced expression in human lung cancer associates with poor prognosis.	(57–60)
miR-145		Reduced expression in colon and breast cancers.	(34,61)



"Epigenetics and Systems Biology"
Spring 2021 (Odd Years)
Biol 476/576
Schedule/Lecture Outline –

Week 1	(Lesson 1)	Systems Biology (History/ Definitions/ Theory)
Week 2	(Lesson 2)	Systems Biology (Networks & Emergence)
Week 3	(Lesson 3)	Systems Biology (Components: DNA to Phenotype)
Week 4	(Lesson 4)	Systems Biology (Genomics / Technology)
Week 5	(Lesson 5)	Epigenetics (History / Molecular Processes)
Week 6	(Lesson 6)	Epigenetics (Molecular Processes & Integration)
Week 7	(Lesson 7)	Epigenetics (Genomics and Technology)
Week 8	(Lesson 8)	Cell & Developmental Biology
Week 9	(Lesson 9)	Epigenetics of Cell & Developmental Biology
Week 10	(Lesson 10)	Environmental Impact on Biology
Week 11	(Lesson 11)	Environmental Epigenetics
Week 12	(Lesson 12)	Disease Etiology
Week 13	(Lesson 13)	Epigenetics & Disease Etiology
Week 14	(Lesson 14)	Evolutionary Biology & Genetics
Week 15	(Lesson 15)	Epigenetics & Evolutionary Biology
Week 16	(Lesson 16)	Grant Review/ Study Section Meeting

Spring 2019 – Epigenetics and Systems Biology
Lecture Outline (Systems Biology)
Michael K. Skinner – Biol 476/576
Weeks 5, 6 and 7 (February 5, 12 and 19)

Epigenetics (History / Molecular Processes/ Genomics)

- Definitions and History
- Molecular Factors (DNA Methylation, Histone Modification, Chromatin Structure, ncRNA)
- Epigenetics Technology and Genomics

Required Reading

Holliday R. Epigenetics: a historical overview. Epigenetics. 2006 Apr-Jun;1(2):76-80.

Books (Reserve in Library)

Kevin V. Morris (2012) Non-coding RNAs and Epigenetic Regulation of Gene Expression: Drivers of Natural Selection. Caister Academic Press.

Russo, V.E.A., Martienssen, A.and Riggs, A.D. (eds.). 1996. Epigenetic Mechanisms of Gene Regulation. Cold Spring Harbor Press. Cold Spring Harbor.

Allis, C.D., Jenuwein, T. & Reinberg, D., Eds. (2007). Epigenetics. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Jeanteur, P. EPIGENETICS AND CHROMATIN. Progress in Molecular and Subcellular Biology, 2005, Volume 38, 151-167, DOI: 10.1007/3-540-27310-7_6

lncRNA and lincRNA

Sequence independent contol of transcription and genome activity

Table 2. ncRNAs and epigenetic modifications

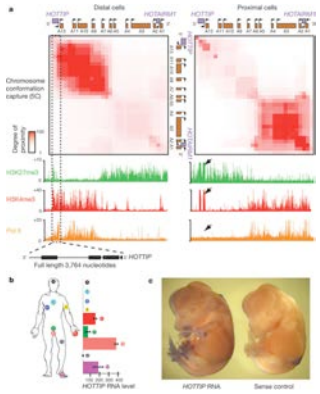
ncRNA(s)	Organism	Function in epigenetics	References
GAL10 ncRNA	Yeast	Influence in the post-translational modification of histones facilitating the repression of overlapping genes	[81, 82]
ICR1 and *PWR1*	Yeast	Cis-acting ncRNAs that modulate the expression of the *FLO11* gene in association to epigenetic mechanisms	[83]
Kcnq1ot1	Mice	Cis-acting silencer that might create a repressive environment for the epigenetic silencing of adjacent genes	[84]
~1600 lincRNAs	Mice	Highly conserved lncRNAs that can associate with chromatin complexes and histone modifications regulating gene expression	[9]
HOTAIR	Human	Regulation of the expression of genes in trans by affecting histone modifications and proteins that bind to DNA	[59]
~4000 lincRNAs	Human	Conserved lncRNAs that are able to modulate gene expression by associating to chromatin complexes with proteins and histone modifications	[10]

LncRNA, long non-coding RNAs; LincRNAs, large or long intergenic ncRNAs.

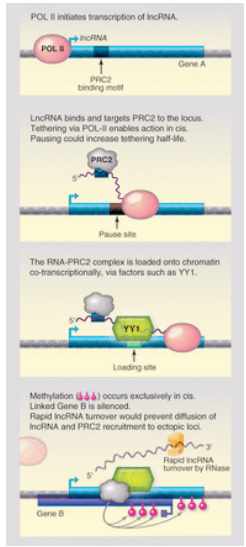
A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression

Kevin C. Wang[1,2], Yul W. Yang[2]*, Bo Liu[3]*, Amartya Sanyal[4], Ryan Corces-Zimmerman[1], Yong Chen[5], Bryan R. Lajoie[4], Angeline Protacio[1], Ryan A. Flynn[1], Rajnish A. Gupta[3], Joanna Wysocka[6], Ming Lei[5], Job Dekker[4], Jill A. Helms[3] & Howard Y. Chang[1]

120 | NATURE | VOL 472 | 7 APRIL 2011



a, Chromatin state map of distal versus proximal cells. Top panels, chromosome conformation capture-carbon copy (5C) analysis of distal (foreskin) and proximal (lung) human fibroblasts. Heat map representations (generated by my5C, ref. 30) of 5C data (bin size 30 kb, step size 3 kb) for *HOXA* in foreskin and lung fibroblasts. Red intensity of each pixel indicates relative interaction between the two points on the genomic coordinates. The diagonal represents frequent cis interactions between regions located in close proximity along the linear genome. 5C signals that are away from the diagonal represent long-range looping interactions. Bottom panels, chromatin occupancy across *HOXA*. x-axis is genomic coordinate; y-axis depicts occupancy of the indicated histone marks or protein (ChIP/input). Box and arrows highlight chromatin states of *HOTTIP* gene. **b**, *HOTTIP* RNA expression in primary human fibroblasts from 11 anatomic sites. Means ± s.d. are shown (n = 2). c, In situ hybridization of *HOTTIP* RNA in E13.5 mouse embryo.



LncRNAs tether epigenetic complexes to chromatin, enabling allele- and locus-specific regulation. LncRNA that is synthesized binds to an epigenetic complex (such as PRC2) and, together, are loaded onto chromatin cotranscriptionally through DNA-bound factors (such as YY1 for Xist RNA). Epigenetic modifications then silence the gene, and rapid lncRNA turnover prevents its diffusion to other loci.

Table 1. Emerging themes in lncRNA regulation. Potential groupings of lncRNA based on proposed interactions, functions, and mechanisms. Representative lncRNAs of each group are shown.

Functions	Examples	Action	Hypothesized mechanisms
Cis-tether cis-targeting	RepA, Xist, Tsix, Gtl2, Kcnq1ot1, Airn, Hottip, ANRIL, Oct4-ps5, COLDAIR, Evf2, BDNF-AS	Cis	Co-transcriptional targeting of chromatin factors; allelic and locus-specific action; repressive or activating
Trans-targeting	pRNA, asOct4-ps5	Trans	RNA:DNA triplex via Hoogsteen base pairing or sense:antisense Watson:Crick base pairing
Enhancer	Xite, ncRNA-a7, eRNAs	Cis	Mediated by RNA, transcriptional force, or chromatin intermediaries
Decoy	PTEN-ps Tsix	Trans Cis	Competitive inhibition of target protein or RNA
Scaffold	MALAT1, TUG1, NEAT1, HOTAIR, roX1, roX2	Trans	RNA subunit of effector complex
Allosteric modification	TLS	Cis (trans also possible)	Alters conformation and activity of protein partners
Coactivator or co-repressor	SRA, SINE B2, Jpx, pRNA	Trans (cis also possible)	Accessory factor for transcriptional activation or repression

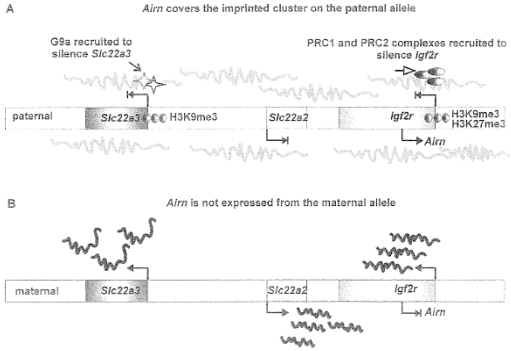


Figure 16.2 Graphical representation of mechanism of imprinting induced by *Airn* macro ncRNAs at the *Igf2r* locus in placenta. *Airn* overlaps partially with the *Igf2r* gene on the cluster containing *Igf2r, Slc22a2* and *Slc22a3* genes. *Airn* RNA is shown as grey wavy lines in (A). The genes in this cluster are expressed from the maternal locus on which *Airn* is silent. The black wavy lines in (B) depict RNA expressed from the three protein coding genes. *Airn* is expressed in the antisense direction to *Igf2r* from the paternal cluster and covers the imprinted region on the paternal locus. *Airn* recruits G9a (stars, open arrow) to mediate H3K9 trimethylation (shown as circles) at the *Slc22a3* locus resulting in silencing. At the *Igf2r* site, *Airn* recruits Polycomb repressive complex 2 (PRC2) (ellipses, closed arrow) to induce trimethylation at H3K9 and H3K27 (shown as circles) to repress *Igf2r*. *Airn* also recruits PRC1 to facilitate H2AK119 mono ubiquitinylation at the *Igf2r* locus and create a repressive environment devoid of RNA polymerase (not shown).

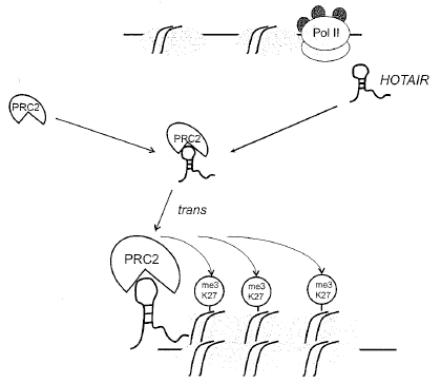


Figure 1.10 Model of regulation of chromatin domains via histone-modification enzymes with *HOTAIR*. *HOTAIR* transcribed from the *HOXC* locus recruits PRC2 to the *HOXD* locus *in trans*. PRC2 recruitment leads to H3K27 methylation and transcriptional silencing of neighbouring *HOX* genes.

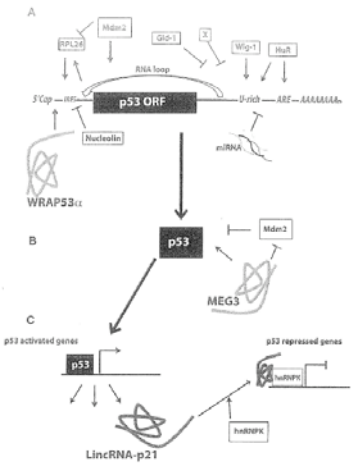
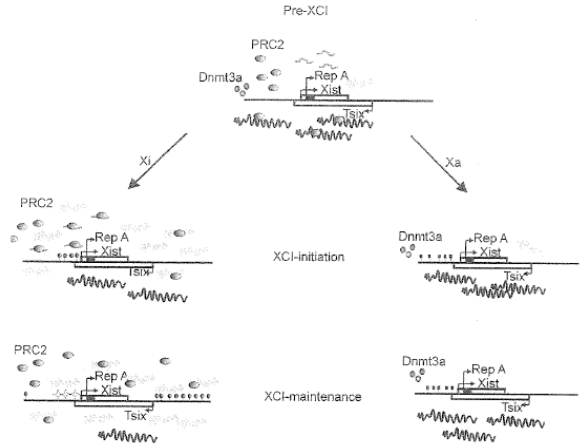
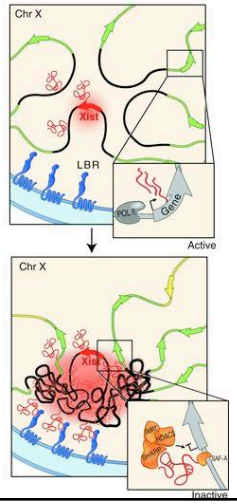


Figure 11.1 (A) Factors regulating mRNA stability and translation efficiency. X denotes a p53-regulating protein of unknown identity. (B) Regulation of p53 protein levels and transcriptional activity by the long ncRNA MEG3. (C) p53 can in turn activate the transcription of the long non-coding lincRNA-p21, which together with hnRNPK mediates transcriptional silencing of p53 transrepressed promoters.



Xist recruits the X chromosome to the nuclear lamina to enable chromosome-wide silencing
Chen CK, Blanco M, Jackson C, et al.
Science. 2016 Oct 28;354(6311):468-472.



Non-canonical RNA-directed DNA methylation.

Cuerda-Gil D, Slotkin RK.
Nat Plants. 2016 Nov 3;2(11):16163.

Abstract

Small RNA-directed DNA methylation (RdDM) has been extensively studied in plants, resulting in a deep understanding of a major 'canonical RdDM' mechanism. However, current models of canonical RdDM cannot explain how this self-perpetuating mechanism is initiated. Recent investigations into the initiation of epigenetic silencing have determined that there are several alternative 'non-canonical RdDM' pathways that function through distinct mechanisms to modify chromatin. This Review aims to illustrate the diversity of non-canonical RdDM mechanisms described to date, recognize common themes within this dizzying array of interconnected pathways, and identify the key unanswered questions remaining in this field.

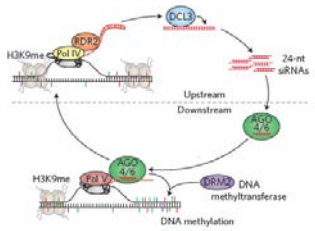


Figure 1 | The canonical RdDM pathway. In the upstream siRNA-generating phase, Pol IV and RDR2 function to produce dsRNA, which is cleaved into 24-nt siRNAs by DCL3. These siRNAs function to direct AGO4 or AGO6 to chromatin-bound transcripts produced by Pol V. In the downstream phase, the interaction of AGO4/6 with the Pol V transcript results in the recruitment and activity of the DNA methyltransferase DRM2. This pathway is reviewed in greater detail in ref. 7. Black strands represent DNA, red strands represent RNA. DNA methylation colours refer to the CG (red), CHG (blue) and CHH (green) sequence contexts, where H = A, C or T.

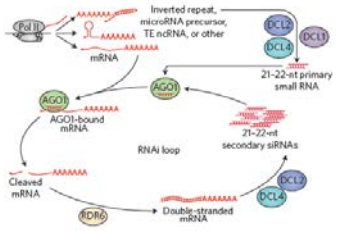
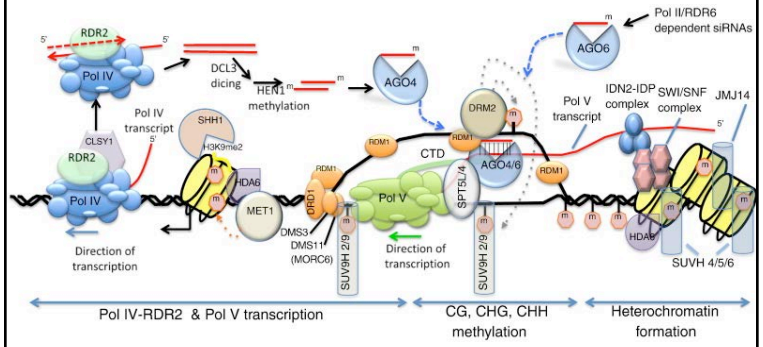
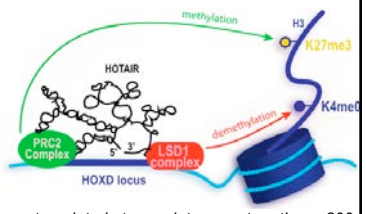


Figure 2 | PTGS in plants. Pol II transcription of regions such as palindromic TEs or microRNA precursors generates primary small RNAs[50,51], which are produced independently of RDR activity. Primary small RNAs target Pol II mRNAs for cleavage, and some of these cleaved mRNAs produce secondary siRNAs through the formation of dsRNA via RDR6 and cleavage by DCL2 and DCL4. Secondary siRNAs can target additional copies of the mRNA for cleavage and production of more siRNAs through the cycle of RNAi. ncRNA, non-coding RNA.

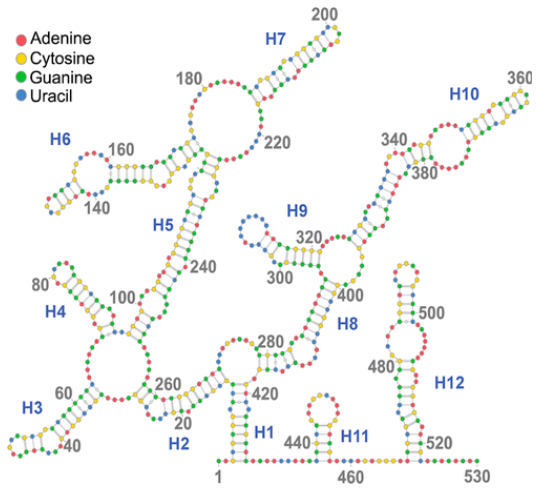
The RNAs of RNA-directed DNA methylation.
Wendte JM, Pikaard CS.
Biochim Biophys Acta. 2017 Jan;1860(1):140-148.



Biochemical Methods To Investigate lncRNA and the Influence of lncRNA:Protein Complexes on Chromatin.
McFadden EJ, Hargrove AE.
Biochemistry. 2016 Mar 22;55(11):1615-30.

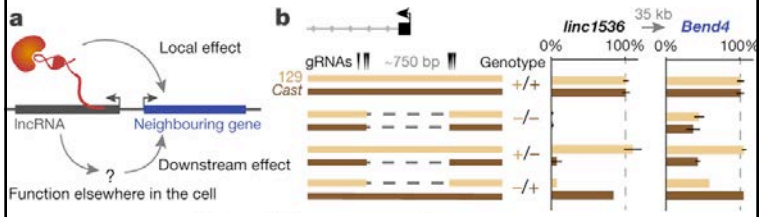


Long noncoding RNAs (lncRNAs), defined as nontranslated transcripts greater than 200 nucleotides in length, are often differentially expressed throughout developmental stages, tissue types, and disease states. The identification, visualization, and suppression/overexpression of these sequences have revealed impacts on a wide range of biological processes, including epigenetic regulation. Biochemical investigations on select systems have revealed striking insight into the biological roles of lncRNAs and lncRNA:protein complexes, which in turn prompt even more unanswered questions. To begin, multiple protein- and RNA-centric technologies have been employed to isolate lncRNA:protein and lncRNA:chromatin complexes. LncRNA interactions with the multi-subunit protein complex PRC2, which acts as a transcriptional silencer, represent some of the few cases where the binding affinity, selectivity, and activity of a lncRNA:protein complex have been investigated. At the same time, recent reports of full-length lncRNA secondary structures suggest the formation of complex structures with multiple independent folding domains and pave the way for more detailed structural investigations and predictions of lncRNA three-dimensional structure. This review will provide an overview of the methods and progress made to date as well as highlight new methods that promise to further inform the molecular recognition, specificity, and function of lncRNAs.



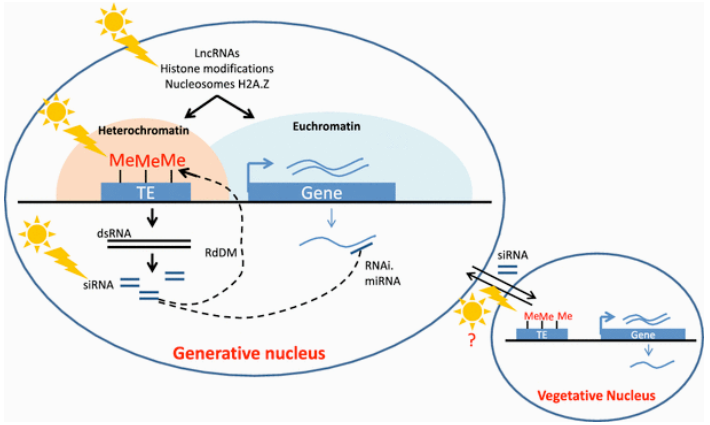
Secondary structure of human *HOTAIR* Domain 1 (1–530 n.t.; GI: 383286742). Pyle and co-workers performed chemical probing on the full-length *HOTAIR* transcript. Domain 1 contains the binding-domain of PRC2. Secondary structure rendered in VARNA.

Local regulation of gene expression by lncRNA promoters, transcription and splicing
Engreitz JM, Haines JE, Perez EM, et al (2016)
Nature. 17;539(7629):452-455.

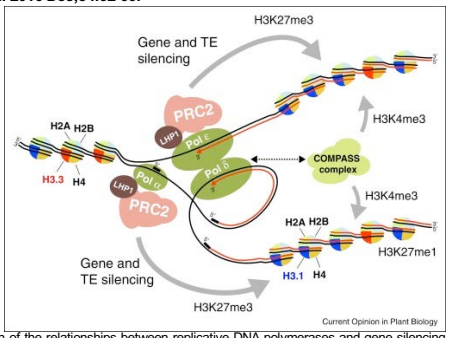


Many lncRNA and mRNA loci influence the expression of neighbouring genes.
a, Knocking out a promoter (black) could affect a neighbouring gene (blue) directly (local) or indirectly (downstream). b, Knockout of the linc1536 promoter. Left, genotypes; right, allele-specific RNA expression for 129 and castaneus (Cast) alleles normalized to 81 control clones (+/+). Error bars, 95% confidence interval for the mean (n = 2 for −/−, 3 for +/−, 1 for −/+).

Epigenetic events in plant male germ cell heat stress responses.
Chen Y, Müller F, Rieu I, Winter P.
Plant Reprod. 2016 Jun;29(1-2):21-9.

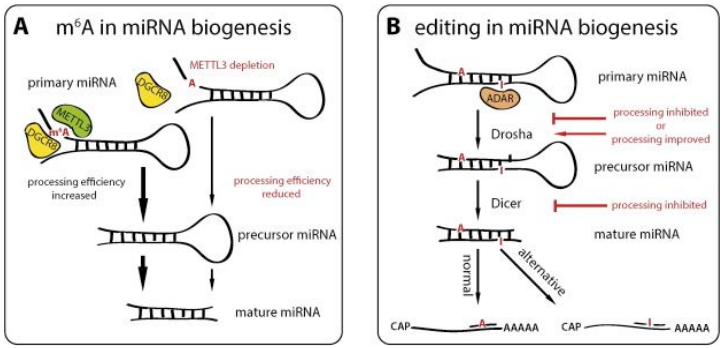


Links of genome replication, transcriptional silencing and chromatin dynamics.
Gutierrez C, Desvoyes B, Vergara Z, Otero S, Sequeira-Mendes J.
Curr Opin Plant Biol. 2016 Dec;34:92-99.



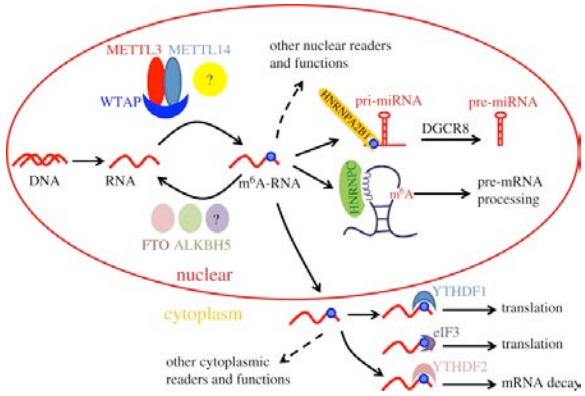
Schematic representation of the relationships between replicative DNA polymerases and gene silencing. During genome replication parental nucleosomes are disassembled and reassembled again past the replication fork. Each nucleosome contains two copies of each histone H2A, H2B, H3 and H4, as indicated. Newly assembled nucleosomes contain exclusively the canonical histone H3.1 (dark blue), which can be exchanged by the H3.3 (red). Histone chaperones responsible for depositing or exchanging histones H3.1 or H3.3 at the replication fork, as well chaperones for other histones, have been omitted for simplicity. Newly synthesized DNA strands are colored in red and the 4–6 nt long RNA primers appear as short thick lines (black). The three replicative DNA polymerases represent the holoenzyme complexes, DNA polymerases Pol δ and Pol ε, and DNA polymerase α-primase (Pol α). They are responsible for the elongation on the lagging DNA strand, on the leading strand and for the synthesis of RNA primers, respectively. Accessory replication proteins, RFC, RPA, PCNA, among others, have been omitted for simplicity. PRC2 and the LHP1 protein associate with Pol α and Pol ε to modify the chromatin past the fork, silencing genes and transposable elements (TEs) in a highly specific manner, as discussed in the text. The COMPASS complex interacts with Pol δ to modulate the H3K4me3 levels of newly formed chromatin.

Epigenetics
RNA Methylation



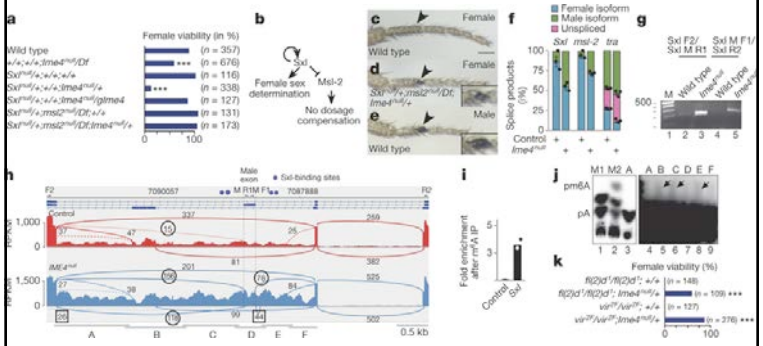
The proposed function of post-transcriptional RNA modifications in miRNA biogenesis. A) m^6A methylation at the base of the pri-miRNA stem-loop is proposed to increase Drosha processing. This was confirmed by METTL3 depletion which results in decreased pri-miRNA processing and consequently reduced accumulation of mature miRNA. B) A-to-I editing effects on miRNA biogenesis are specific to each pri-miRNA transcript. Editing can inhibit either Drosha or Dicer processing, but may also enhance Drosha processing of the pri-miRNA. Editing sites present within the mature miRNA can lead to the targeting of a distinct alternative set of transcripts.

Recent advances in dynamic m6A RNA modification.
Cao G, Li HB, Yin Z, Flavell RA.
Open Biol. 2016 Apr;6(4):160003.



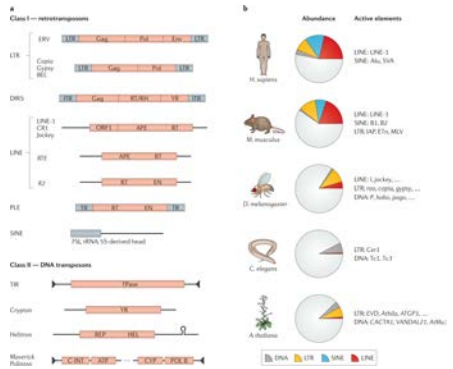
Dynamic m^6A RNA modifications and mediated functions. m^6A mRNA methylation is mediated by a multiprotein complex that includes METTL3, METTL14 and WTAP, whereas demethylases, such as FTO and ALKBH5, erase m^6A. Recognition of m^6A by HNRNPC in the nucleus mediates alternative splicing of pre-mRNA, and HNRNPA2B1 promotes pri-miRNA processing to pre-miRNA. In cytoplasm, binding of m^6A sites with different readers mediates divergent functions. YTHDF1 binds m^6A-modified mRNAs through interactions with initiation factors and ribosomes to increase translational output, and eIF3 can also directly bind to 5′UTR m^6A to initiate translation, whereas m^6A recognition by YTHDF2 leads to mRNA decay. More nuclear and cytoplasmic readers need to be defined to illuminate the functions of m^6A in mRNA export, translation and storage.

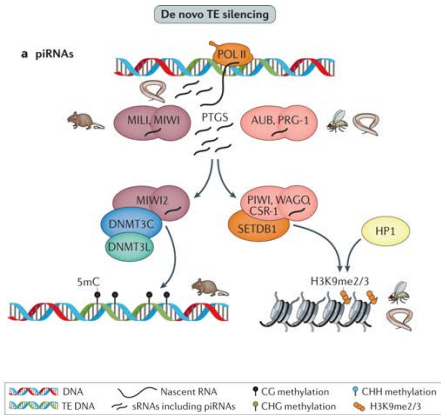
m^{6}A potentiates *Sxl* alternative pre-mRNA splicing for robust *Drosophila* sex determination.
Haussmann IU, Bodi Z, Sanchez-Moran E, et al, (2016)
Nature. 8;540(7632):301-304.

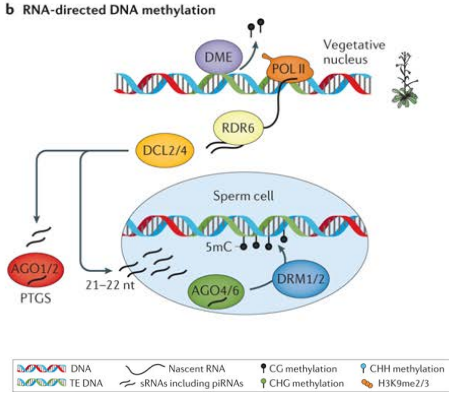


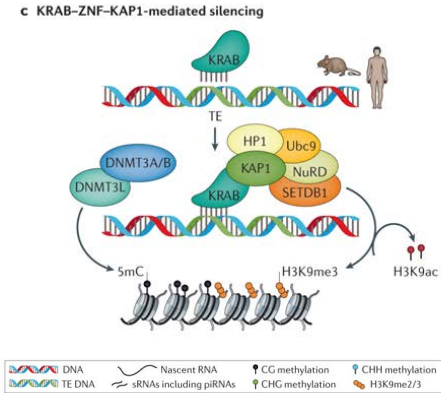
Epigenetics
Molecular Integration

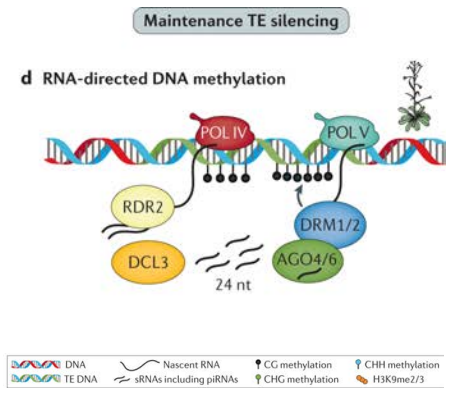
Regulation of transposable elements by DNA modifications.
Deniz Ö, Frost JM, Branco MR.
Nat Rev Genet. 2019 Jul;20(7):417-431.

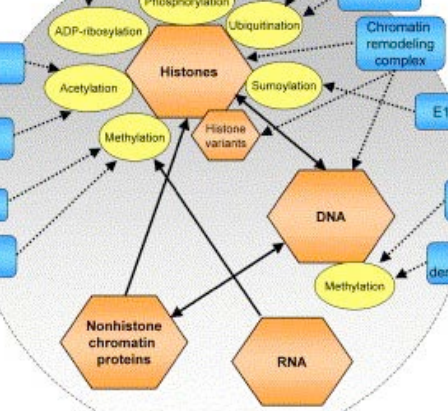





b RNA-directed DNA methylation
DME
POL II
Vegetative nucleus
RDR6
DCL2/4
Sperm cell
5mC
AGO1/2
PTGS
21–22 nt
AGO4/6
DRM1/2
DNA
TE DNA
Nascent RNA
sRNAs including piRNAs
CG methylation
CHG methylation
CHH methylation
H3K9me2/3


c KRAB–ZNF–KAP1-mediated silencing
KRAB
TE
HP1
Ubc9
DNMT3A/B
KAP1
NuRD
DNMT3L
KRAB
SETDB1
5mC
H3K9me3
H3K9ac
DNA
TE DNA
Nascent RNA
sRNAs including piRNAs
CG methylation
CHG methylation
CHH methylation
H3K9me2/3


Maintenance TE silencing
d RNA-directed DNA methylation
POL IV
POL V
RDR2
DRM1/2
DCL3
AGO4/6
24 nt
DNA
TE DNA
Nascent RNA
sRNAs including piRNAs
CG methylation
CHG methylation
CHH methylation
H3K9me2/3


Kinase
PARP
DUB
E1,E2,E3
Phosphorylation
ADP-ribosylation
Ubiquitination
Chromatin remodeling complex
HAT
Histones
Acetylation
Sumoylation
E1, E2
HDAC
Methylation
Histone variants
HMT
DNA
DNMT
PAD
DNA demethylase
Methylation
Nonhistone chromatin proteins
RNA

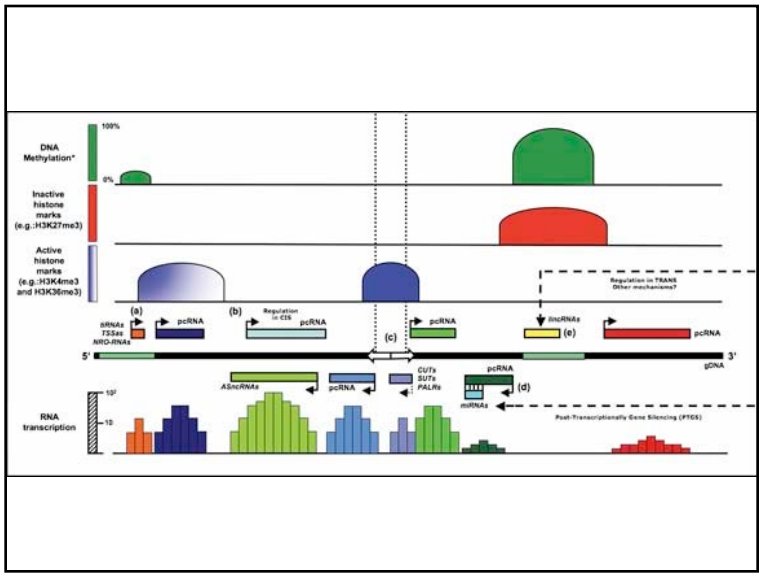
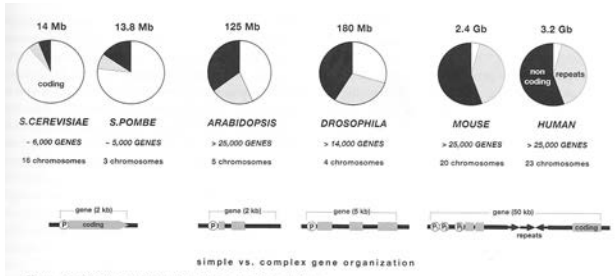
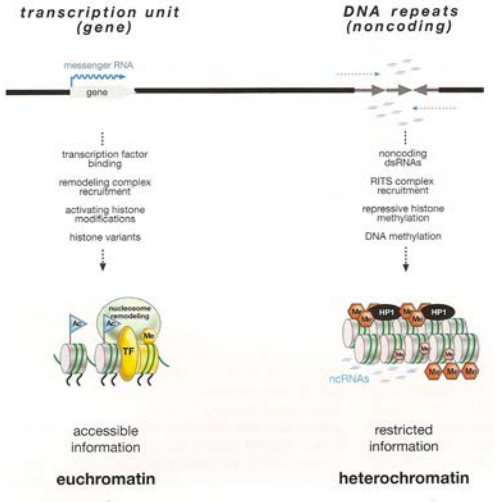
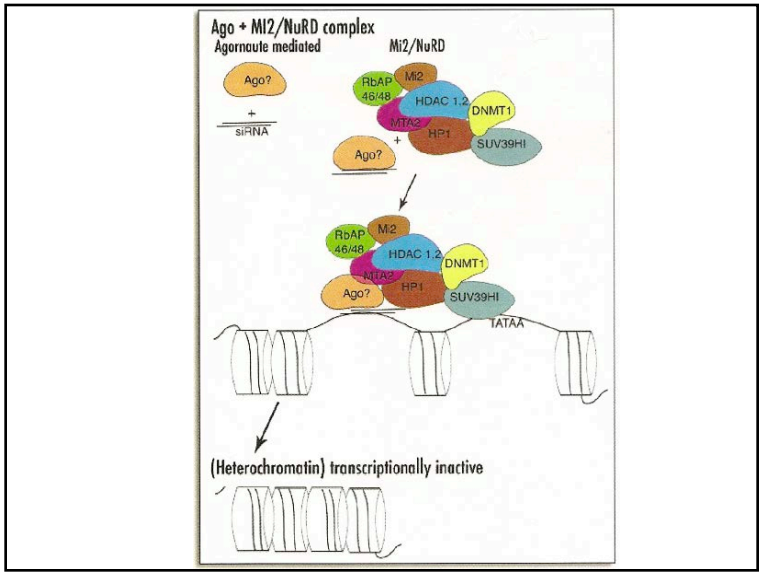


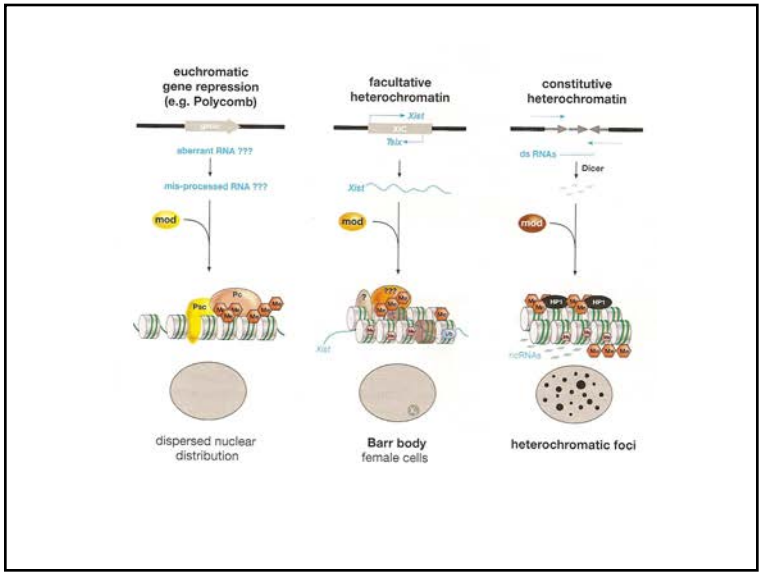
Table 16.1 Genome size increases with complexity of organisms but the gene density is not proportional to genome size (http://www.ornl.gov/sci/techresources/Human_Genome/faq/compgen.shtml)

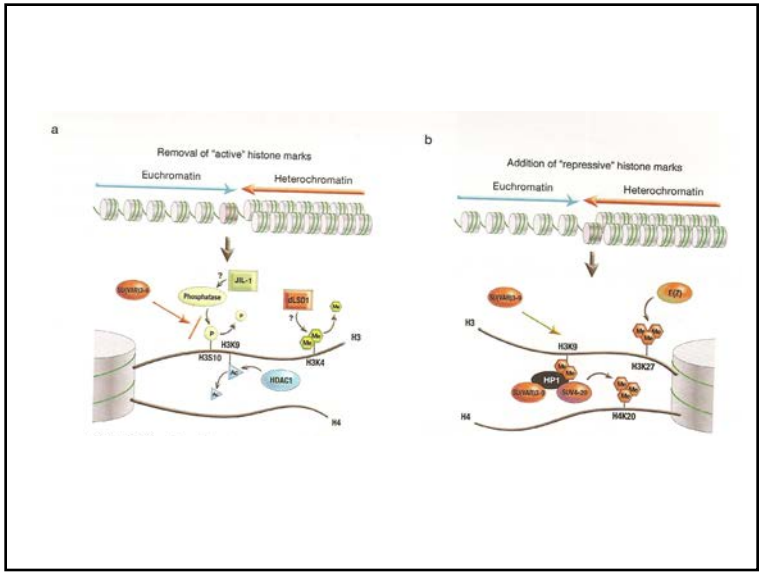
Organism	Genome size (million bps)	Number of chromosomes	Number of genes	Genes per 100,000 bp	Reference
Homo sapiens	3200	46	~25,000	~1	International Human Genome Sequencing Consortium (2004)
Mus. musculus	2600	40	~25,000	~1	Waterston *et al.* (2002)
D. melanogaster	137	8	~13,000	~10	Adams *et al.* (2000)
A. thaliana	100	10	~25,000	~25	The *Arabidopsis* Genome Initiative (2000)
C. elegans	97	12	~19,000	~20	The *C. elegans* Sequencing Consortium (1998)
S. cerevisiae	12.1	32	~6000	~50	Goffeau *et al.* (1996)
E. coli	4.6	1	~3200	~70	Blattner *et al.* (1997)
H. influenzae	1.8	1	~1700	~95	Fleischmann *et al.* (1995)

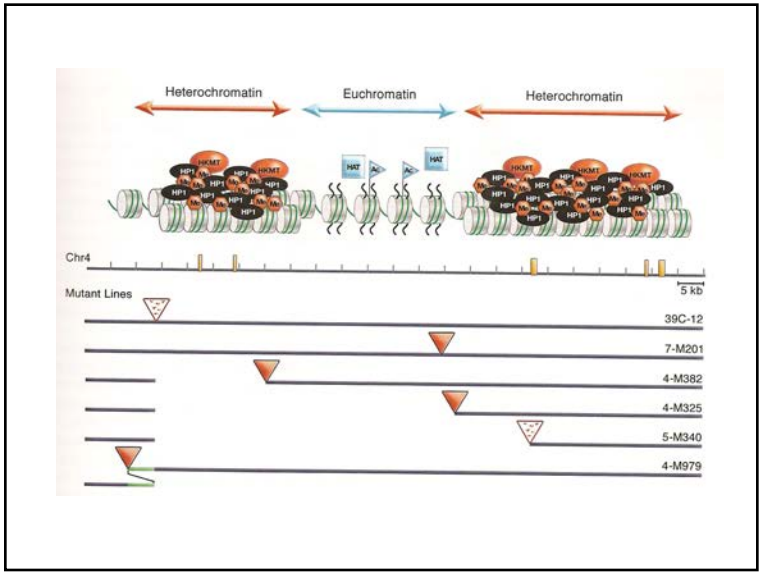


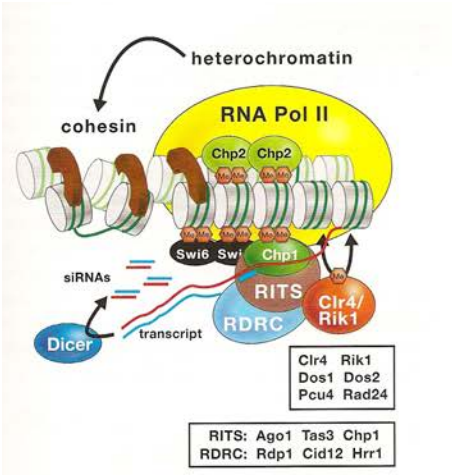


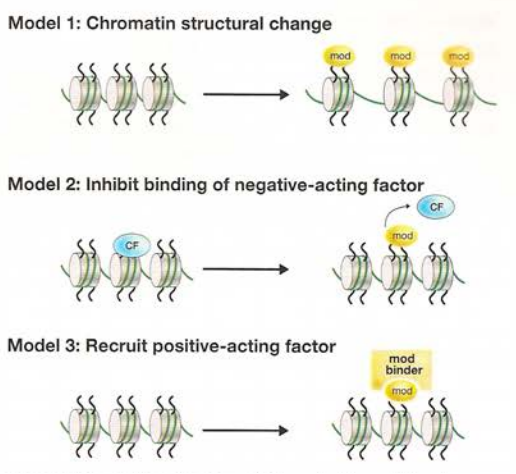

Ago + MI2/NuRD complex
Agornaute mediated
Mi2/NuRD
Ago?
siRNA
RbAP 46/48
Mi2
HDAC 1,2
DNMT1
MTA2
HP1
SUV39HI
TATAA
(Heterochromatin) transcriptionally inactive

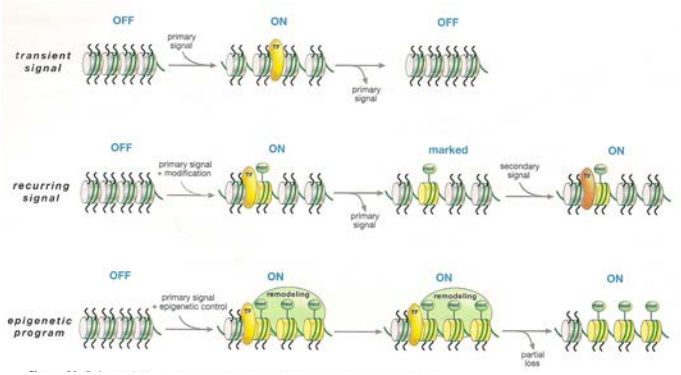

euchromatic gene repression (e.g. Polycomb)
facultative heterochromatin
constitutive heterochromatin
aberrant RNA ???
mis-processed RNA ???
ds RNAs
Dicer
mod
dispersed nuclear distribution
Barr body female cells
heterochromatic foci

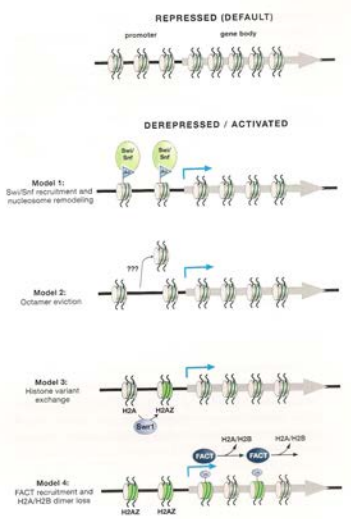

a
Removal of "active" histone marks
Euchromatin
Heterochromatin
JIL-1
Phosphatase
H3K9
H3S10
H3K4
HDAC1
H3
H4
b
Addition of "repressive" histone marks
Euchromatin
Heterochromatin
H3K9
H3K27
HP1
H4K20

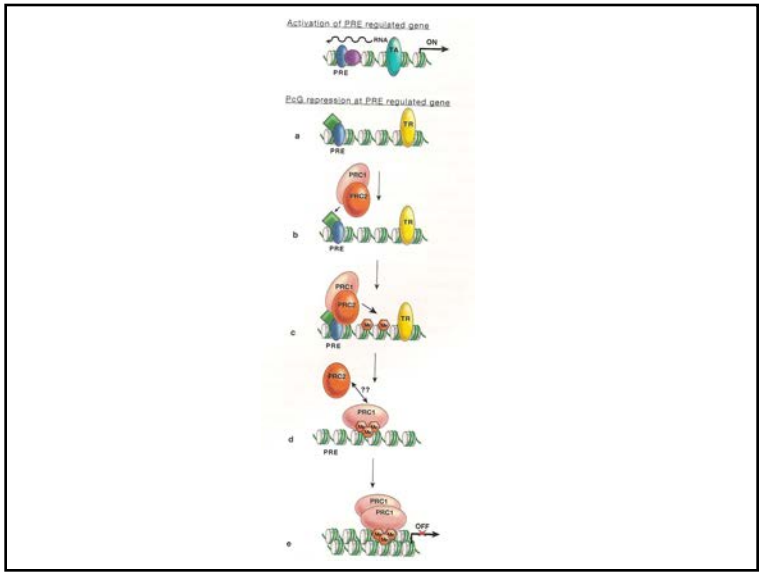

Heterochromatin
Euchromatin
Heterochromatin
HKMT
HP1
HAT
Chr4
5 kb
Mutant Lines
39C-12
7-M201
4-M382
4-M325
5-M340
4-M979

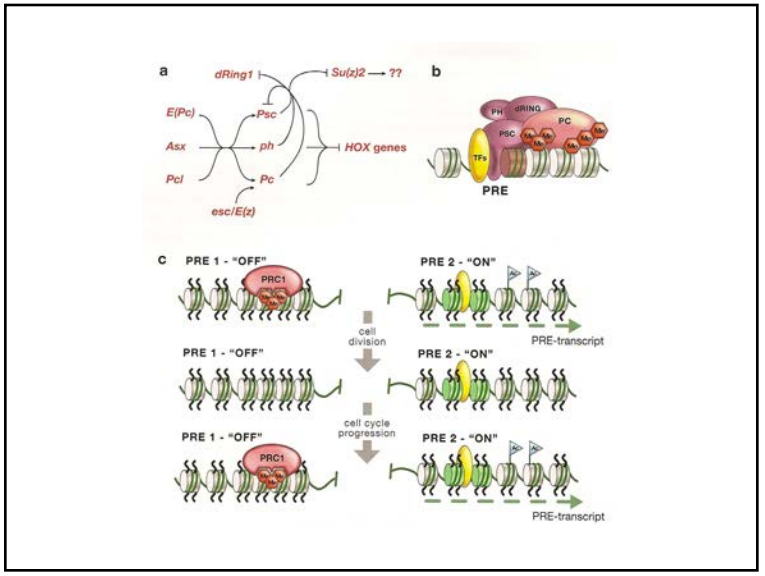

heterochromatin
cohesin
RNA Pol II
Chp2
Chp2
Swi6
Swi
Chp1
siRNAs
RITS
Clr4/
Rik1
RDRC
Dicer
transcript
Clr4 Rik1
Dos1 Dos2
Pcu4 Rad24
RITS: Ago1 Tas3 Chp1
RDRC: Rdp1 Cid12 Hrr1

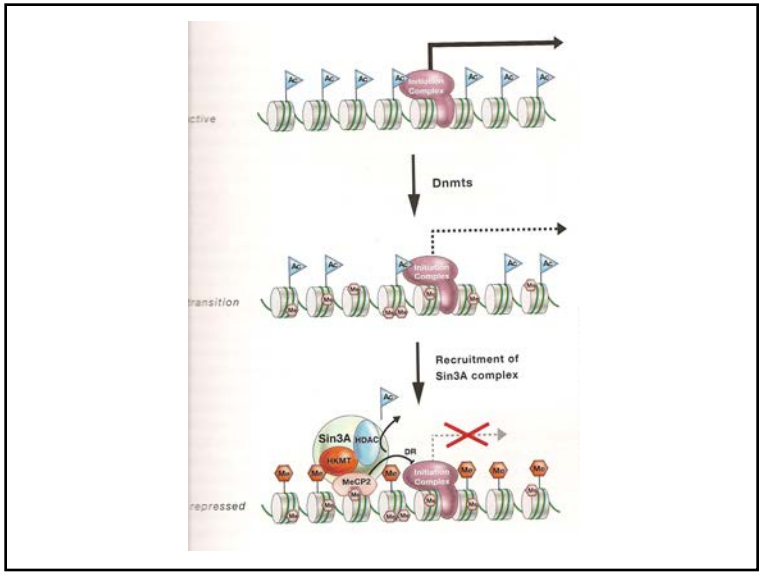

Model 1: Chromatin structural change
mod
mod
mod
Model 2: Inhibit binding of negative-acting factor
CF
CF
mod
Model 3: Recruit positive-acting factor
mod
binder
mod

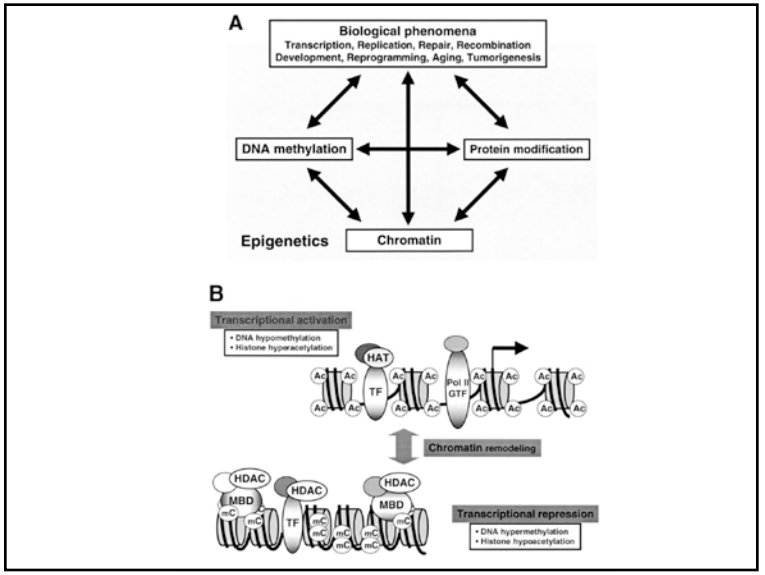

transient signal
OFF
primary signal
ON
primary signal
OFF
recurring signal
OFF
primary signal + modification
ON
primary signal
marked
secondary signal
ON
epigenetic program
OFF
primary signal + epigenetic control
ON
remodeling
ON
remodeling
partial loss
ON


REPRESSED (DEFAULT)
promoter
gene body
DEREPRESSED / ACTIVATED
Model 1:
Swi/Snf recruitment and nucleosome remodeling
Model 2:
Octamer eviction
???
Model 3:
Histone variant exchange
H2A
H2AZ
Swr1
Model 4:
FACT recruitment and H2A/H2B dimer loss
FACT
FACT
H2AZ
H2AZ

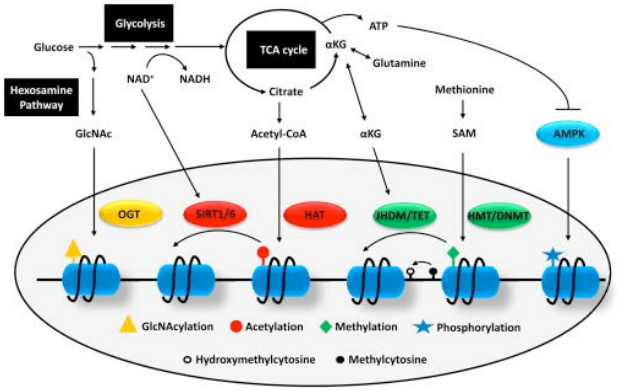

Activation of PRE regulated gene
PRE
RNA
ON
PcG repression at PRE regulated gene
a
TR
PRE
b
PRC1
PRC2
c
d
??
e
OFF


a
dRing1
Su(z)2 → ??
E(Pc)
Psc
Asx
ph
HOX genes
Pcl
Pc
esc/E(z)
b
PH
dRING
PC
PSC
TFs
PRE
c
PRE 1 - "OFF"
PRC1
PRE 2 - "ON"
cell division
PRE-transcript
cell cycle progression


ctive
Dnmts
transition
Recruitment of Sin3A complex
Sin3A
HDAC
HKMT
MeCP2
DR
repressed

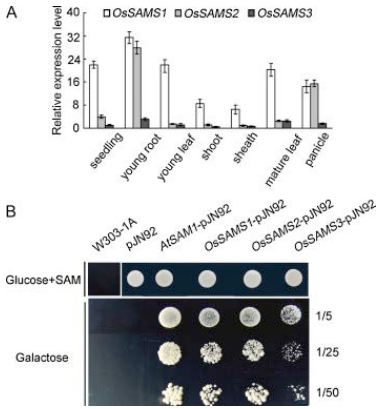

A
Biological phenomena
Transcription, Replication, Repair, Recombination
Development, Reprogramming, Aging, Tumorigenesis
DNA methylation
Protein modification
Epigenetics
Chromatin
B
Transcriptional activation
• DNA hypomethylation
• Histone hyperacetylation
HAT
TF
Pol II GTF
Chromatin remodeling
HDAC
MBD
Transcriptional repression
• DNA hypermethylation
• Histone hypoacetylation

Metabolic regulation of epigenetics.
Lu C, Thompson CB. Cell Metab. 2012 Jul 3;16(1):9-17.

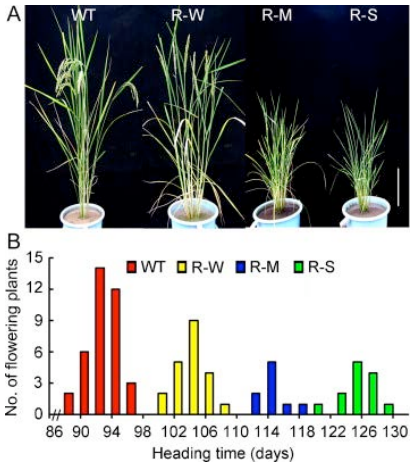


Crosstalk between Metabolism and Epigenetics As glucose enters the glycolytic pathway, a minor portion is branched to hexosamine biosynthetic pathway to produce GlcNAc which can be used as substrate for histone GlcNAcylation by OGT. Flux through glycolysis determines the NAD+/NADH ratio which is important for the activities of sirtuin histone deacetylases. Several TCA cycle intermediates can be exported out of mitochondria including citrate and αKG. Cytosolic citrate is converted to acetyl-CoA which is used as a donor for HAT-mediated histone acetylation. αKG is used as cofactor for histone and DNA demethylation reactions by JHDM and TET, respectively. The substrate for HMT and DNMT is SAM, which is synthesized from essential amino acid methionine. Finally, a low ATP/AMP ratio can activate AMPK, a kinase that phosphorylates histones.

Knockdown of SAMS genes encoding S-adenosyl-l-methionine synthetases causes methylation alterations of DNAs and histones and leads to late flowering in rice.
Li W, et al. J Plant Physiol. 2011 Oct 15;168(15):1837-43.

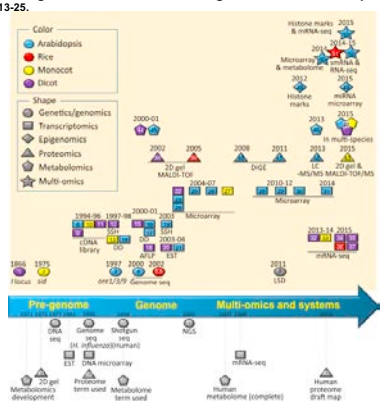


Biochemical characterization of Oryza sativa S-adenosylmethionine synthetase (OsSAMS1, 2, 3) and expression patterns. (A) Expression profile of OsSAMS1, 2, 3 in different ZH 10 organs detected by real-time RT-PCR. Error bars represent ± SD. (B) OsSAMS1, 2, 3 complemented yeast sam1 sam2 double mutant W744-1A. Yeast transformants were grown as patches on uracil-less synthetic glucose media (top panel), and then were diluted and replicated to uracil-less media containing galactose (bottom panel). 1/5, 1/25 and 1/50 meant dilution ratio. W303-1A was used as WT yeast, AtSAM1 was used as a positive control.



Phenotypic comparison of the OsSAMS1-RNAi transgenic plants was shown during the heading period. (A) Transgenic plants showed late-flowering phenotype in the field. Rice plants were photographed at 114 DAG. Bar = 20 cm (B) Heading time distributions of the transgenic rice plants grown in the field. OsSAMS1-RNAi-weak, medium-strength and strong transgenic plants were abbreviated to R-W, M, S, respectively.

Toward Systems Understanding of Leaf Senescence: An Integrated Multi-Omics Perspective on Leaf Senescence Research.
Mol Plant. 2016 Jun 6;9(6):813-25.
Kim J, Woo HR, Nam HG.

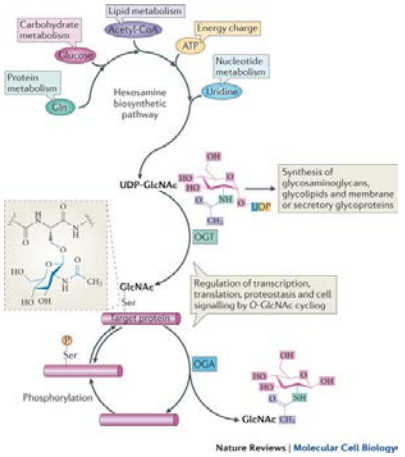


Bittersweet memories: linking metabolism to epigenetics through O-GlcNAcylation.

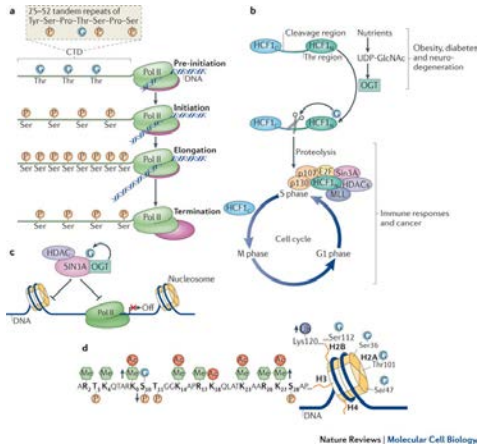
Hanover JA, et al. Nat Rev Mol Cell Biol. 2012 23;13(5):312-21

Abstract

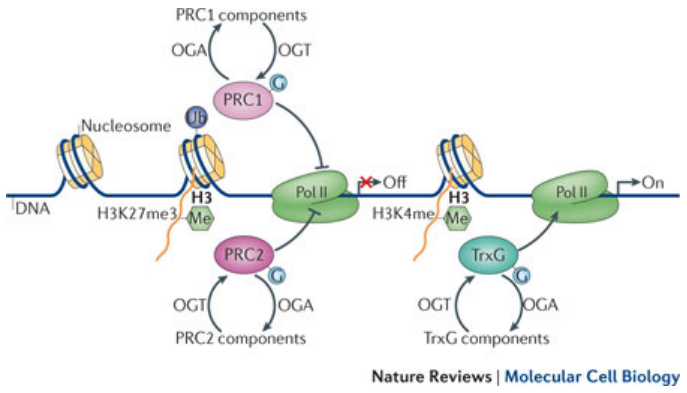
O-GlcNAcylation, which is a nutrient-sensitive sugar modification, participates in the epigenetic regulation of gene expression. The enzymes involved in O-linked β-D-N-acetylglucosamine (O-GlcNAc) cycling - O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) - target key transcriptional and epigenetic regulators including RNA polymerase II, histones, histone deacetylase complexes and members of the Polycomb and Trithorax groups. Thus, O-GlcNAc cycling may serve as a homeostatic mechanism linking nutrient availability to higher-order chromatin organization. In response to nutrient availability, O-GlcNAcylation is poised to influence X chromosome inactivation and genetic imprinting, as well as embryonic development. The wide range of physiological functions regulated by O-GlcNAc cycling suggests an unexplored nexus between epigenetic regulation in disease and nutrient availability.



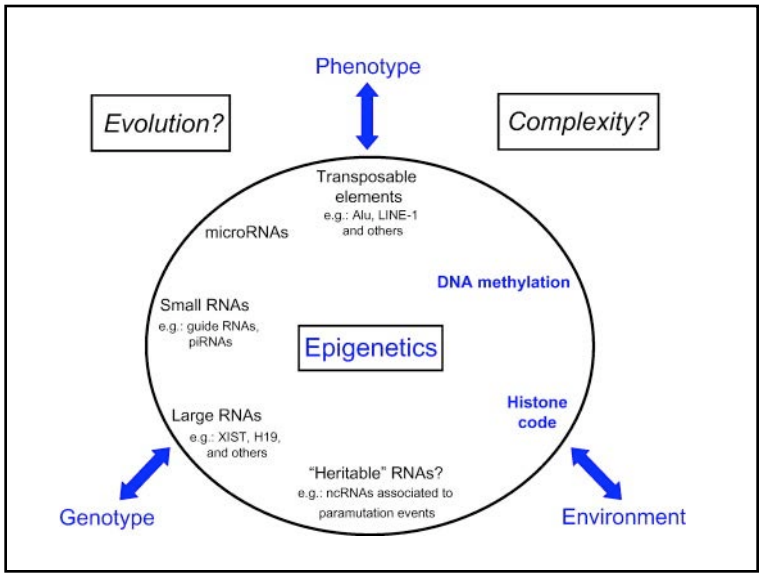
The synthesis of UDP-GlcNAc via the hexosamine biosynthetic pathway is nutrient responsive.
The hexosamine biosynthetic pathway (HBP) integrates the metabolism of carbohydrates, amino acids, fat and nucleotides in the synthesis of uridine diphosphate N-acetylglucosamine (UDP-GlcNAc). UDP-GlcNAc is utilized for the synthesis of glycosaminoglycans, glycolipids and membrane and secretory glycoproteins. In addition, it is utilized by O-GlcNAc transferase (OGT) in the nucleus and the cytoplasm for the addition of GlcNAc at Ser or Thr residues (not shown) of target proteins (O-GlcNAcylation). Addition and removal of O-GlcNAc by OGT and O-GlcNAcase (OGA), respectively, contributes to continuous O-GlcNAc cycling, which dynamically modifies numerous intracellular proteins. O-GlcNAcylation often occurs at sites that are also targeted by phosphorylation. Thus, the resulting diversity of O-GlcNAcylated and phosphorylated proteins might contribute to the regulation of several complex cellular processes, including transcription, translation, proteostasis and cell signalling.



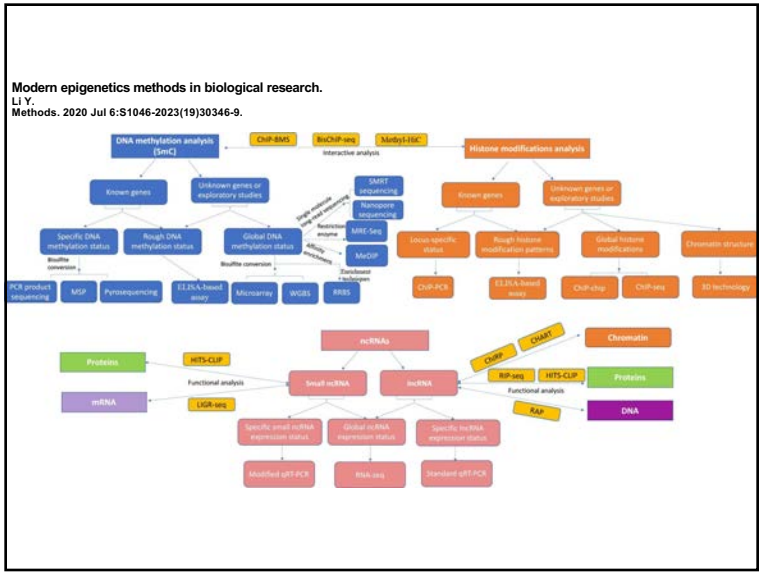
The enzymes of O-GlcNAc cycling interact with and modify known epigenetic regulators. a | The carboxy-terminal domain (CTD) of RNA polymerase II (Pol II) consists of tandem heptad repeats that are phosphorylated and O-GlcNAcylated. Changes in the sites of phosphorylation (P, shown in orange) are associated with the indicated transcriptional activity of Pol II. An O-GlcNAcylation site (G, shown in blue) has been mapped to Thr4 and may inhibit Ser5 and Ser2 phosphorylation of Pol II. b | O-GlcNAc transferase (OGT) interacts with host cell factor 1 (HCF1) and promotes cleavage and activation of HCF1, which allows cell cycle progression. The HCF1 amino-terminal subunit (HCF1N) acts in complex with other factors, including the transcription factors p130, p107 and E2F, the co-repressor switch-independent 3A (SIN3A)–histone deacetylase (HDAC) complex and the Trithorax group-related protein MLL, to promote transition from G1 phase to S phase, whereas the HCF1 carboxy-terminal subunit (HCF1C) promotes entry to M phase. O-GlcNAcylation sites have been mapped throughout the HCF1 protein. Disruption of the regulation of HCF1 by O-GlcNAcylation may have important implications for human disease. c | OGT associates with and modifies the co-repressor SIN3A–HDAC complex to repress transcription. d | The core histones H2A, H2B, H3 and H4 are modified by O-GlcNAcylation. The mapped target sites for O-GlcNAcylation in H4 (Ser47), H2A (Thr101) and H2B (Ser36) are also phosphorylation sites that are important for nucleosome assembly. The histone tails of H3 and H2B contain O-GlcNAcylation sites at Ser10 and Ser112, respectively. Arrows indicate the effect of O-GlcNAcylation on the other histone post-translational modifications. Me, methylation; Ub, ubiquitylation.



The influence of O-GlcNAc cycling on the activity of the PcG and TrxG complexes.
Epigenetic regulation is maintained by the balance of the activity of Trithorax group (TrxG) complexes and Polycomb group (PcG) complexes (Polycomb group repressive complex 1 (PRC1) and PRC2), and epigenetic regulation is associated with the indicated histone modifications. O-linked β-D-N-acetylglucosamine (O-GlcNAc) cycling resulting from the catalytic activity of O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) is essential for PcG-mediated repression, but also promotes the activity of some TrxG members, which are associated with active transcription. The role of O-GlcNAc cycling in PcG and TrxG function is likely to be complex, and a generic protein target is shown for simplicity. As a homeostatic mechanism, O-GlcNAc cycling on the proteins that make up the PcG and TrxG complexes is likely to be in a state of constant flux. A known O-GlcNAcylation (shown as G) target in the PcG complex is the protein Polyhomeotic. Pol II, RNA polymerase II; Ub, ubiquitylation.



Epigenetics
Genomics Technology



General Approach to Method

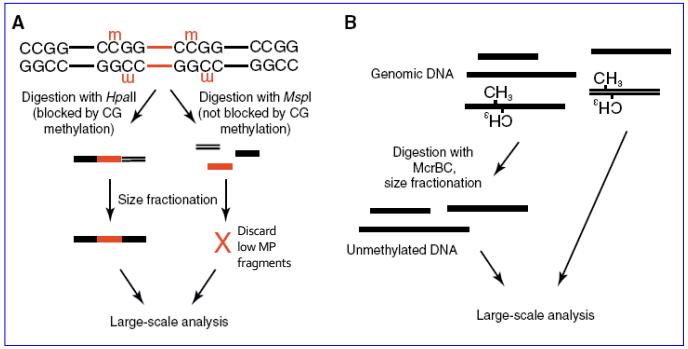
- Sample collection
- Sample enrichment to reduce genome complexity
- Biochemical modification of DNA
- Genomic analyses
  - Array-based
  - Sequencing-based
- Bioinformatic analysis

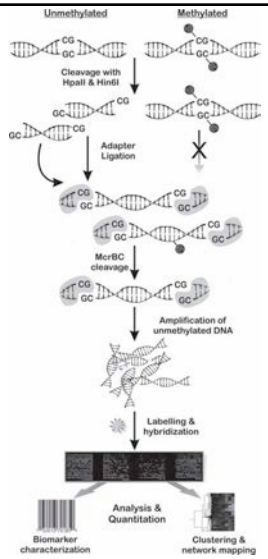
Reduction of genome complexity

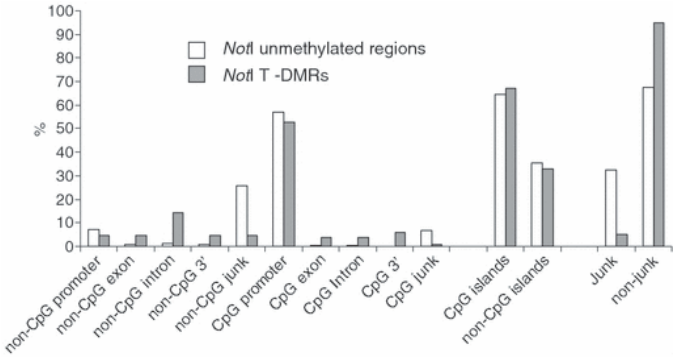
- Restriction enzymes followed by size fractionation
  - *Hpa*II, *Msp*I treatment; discard or use low MW fragments
- Affinity purification
  - E.g., Me-DIP or MBD protein
  - Possibly followed by bisulfite conversion
- Hybridization to microarray
  - E.g., capture targeted portion of genome on a microarray > elute from array > RE or bisulfite conversion > down stream assay (eg, sequencing)
- Solution-based capture
  - E.g., capture targeted portion of genome using "liquid array" > elute from oligos > bisulfate sequencing

Sample Preparation With Restriction Enzymes

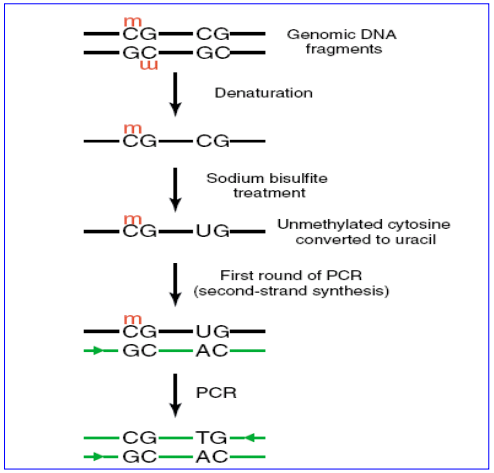
▪Reduction of complexity using size fractionation







Sample Preparation with Sodium Bisulfite



- Most popular method
- Less sample input
- Suitable for many down-stream assay platforms.

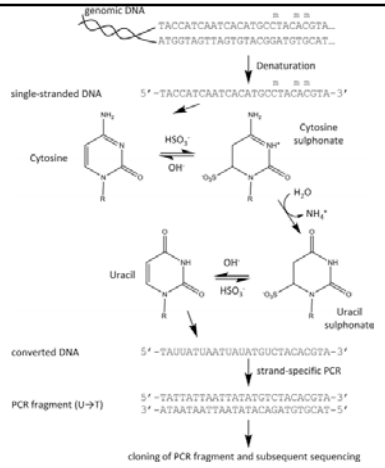


Fig. 29.1. Scheme of bisulphite conversion. Unmethylated cytosine residues of single-stranded DNA are sulphonated, followed by deamination to uracil sulphonate and desulphonation to uracil. Methylated cytosine residues are protected from this reaction. As the converted DNA is not complementary any more, the PCR has to be strand specific.

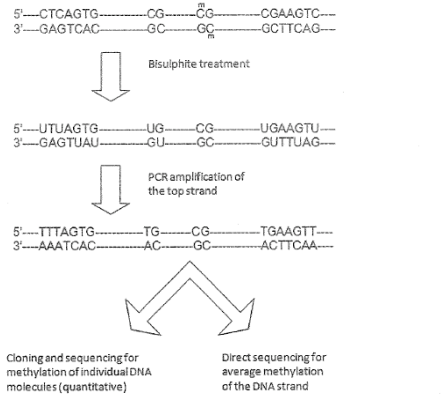


Figure 17.2 Bisulfite sequencing. Bisulfite treatment transforms the information of CpG methylation into nucleotide sequence information (C to T changes), which is easy to detect. After bisulfite conversion, the top and bottom DNA strands have different nucleotide sequence and will be amplified independently in the subsequent polymerase chain reaction (PCR) (amplification of the top strand is shown here). During the PCR all uracils are amplified as thymine by DNA polymerase. Amplified products can be sequenced directly or cloned prior to sequencing.

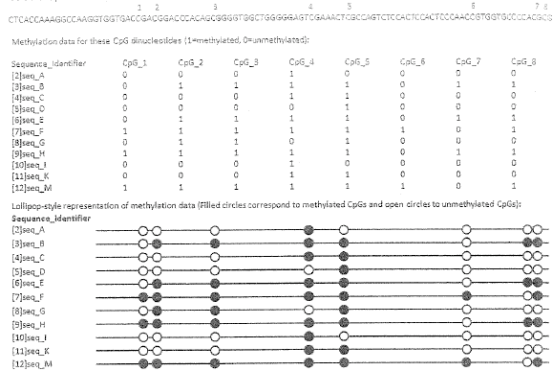
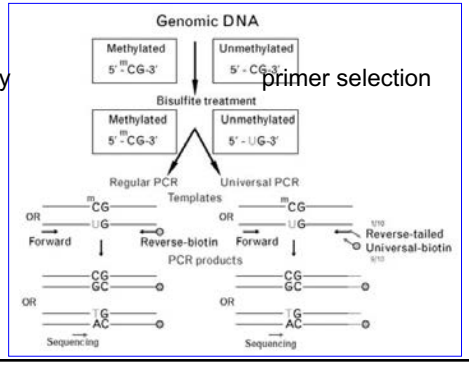


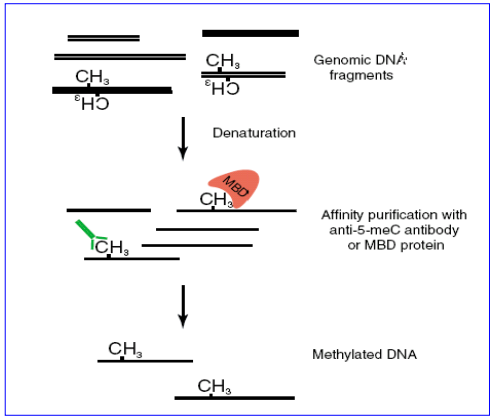
Figure 17.3 Clonal bisulfite sequencing data presentation by BiQ Analyzer. Genomic sequence of the analysed DNA fragment is given with the target CpG sites numbered. Derived methylation data are presented in table format where each row corresponds to each individual DNA sequence and each column to CpG site numbered according to the above sequence. Each data point is given either a value of 1 if methylated or 0 if unmethylated. Lower part of the figure shows a visual representation of methylation as lollipop diagrams. Each row corresponds to each individual DNA sequence, and black (methylated) and white (unmethylated) circles represent each CpG site showing the methylation status of the corresponding CpG site.

DNA Methylation Profile by Pyrosequencing

- Bisulfite DNA treatment
- Amplification of selected genes by PCR
- Measure methylation by sequencing-by-synthesis
- + Cheap
- + High resolution
- - Regions limited by primer selection



Sample Preparation with Anti- ^{5}mC antibodies

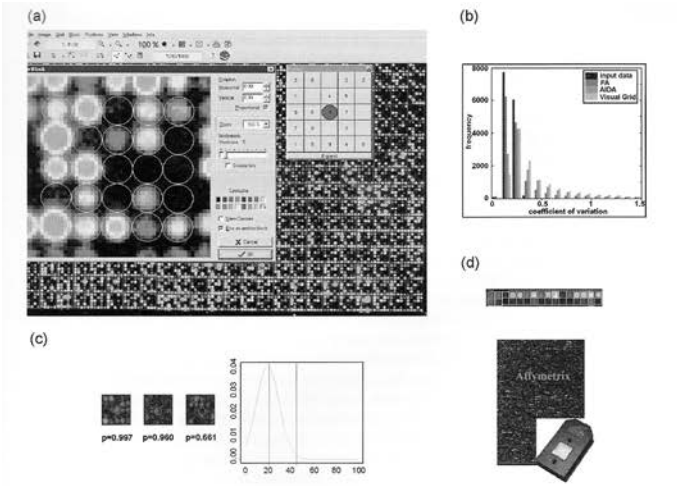


The most effective way to enrich methylated DNA and reduce complexity

Table 1 | **Main principles of DNA methylation analysis**

Pretreatment	Analytical step			
	Locus-specific analysis	Gel-based analysis	Array-based analysis	NGS-based analysis
Enzyme digestion	• *Hpa*II-PCR	• Southern blot • RLGS • MS-AP-PCR • AIMS	• DMH • MCAM • HELP • MethylScope • CHARM • MMASS	• Methyl–seq • MCA–seq • HELP–seq • MSCC
Affinity enrichment	• MeDIP-PCR		• MeDIP • mDIP • mCIP • MIRA	• MeDIP–seq • MIRA–seq
Sodium bisulphite	• MethyLight • EpiTYPER • Pyrosequencing	• Sanger BS • MSP • MS-SNuPE • COBRA	• BiMP • GoldenGate • Infinium	• RRBS • BC–seq • BSPP • WGSBS

AIMS, amplification of inter-methylated sites; BC–seq, bisulphite conversion followed by capture and sequencing; BiMP, bisulphite methylation profiling; BS, bisulphite sequencing; BSPP, bisulphite padlock probes; CHARM, comprehensive high-throughput arrays for relative methylation; COBRA, combined bisulphite restriction analysis; DMH, differential methylation hybridization; HELP, *Hpa*II tiny fragment enrichment by ligation-mediated PCR; MCA, methylated CpG island amplification; MCAM, MCA with microarray hybridization; MeDIP, mDIP and mCIP, methylated DNA immunoprecipitation; MIRA, methylated CpG island recovery assay; MMASS, microarray-based methylation assessment of single samples; MS-AP-PCR, methylation-sensitive arbitrarily primed PCR; MSCC, methylation-sensitive cut counting; MSP, methylation-specific PCR; MS-SNuPE, methylation-sensitive single nucleotide primer extension; NGS, next-generation sequencing; RLGS, restriction landmark genome scanning; RRBS, reduced representation bisulphite sequencing; –seq, followed by sequencing; WGSBS, whole-genome shotgun bisulphite sequencing.



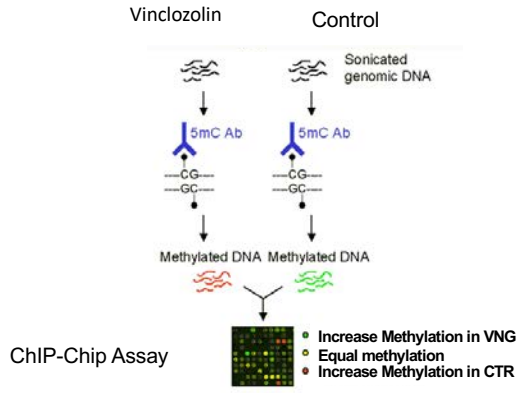
Microarray-based Methylation assays: Array type is a key consideration

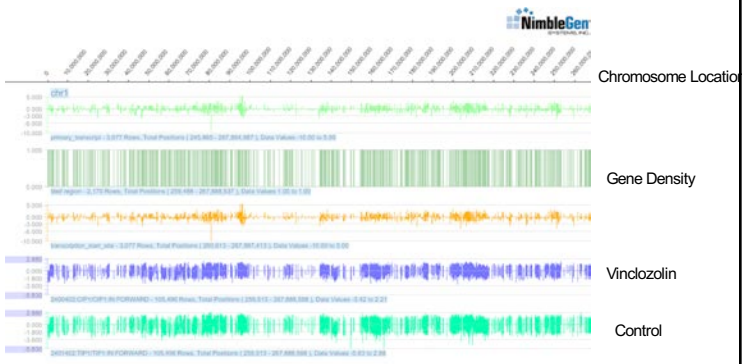
- **SNP genotyping arrays**
  - Screening assay uses methylation-sensitive enzymes > hyb to Affy SNP chips sets.. FFS at SeqWright. Cancer Res 66, 3443 (2006)
- **Promoter arrays**
  - Illumina
    - Cancer Panel I: 1,505 CpG loci from 807 genes; Golden Gate assay
    - Humanmethylation27 array: 27,578 promoter CpG sites
  - Affymetrix
    - GeneChip® Human Promoter 1.0R Array - designed for ChIP experiments
    - >4.6 million probes tiled through over 25,500 human promoter regions.
    - Average resolution of 35 bp. Approx 7.5 kb upstream through 2.45 kb downstream of 5' transcription start sites
  - NimbleGen
    - RefSeq Promoters; single array; probes with ~ 100bp spacing
    - Two-Array Sets; for splice variants and alternative transcription start sites.
    - RefSeq XM Promoters -- with predicted transcripts
    - CpG Island-Plus-Promoter Arrays
- **Tiling arrays**
  - Affymetrix
    - GeneChip® Human Tiling 1.0R Array Set --designed for transcript mapping
    - >45 million probes, 14-array set covers entire genome.
    - Average resolution of 35 bp.
  - NimbleGen
    - 7-10 array set tiling the genome at an average probe spacing of 100bp or less

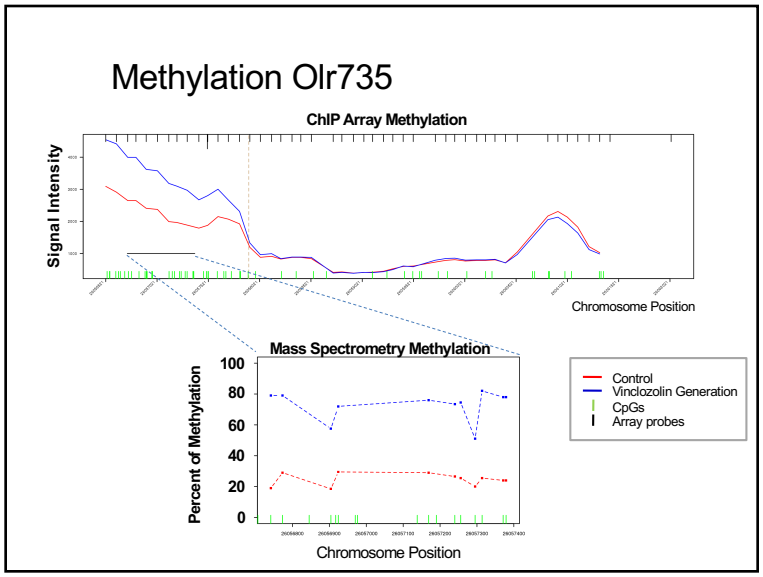
ChIP-chip

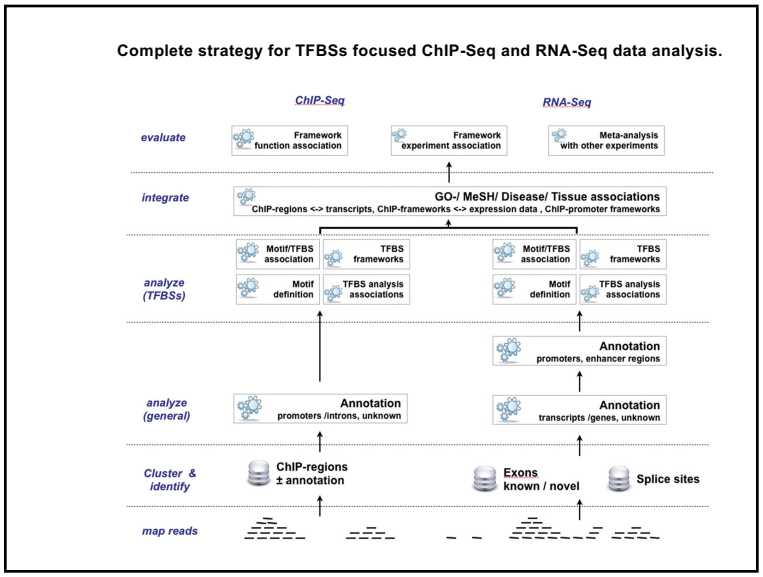
A high-throughput experimental technique that combines chromatin immunoprecipitation (ChIP) and microarray technology (chip) that directly identifies protein-DNA interactions.

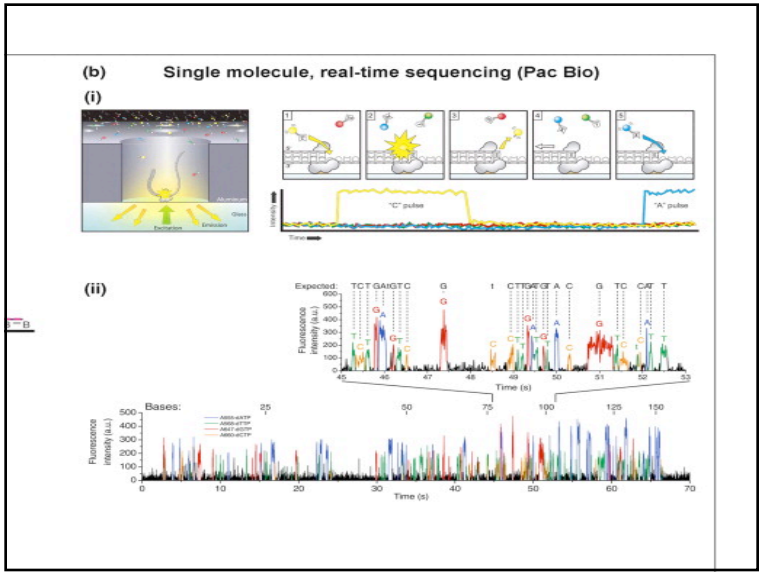
Comparative Methylation, MeDIP Chip, F3 Generation Sperm DNA pools

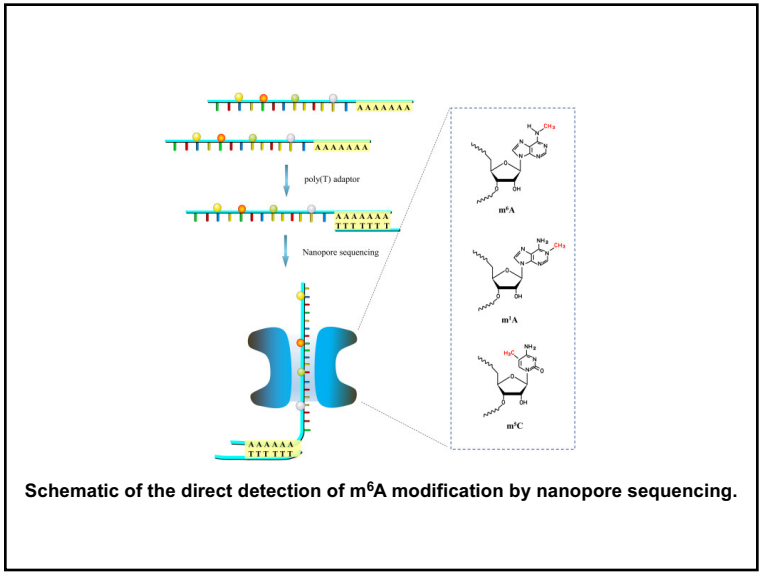






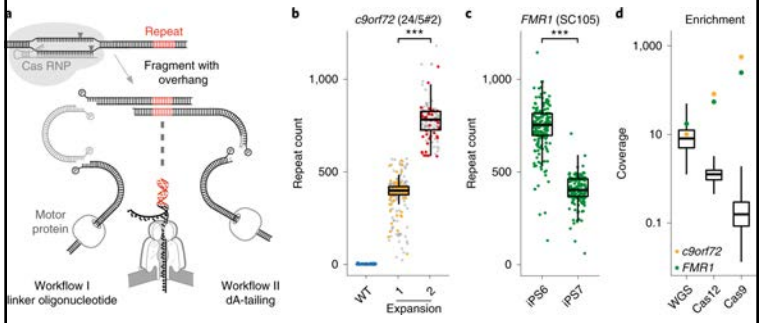


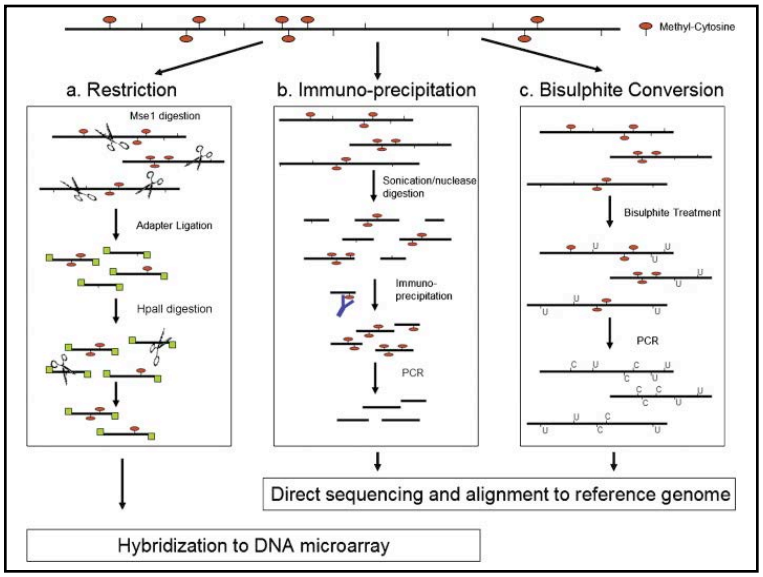




Schematic of the direct detection of m^{6}A modification by nanopore sequencing.

Analysis of short tandem repeat expansions and their methylation state with nanopore sequencing
Giesselmann P, Brändl B, Raimondeau E, et al.
Nat Biotechnol. 2019 Dec;37(12):1478-1481.





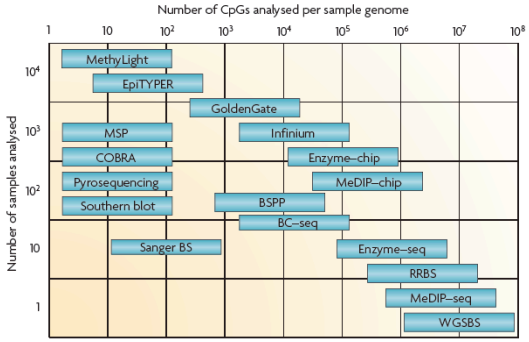
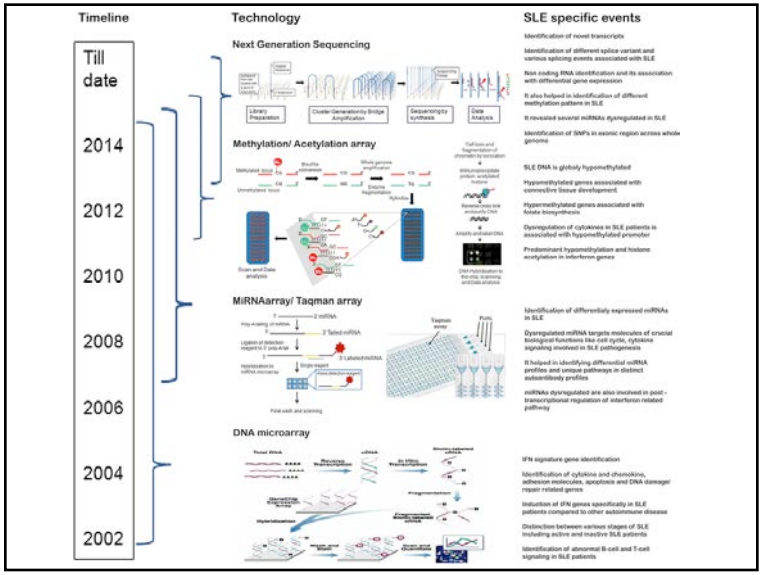
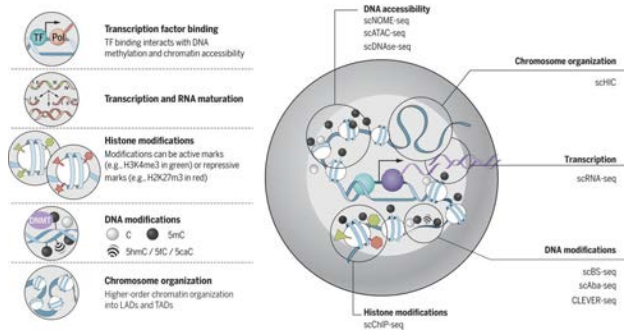


Figure 1 | **Sample throughput versus genome coverage.** A plot of sample throughput against genome coverage for various DNA methylation techniques. Throughput is determined by the number of samples that can be analysed per experiment, based on large eukaryotic genomes. Coverage is determined by the number of CpGs in the genome that can be analysed per experiment. BC–seq, bisulphite conversion followed by capture and sequencing; BS, bisulphite sequencing; BSPP, bisulphite padlock probes; –chip, followed by microarray; COBRA, combined bisulphite restriction analysis; MeDIP, methylated DNA immunoprecipitation; MSP, methylation-specific PCR; RRBS, reduced representation bisulphite sequencing; –seq, followed by sequencing; WGSBS, whole-genome shotgun bisulphite sequencing.

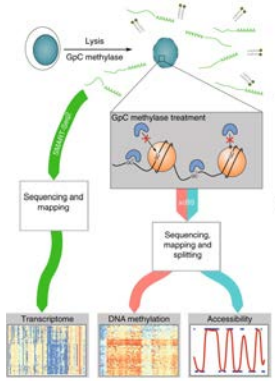


Single-cell epigenomics: Recording the past and predicting the future.
Science. 2017 Oct 6;358(6359):69-75.
Kelsey G, Stegle O, Reik W.

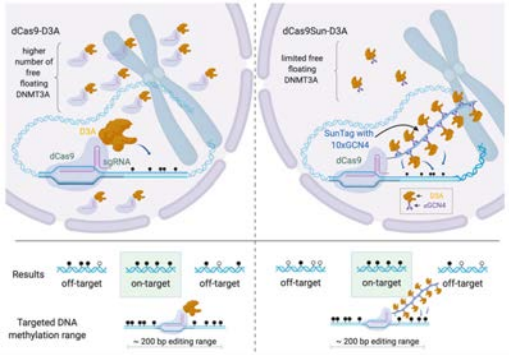


Single-cell methods and heterogeneity of different molecular layers. (Left) Overview of different molecular layers that can be assayed using single-cell protocols. **(Right)** A cell with different layers of multi-omics measurements, as defined on the left. Concordance or heterogeneity respectively may exist between the different layers, and this can be recorded by single-cell sequencing and computationally evaluated.

scNMT-seq enables joint profiling of chromatin accessibility DNA methylation and transcription in single cells
Clark SJ, Argelaguet R, Kapourani A, et al.
Nat Commun. 2018 Feb 22;9(1):781.



Harnessing targeted DNA methylation and demethylation using dCas9
Pflueger C, Swain T, Lister R.
Essays Biochem. 2019 Dec 20;63(6):813-825.

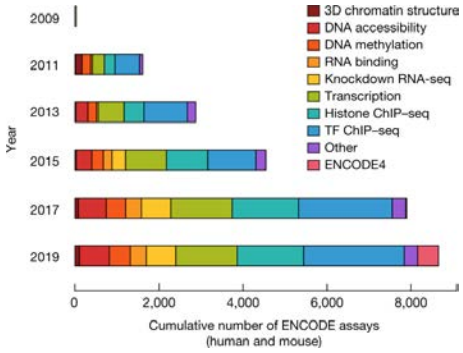


Expanded encyclopaedias of DNA elements in the human and mouse genomes
ENCODE Project Consortium; Moore JE, Purcaro MJ, Pratt HE, et al.
Nature. 2020 Jul;583(7818):699-710.

All data are available through the ENCODE data portal (https://www.encodeproject.org), including phase II ENCODE1 and Roadmap Epigenomics2 data. We have developed a registry of 926,535 human and 339,815 mouse candidate cis-regulatory elements, covering 7.9 and 3.4% of their respective genomes, by integrating selected datatypes associated with gene regulation, and constructed a web-based server (SCREEN; http://screen.encodeproject.org) to provide flexible, user-defined access to this resource. Collectively, the ENCODE data and registry provide an expansive resource for the scientific community to build a better understanding of the organization and function of the human and mouse genomes.

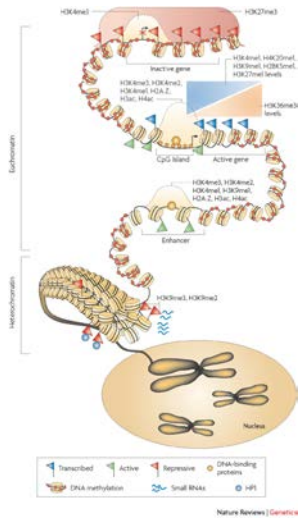
Perspectives on ENCODE.
ENCODE Project Consortium, Snyder MP, Gingeras TR, et al.
Nature. 2020 Jul;583(7818):693-698.

The Encylopedia of DNA Elements (ENCODE) Project launched in 2003 with the long-term goal of developing a comprehensive map of functional elements in the human genome. These included genes, biochemical regions associated with gene regulation (for example, transcription factor binding sites, open chromatin, and histone marks) and transcript isoforms. The marks serve as sites for candidate cis-regulatory elements (cCREs) that may serve functional roles in regulating gene expression[1]. The project has been extended to model organisms, particularly the mouse. In the third phase of ENCODE, nearly a million and more than 300,000 cCRE annotations have been generated for human and mouse, respectively, and these have provided a valuable resource for the scientific community.

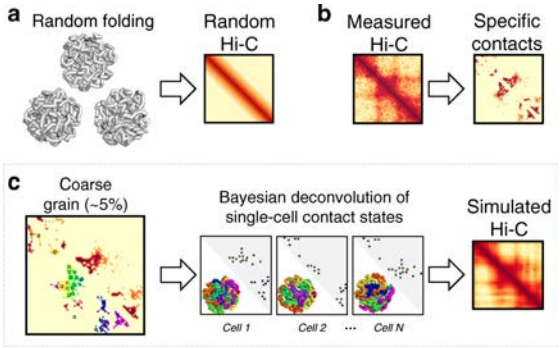


Epigenetic Mechanisms of Gene Regulation

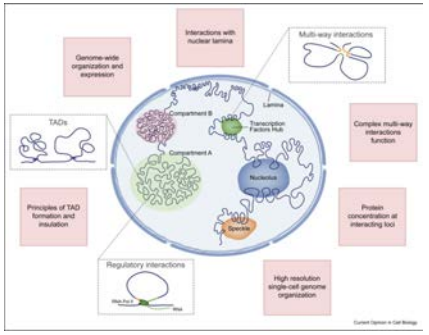
-DNA Methylation
-Histone Modification
-Chromatin Structure
-DNA Organization into Domains (eg Loops)
-Nuclear Compartmentalization (eg nuclear matrix)
-Noncoding functional RNAs
-RNA Methylation



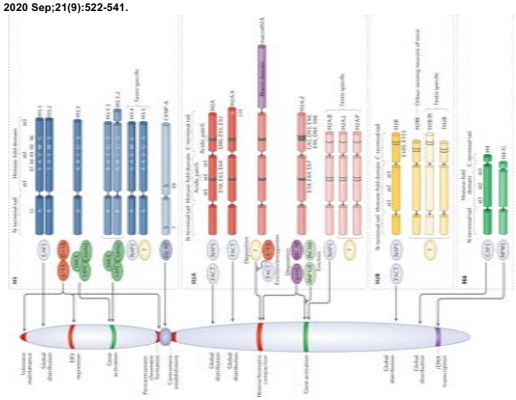
CHROMATIX: computing the functional landscape of many-body chromatin interactions in transcriptionally active loci from deconvolved single cells
Perez-Rathke A, Sun Q, Wang B, Boeva V, Shao Z, Liang J.
Genome Biol. 2020 Jan 16;21(1):13.



Evolving methodologies and concepts in 4D nucleome research
Sparks TM, Harabula I, Pombo A.
Curr Opin Cell Biol. 2020 Jun;64:105-111.



The roles of histone variants in fine-tuning chromatin organization and function
Martire S, Banaszynski LA.
Nat Rev Mol Cell Biol. 2020 Sep;21(9):522-541.

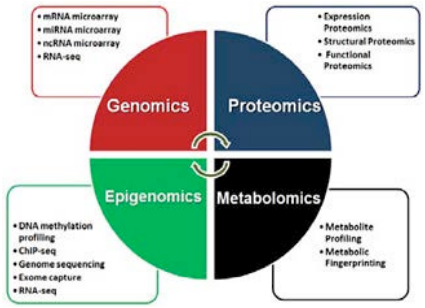


CRISPR-Mediated Epigenome Editing.
Enríquez P.
Yale J Biol Med. 2016 Dec 23;89(4):471-486.

Mounting evidence has called into question our understanding of the role that the central dogma of molecular biology plays in human pathology. The conventional view that elucidating the mechanisms for translating genes into proteins can account for a panoply of diseases has proven incomplete. Landmark studies point to epigenetics as a missing piece of the puzzle. However, technological limitations have hindered the study of specific roles for histone post-translational modifications, DNA modifications, and non-coding RNAs in regulation of the epigenome and chromatin structure. This feature highlights CRISPR systems, including CRISPR-Cas9, as novel tools for targeted epigenome editing. It summarizes recent developments in the field, including integration of optogenetic and functional genomic approaches to explore new therapeutic opportunities, and underscores the importance of mitigating current limitations in the field. This comprehensive, analytical assessment identifies current research gaps, forecasts future research opportunities, and argues that as epigenome editing technologies mature, overcoming critical challenges in delivery, specificity, and fidelity should clear the path to bring these technologies into the clinic.

Strategies for precision modulation of gene expression by epigenome editing: an overview.
Laufer BI, Singh SM.
Epigenetics Chromatin. 2015 Sep 17;8:34.

Genome editing technology has evolved rather quickly and become accessible to most researchers. It has resulted in far reaching implications and a number of novel designer systems including epigenome editing. Epigenome editing utilizes a combination of nuclease-null genome editing systems and effector domains to modulate gene expression. In particular, Zinc Finger, Transcription-Activator-Like Effector, and CRISPR/Cas9 have emerged as modular systems that can be modified to allow for precision manipulation of epigenetic marks without altering underlying DNA sequence. This review contains a comprehensive catalog of effector domains that can be used with components of genome editing systems to achieve epigenome editing. Ultimately, the evidence-based design of epigenome editing offers a novel improvement to the limited attenuation strategies. There is much potential for editing and/or correcting gene expression in somatic cells toward a new era of functional genomics and personalized medicine.



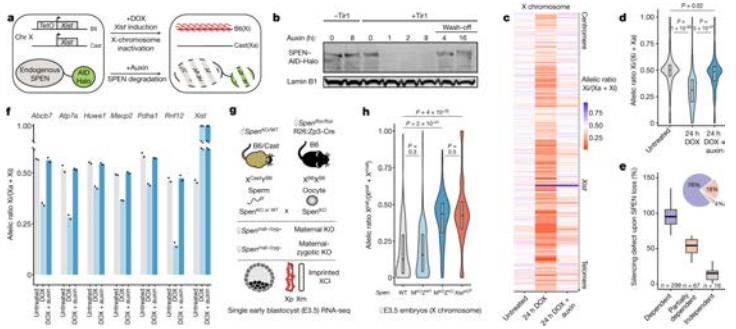
DeepH&M: Estimating single-CpG hydroxymethylation and methylation levels from enrichment and restriction enzyme sequencing methods
He Y, Jang HS, Xing X, Li D, Vasek MJ, Dougherty JD, Wang T.
Sci Adv. 2020 Jul 1;6(27):eaba0521.

Increased appreciation of 5-hydroxymethylcytosine (5hmC) as a stable epigenetic mark, which defines cell identity and disease progress, has engendered a need for cost-effective, but high-resolution, 5hmC mapping technology. Current enrichment-based technologies provide cheap but low-resolution and relative enrichment of 5hmC levels, while single-base resolution methods can be prohibitively expensive to scale up to large experiments. To address this problem, we developed a deep learning-based method, "DeepH&M," which integrates enrichment and restriction enzyme sequencing methods to simultaneously estimate absolute hydroxymethylation and methylation levels at single-CpG resolution. Using 7-week-old mouse cerebellum data for training the DeepH&M model, we demonstrated that the 5hmC and 5mC levels predicted by DeepH&M were in high concordance with whole-genome bisulfite-based approaches. The DeepH&M model can be applied to 7-week-old frontal cortex and 79-week-old cerebellum, revealing the robust generalizability of this method to other tissues from various biological time points.

Bayesian Data Fusion of Gene Expression and Histone Modification Profiles for Inference of Gene Regulatory Network
Chen H, Maduranga DAK, Piyushkumar A Mundra PA, Zheng J.
IEEE/ACM Trans Comput Biol Bioinform. Mar-Apr 2020;17(2):516-525.

Accurately reconstructing gene regulatory networks (GRNs) from high-throughput gene expression data has been a major challenge in systems biology for decades. Many approaches have been proposed to solve this problem. However, there is still much room for the improvement of GRN inference. Integrating data from different sources is a promising strategy. Epigenetic modifications have a close relationship with gene regulation. Hence, epigenetic data such as histone modification profiles can provide useful information for uncovering regulatory interactions between genes. In this paper, we propose a method to integrate epigenetic data into the inference of GRNs. In particular, a dynamic Bayesian network (DBN) is employed to infer gene regulations from time-series gene expression data. Epigenetic data (histone modification profiles here) are integrated into the prior probability distribution of the Bayesian model. Our method has been validated on both synthetic and real datasets. Experimental results show that the integration of epigenetic data can significantly improve the performance of GRN inference. As more epigenetic datasets become available, our method would be useful for elucidating the gene regulatory mechanisms driving various cellular activities. The source code and testing datasets are available at https://github.com/Zheng-Lab/MetaGRN/tree/master/histonePrior.

SPEN integrates transcriptional and epigenetic control of X-inactivation
Dossin F, Pinheiro I, Żylicz JJ, et al.
Nature. 2020 Feb;578(7795):455-460.



MethHaplo: combining allele-specific DNA methylation and SNPs for haplotype region identification.
Zhou Q, Wang Z, Li J, Sung WK, Li G.
BMC Bioinformatics. 2020 Oct 12;21(1):451.

This study illustrates the usefulness of methylation haplotypes. By constructing methylation haplotypes for various cell lines, we provide a clearer picture of the effect of DNA methylation on gene expression, histone modification and three-dimensional chromosome structure at the haplotype level. Our method could benefit the study of parental inheritance-related disease and hybrid vigor in agriculture.

Reference-free deconvolution, visualization and interpretation of complex DNA methylation data using DecompPipeline, MeDeCom and FactorViz

Scherer M, Nazarov PV, Toth R, Sahay S, Kaoma T, Maurer V, Vedeneev N, Plass C, Lengauer T, Walter J, Lutsik P.
Nat Protoc. 2020 Oct;15(10):3240-3263.

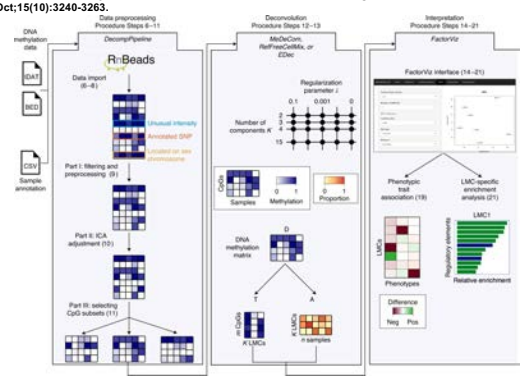


Table 2. List of databases and tools for analyzing miRNA

Databases	Web Links
1. miRBase	http://www.mirbase.org/
2. miRNAmap	http://mirnamap.mbc.nctu.edu.tw/
3. miRWalk	http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/index.html
4. miRDB	http://mirdb.org/miRDB/
5. TarBase V.5c	http://diana.cslab.ece.ntua.gr/tarbase/
6. miRGen	http://www.diana.pcbi.upenn.edu/miRGen.html
7. miROrtho	http://cegg.unige.ch/mirortho
8. mir2disease	http://www.mir2disease.org/
9. miRanda	http://www.microrna.org/microrna/home.do
10.TargetScan 6.2	http://www.targetscan.org/
11.miRanda	http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/
12.DIANAmicroT	www.microrna.gr/microT
13.PicTar	http://pictar.mdc-berlin.de/
14.miRecords	http://mirecords.umn.edu/miRecords/
15.mirTarBase	http://mirtarbase.mbc.nctu.edu.tw/
16.miRWalk	http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/
17.miRNAMap	http://mirnamap.mbc.nctu.edu.tw/

Table 5. Software tools for epigenomics data processing and analyses

Software Tools	Methylation Analysis
1. Batman	http://td-blade.gurdon.cam.ac.uk/software/batman
2. BDPC	http://biochem.jacobs-university.de/BDPC
3. Epinexus	http://epinexus.net/home.html
4. MethDB	http://www.methdb.de
5. methPrimerDB	http://medgen.ugent.be/methprimerdb
6. MethyCancer Database	http://methycancer.psych.ac.cn
7. Methylumi	http://www.bioconductor.org/packages/bioc/html/methylumi.html
8. mPod	http://www.compbio.group.cam.ac.uk/Projects/p4meth.html
9. PubMeth	http://www.pubmeth.org
10.QUMA	http://quma.cdb.riken.jp
11.TCGA Data Portal	http://cancergenome.nih.gov/dataportal
Software Tools	**Bisulphite Sequencing Analysis**
1. BSMAP	http://code.google.com/p/bsmap
2. methBLAST	http://medgen.ugent.be/methBLAST
3. methPrimer	http://www.urogene.org/methprimer
4. MethyI Primer Express	http://www.appliedbiosystems.com/methylprimerexpress
5. CpGviewer	http://dna.leeds.ac.uk/cpgviewer
Software Tools	**CpG Island Analysis**
1. CpGcluster	http://bioinfo2.ugr.es/CpGcluster
2. CpGFinder	http://linux1.softberry.com
3. CpG Island Explorer	http://bioinfo.hku.hk/cpgieintro.html
4. CpG Island Explorer	http://cpgislands.usc.edu
5. CpG Promoter	http://www.cshl.edu/OTT/html/cpg_promoter.html
6. CpG ratio/GC content Plotter	http://mwsross.bms.ed.ac.uk/public/cgi-bin/cpg.pl
7. EMBOSS CpGPlot/CpGReport/Isochore	http://www.ebi.ac.uk/Tools/emboss/cpgplot/index.html

Table 6. Software tools for analyzing and interpreting metabolomics data

Software Tools	Web Links
1. BioSpider	http://www.biospider.ca/
2. COLMAR	http://spinportal.magnet.fsu.edu/
3. FiD	http://www.cs.helsinki.fi/group/sysfys/software/fragid/
4. HORA suite	http://www.paternostrolab.org/
5. MeltDB 2.0	https://meltdb.cebitec.uni-bielefeld.de/cgi-bin/login.cgi
6. MetaboAnalyst 20	http://www.metaboanalyst.ca/MetaboAnalyst/faces/Home.jsp
7. MetaboMiner	http://wishart.biology.ualberta.ca/metabominer/
8. MolFind	http://metabolomics.pharm.uconn.edu/Software.html
9. OpenMS 1.11.1	http://open-ms.sourceforge.net/openms-1-11-1-released/
10. SetupX	http://fiehnlab.ucdavis.edu/projects/binbase_setupx/
11. Seven Golden Rules	http://fiehnlab.ucdavis.edu/projects/Seven_Golden_Rules/Software/
12. XCMS	http://metlin.scripps.edu/xcms/
13. MSEA	http://www.msea.ca
14. MBRole	http://csbg.cnb.csic.es/mbrole
15. MPEA	http://ekhidna.biocenter.helsinki.fi/poxo/mpea/
16. IMPaLA	http://impala.molgen.mpg.de
17. BioCyc – Omics Viewer	http://biocyc.org
18. MetPA	http://metpa.metabolomics.ca
19. Reactome	http://www.reactome.org
20. Cytoscape	http://www.cytoscape.org/

"Epigenetics and Systems Biology"

Spring 2021 (Odd Years)
Biol 476/576
Schedule/Lecture Outline –

Week 1	(Lesson 1)	Systems Biology (History/ Definitions/ Theory)
Week 2	(Lesson 2)	Systems Biology (Networks & Emergence)
Week 3	(Lesson 3)	Systems Biology (Components: DNA to Phenotype)
Week 4	(Lesson 4)	Systems Biology (Genomics / Technology)
Week 5	(Lesson 5)	Epigenetics (History / Molecular Processes)
Week 6	(Lesson 6)	Epigenetics (Molecular Processes & Integration)
Week 7	(Lesson 7)	Epigenetics (Genomics and Technology)
Week 8	(Lesson 8)	Cell & Developmental Biology
Week 9	(Lesson 9)	Epigenetics of Cell & Developmental Biology
Week 10	(Lesson 10)	Environmental Impact on Biology
Week 11	(Lesson 11)	Environmental Epigenetics
Week 12	(Lesson 12)	Disease Etiology
Week 13	(Lesson 13)	Epigenetics & Disease Etiology
Week 14	(Lesson 14)	Evolutionary Biology & Genetics
Week 15	(Lesson 15)	Epigenetics & Evolutionary Biology
Week 16	(Lesson 16)	Grant Review/ Study Section Meeting