

Heterochromatin, from Chromosome to Protein

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I The concept of heterochromatin

Definition of Chromatin

In eukaryotes, on the contrary of prokaryotes, the DNA is packaged in the form of a nucleoprotein complex called "[chromatin](#)", which carries the hereditary message. It is located in a nucleus and is organised in several separate entities, the chromosomes.

The Concept of Heterochromatin

In 1928, based on histological observations, Emil HEITZ defined heterochromatin (HC) as being the chromosomal segments which appear extremely condensed and dark in colour in the interphase nucleus. In fact, chromatin consists of a tangle of fibres, the diameter of which not only vary during the cell cycle, but also depend on the region of the chromosome observed.

The active euchromatin consists of a fibre with a diameter corresponding to that of a nucleosome, a double strand DNA segment, wound around homodimers of the histones H2A, H2B, H3, and H4. In inactive euchromatin, this fibre can wind itself into a solenoid thanks to histones H1. It is further organised through interactions with non-histone proteins (topoisomerase II, scaffold protein 2, lamins...). As regards the heterochromatin, as defined above, its constituent fibre is more condensed and often appears to be composed of aggregates. It involves numerous additional proteins, including the HP1 proteins (Heterochromatin Protein 1).

II Two types of heterochromatin

There are two types of heterochromatin, *constitutive HC* and *facultative HC*, which differ slightly, depending on the DNA that they contain. The richness in satellite DNA determines the permanent or reversible nature of the heterochromatin, its polymorphism and its staining properties.

constitutive HC	facultative HC
stable	reversible
contains satellite DNA	enriched in LINES sequences
polymorphism +	polymorphism -
C bands +	C bands -

Table I: Properties which allow to differentiate constitutive from facultative heterochromatin

II.1 Constitutive heterochromatin

- *Constitutive* HC contains a particular type of DNA called satellite DNA, which consists of large numbers of short tandemly repeated sequences : *Alpha-satellite DNA*, *DNA satellite I*, *II* and *III*. These satellite DNA sequences are able to fold on themselves and may have an important role in the formation of the highly compact structure of the constitutive HC.
- *Constitutive* HC is stable and conserves its heterochromatic properties during all stages of development and in all tissues.
- *Constitutive* HC is highly polymorphic, probably because of the instability of the satellite DNA. This polymorphism can affect not only the size but also the localisation of the heterochromatin, and apparently has no phenotypic effect.
- *Constitutive* HC is strongly stained by the C-band technique, which is the result of the very rapid renaturation of the satellite DNA following denaturation.

II.2 Facultative heterochromatin

- *Facultative* HC is characterised by the presence of *LINE*-type repeated sequences. These sequences, dispersed throughout the genome, could promote the propagation of a condensed chromatin structure.
- *Facultative* HC is reversible, its heterochromatic state depending on the stage of development or the cell type examined. The inactive X (Barr body) in the somatic cells females and the inactive sex vesicle at the pachytene stage of male meiosis provide two examples of facultative HC.
- *Facultative* HC is not particularly rich in satellite DNA, and is therefore not polymorphic.
- *Facultative* HC is never stained by the C-band technique.

III Properties of heterochromatin

Despite the differences described above, constitutive HC and facultative HC have very similar properties.

III.1 Heterochromatin is condensed

This is in fact what defines heterochromatin, and it is applicable to both *constitutive* HC and *facultative* HC. This high condensation renders it strongly chromophilic and inaccessible to DNase 1 and to other restriction enzymes in general.

III.2 Heterochromatin DNA is late replicating

The incorporation of various nucleotide analogues shows that the DNA from both *constitutive* and *facultative* HC, is late replicating. HC late replication results, on the one hand, from its high degree of condensation, which prevents the replicating machinery from easily accessing the DNA, and, on the other hand, from its location in a peripheral nuclear domain that is poor in active elements.

III.3 Heterochromatin DNA is methylated

- The DNA of *constitutive* HC is highly methylated on the cytosines. An anti-5-methyl cytosine antibody therefore strongly labels all the regions of *constitutive* HC.
- As regards *facultative* HC, the methylation of the DNA is more discrete, but restriction enzymes sensitive to methylation reveals strong methylation of the CpG islands, which are specifically located in the control regions of the genes.

III.4 In heterochromatin, histones are hypo-acetylated

Histones may undergo post-translational modifications of their N-terminal ends which may affect the genetic activity of the chromatin.

- The hypo-acetylation of histone N-terminal tails, principally on the lysines, is associated with an inactive chromatin. In contrast, hyper-acetylated histones characterise the active chromatin.
- Acetylation/de-acetylation of histones is a mechanism that is absolutely essential for the control of gene expression. Numerous transcription factors have been shown to have, either an activity Histone Acétyl Transférase or Histone De-Acétylases.

III.5 Histones from heterochromatin are methylated on lysine 9

Methylation of the histone H3 lysine 9 (H3-K9) has only very recently been found to be involved in the process of heterochromatinisation of the genome, both in *constitutive* and *facultative* HC.

III.6 Heterochromatin is transcriptionally inactive

- Unlike in *Drosophila*, human *constitutive* HC does not contain any genes and incorporating tritiated uridine into a cell culture does not result in any labelling at its level .
- The *facultative* HC is relatively poor in genes, and its genes are not usually transcribed in a heterochromatic context.

III.7 Heterochromatin does not participate in genetic recombination

- It is generally accepted that *constitutive* HC does not participate in genetic recombination. There is no preliminary pairing of the homologous heterochromatic regions probably because the polymorphism that characterises the heterochromatic regions render it difficult if not impossible. *Constitutive* HC also acts to repress recombination in adjacent euchromatic regions.
- As regards the *facultative* HC, it does not participate in meiotic recombination when it is in its inactive form.

III.8 Heterochromatin has a gregarious instinct

The study of various organisms has shown that *constitutive* HC has a genuine tendency to aggregate during interphase.

- In *Drosophila* larvae, the centromeres of polytene chromosomes, which are rich in heterochromatin, can aggregate to form the chromocentres during interphase.
- In the mouse, the number of heterochromatic blocks that can be observed in interphase nuclei is always lower than the number of heterochromatic regions visualised on the metaphase chromosomes.
- In humans, the short arms of the acrocentric chromosomes, mainly formed from heterochromatin, are frequently associated in the

interphase nucleus with other chromosomes having a large HC block (1, 9 and 16).

This tendency of the heterochromatin to aggregate appears to be strongly linked to the presence of satellite DNA sequences, but it may also involve other additional sequences.

IV Factors involved in heterochromatinisation

Certain observations have led to the identification of various elements that have an important role in the formation of heterochromatin, be it *constitutive* or *facultative*.

IV.1 Large arrays of tandemly repeated sequences.

- The satellite DNA visualised by FISH colocalises exactly with *constitutive* heterochromatin. Moreover, satellite DNA has the distinctive feature of bending and folding upon itself, and this may be an important factor in determining the extremely compact structure of the constitutive heterochromatin.
- However, this does not only concern satellite DNA. In plants, *Drosophila*, and also in the mouse, certain multicopy transgenes are barely expressed, or are not expressed at all, even when they are not subject to centromere repression.

These different observations suggest that the tandem repetition of a DNA sequence in a large number of copies is sufficient on its own to direct the formation of heterochromatin. Such repeated sequences could allow the chromatin to be compacted to a greater extent, by forming characteristic structures. These structures could be recognised by specific proteins, such as the HP1 proteins, which in turn direct the formation of a higher-order chromatin.

IV.2 Methylation of DNA

Large repetitions of transgenes do not all lead to a transcriptional inactivation of the transgene. The silencing induced by tandem repeats appears to be linked to the presence of prokaryotic DNA sequences, rich in CpG, likely to be methylated. Then the base composition of the tandem repeats could therefore play an important role in the formation of heterochromatin.

- Interestingly, it has recently been shown that the methyl binding protein MeCP2, which normally binds to DNA methylated cytosines, has thus been shown to be able to recruit histone de-acetylases (Figure 1). Methylation of the DNA could therefore induce a de-acetylation of histones and thus promote heterochromatinisation.
- However, the methylation of DNA is not indispensable for the formation of heterochromatin. It could be an element involved in stabilisation. Indeed in marsupials, the inactive X is not methylated and is much less stable than in eutherian mammals.

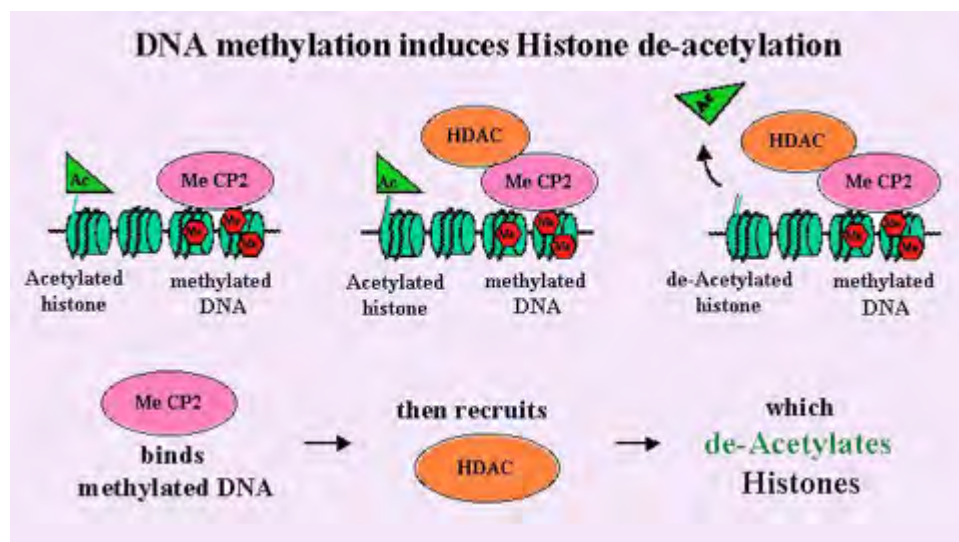


Figure 1: DNA methylation induces Histone de-acetylation, modification which characterizes histones in both heterochromatin and repressed euchromatin.

MeCP2 specifically binds to methylated DNA, and recruits an HDAC which de-acetylates

histones (Ac= Acetyl; Me= Methyl; MeCP2= Methyl-CpG binding Protein 2; HDAC= Histone De-Acetylase).

IV.3 Hypo-acetylation of Histones

We have seen that hypo-acetylation of histones is a characteristic of silent chromatin, whether it is heterochromatin or not. Thus, blocking the de-acetylation of the histones by adding trichostatine A induces hyper-acetylation of the histones, which causes a more open chromatin structure

- In fact, acetylation of the lysines removes the positive charge from the histones, thus reducing the force of attraction with the negative charge of DNA phosphate and leading to a wider opening of the chromatin.
- In contrast, de-acetylation of the lysines restores their positive charges and thus promotes a close attraction with the DNA, leading to a condensed chromatin.

IV.4 Methylation of H3-K9

Methylation of the histone H3 on lysine 9 is an epigenetic modification that has recently been shown to be involved in the process of heterochromatinisation, not only in *constitutive* HC but also on the inactive X. The enzyme responsible for this methylation is the histone methyltransferase SUV39H1.

- On H3-K9, acetylation and methylation appear to be mutually exclusive. In *Drosophila*, therefore, the methyltransferase Suv39h is associated with a histone de-acetylase, suggesting a *single molecular mechanism* that allows the direct conversion of an acetylated lysine 9 into a methylated lysine 9.
- In addition, the methylation of H3-K9 creates a high-affinity binding site for the heterochromatin protein HP1. Co-immuno precipitation of Suvar39h with HP1 suggests a heterochromatinisation mechanism based on the interaction of these two proteins and lysine 9.
- Lastly, in *Neurospora crassa*, it has recently been shown that methylation of H3-K9 can cause methylation of DNA (Figure 2).

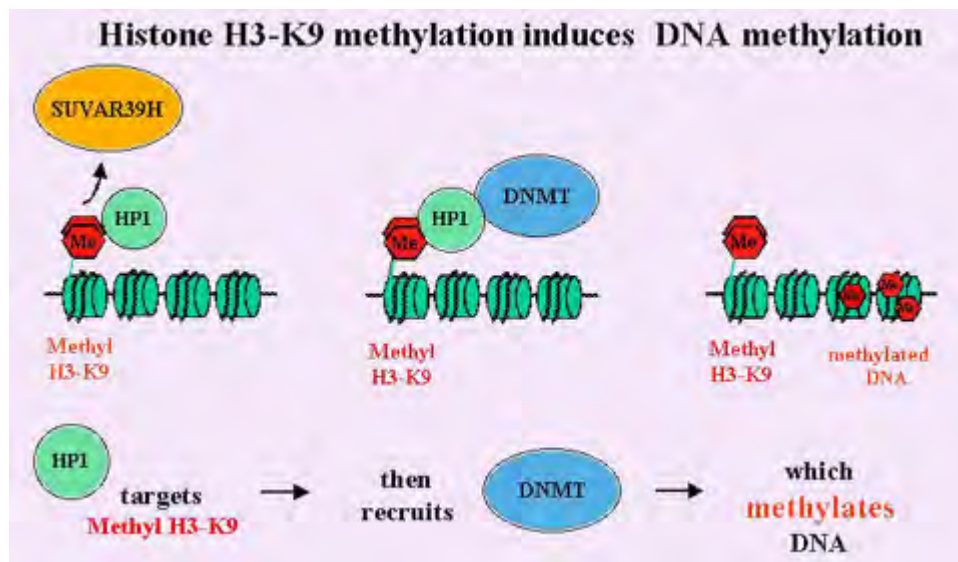


Figure 2: Histone H3-K9 methylation induces DNA methylation, modification which characterizes DNA in heterochromatin or repressive euchromatin.

SUVAR39H is a methyltransferase which specifically methylates the Lysine 9 of histone H3. Such a methylation creates a binding site for the Heterochromatin Protein HP1 which recruits a DNA methyl transferase, capable to methylate the CpG in DNA (Me= Methyl; Methyl H3-K9= Methyl on Lysine 9 of Histone H3; HP1=Heterochromatin Protein 1; DNMT=DNA Methyl transferase).

IV.5 HP1 proteins

The HP1 proteins do appear to have a particular role in the organisation of heterochromatin. Studies of the variegation by position effect (PEV effect) in *Drosophila* and studies of transgenes in *Drosophila* and mouse have allowed a better understanding of the role of these proteins.

- In *Drosophila*, the HP1 protein is coded for by the Su(var)205 gene, which is a suppresser of variegation that can modify the PEV effect. The variegation by position effect can be described as follows: genes that are normally localised in active euchromatin are, following a chromosome rearrangement, placed close to a centromeric region that is heterochromatic. Then, newly translocated chromatin become much more compact, and it becomes associated with HP1 proteins that are normally confined to centromeres. Moreover, the genes contained in the translocated chromatin become repressed.
- In mouse, the insertion of a transgene close to the centromere may have similar consequences.

It is interesting to note that even where a transgene is repressed, not as a result of a centromeric effect but as a result of its presence in multiple copies, HP1 proteins are also found to be associated with the repressed chromatin.

HP1 proteins appear to be an essential link in the formation of heterochromatin, and could have the role of *chromatin domain organisers*. These proteins appear to be able to recognise particular structures that are created by the pairing and/or the association of repeated DNA sequences. In addition, thanks to the chromodomain (CD) and the chromoshadow domain (CSD), they are able to establish secondary interactions with a large number of other proteins.

IV.6 Nuclear RNAs

- It is already well established that certain nuclear RNAs are able to contribute to the formation of facultative HC. The transcripts of the XIST gene have an essential role in the initiation of facultative inactivation of one X chromosome, in the somatic cells in female mammals.
- Some recent studies in mouse have suggested that nuclear transcripts may also be involved in the formation of constitutive HC. In mouse cells, the centromeric HC is characterised by a high concentration of methylated H3-K9 histone and heterochromatic HP1 proteins, which are rapidly de-localized after incubation with RNAse A. This suggests that a nuclear RNA may be an essential *structural component* of constitutive HC.

V Functions of heterochromatin

The precise role of heterochromatin in the human genome long remained a mystery, as its frequent polymorphisms did not appear to have any functional or phenotypic effect.

V.1 Role of HC in the organisation of nuclear domains

- Heterochromatin and euchromatin occupy different nuclear domains. HC is usually localised in the periphery of the nucleus and is attached to the nuclear membrane. In contrast, the active chromatin occupies a more central position.
- The preferential localisation of HC against the nuclear membrane may be due to the interaction of the protein HP1 with the lamin B receptor, which is an integral component of the inner membrane of the nucleus.
- The peripheral localisation of HC concentrates the active elements towards the centre of the nucleus, allowing the active euchromatin to replicate and be transcribed with maximum efficiency.

V.2 Role of HC in the centromeric function

In most eukaryotes, the centromeres are loaded with a considerable mass of heterochromatin. It has been suggested that centromeric HC is necessary for the cohesion of sister chromatids and that it allows the normal disjunction of mitotic chromosomes.

- In the yeast *Schizosaccharomyces pombe*, the homologue of the HP1 protein Swi6 is absolutely essential for efficient cohesion of sister chromatids during cell division.
- Moreover, experiments involving the deletion of satellite DNA show that a large region of satellite DNA repeats is indispensable for the correct functioning of the centromere.

It is supposed that centromeric HC might, *de facto*, create a compartment by increasing the local concentration of the centromeric histone variant, CENP-A, and by promoting the incorporation of CENP-A rather than the histone H3 during replication.

V.3 Role of HC in gene repression (epigenetic regulation)

Gene expression may be controlled at two levels:

- Firstly, at the local level which is *transcription control*, thanks to the formation of local transcription complexes. This level involves relatively small DNA sequences linked to individual genes.
- At a more global level, in which case it is the *transcriptability* that is controlled. It involves much larger sequences that represent a large chromatin domain, which can be either in an active or an inactive state. Heterochromatin appears to be involved in controlling the *transcriptability* of the genome. Genes that are usually located in the euchromatin can, therefore, be silenced when they are placed close to a heterochromatic domain.

Mechanism of inactivation in *cis* :

Following a chromosomal rearrangement, a euchromatic region may be juxtaposed with a heterochromatic region. Where the rearrangement removes certain normal barriers that protect the euchromatin, the heterochromatic structure is able to propagate *in cis* to the adjacent euchromatin, thus inactivating the genes contained therein. This mechanism has been observed in position effect variegation (PEV) in *Drosophila* and also in the inactivation of certain transgenes in mouse.

Mechanism of inactivation in *trans*:

During cell differentiation, certain active genes are likely to be transposed into a heterochromatic nuclear domain, thus causing them to become inactive. Such a mechanism has been proposed as an explanation for the co-localisation in lymphocyte nuclei of the protein IKAROS and the genes of which it controls the expression, with centromeric heterochromatin.

VI Heterochromatin diseases

VI.1 Diseases of the constitutive heterochromatin

These diseases are generally the result of an alteration in the process of cell differentiation.

- They may be **constitutional**, as in the case of the ICF syndrome or the Roberts syndromes. The ICF syndrome associates Immunodeficiency, Centromeric instability and Facial anomalies. It is a rare recessive disease that is linked to mutations of the gene DNMT3B, a DNA methyl transferase. The G-C rich satellite DNAs II and III are particularly demethylated, which can cause abnormal segregation of the sister chromatids, formation of multiradial figures, deletions, micronuclei, etc.
- They may be **acquired**: anomalies of the constitutive heterochromatin, involving either the DNA or the heterochromatin proteins, have been found in many types of cancer.
 - In particular, non-Hodgkin's lymphoma and multiple myeloma have been shown to be associated with anomalies of the secondary constriction of chromosome 1, these anomalies being similar to those observed in the ICF syndrome. Indeed, it has been shown that there is a global hypomethylation of the genome, associated, in particular, with a hypomethylation of DNA satellite II.
 - In metastatic breast cancer, it has been shown that there is a decrease in the HP1 alpha protein, which is a protein that is usually localised in the heterochromatic regions of the chromosomes.

VI.2 Diseases of the facultative heterochromatin

- They can result from a defect in the inactivation of an X chromosome in female somatic cells (mutation in the XIST gene) and may lead to the expression of an X-linked recessive disease in females.
- They can result from a defect in the condensation of the sex vesicle in male germ cells, leading to a sterility due to pachytene arrest of the meiosis.

VII Conclusion

In conclusion, although heterochromatin is apparently amorphous and isolated at the periphery of the nucleus, it appears to have an absolutely essential role in the organisation and function of the genome.

Throughout this review we have mainly presented the characteristics linked with heterochromatin, be it *constitutive* or *facultative*. We have

shown that the properties of *constitutive* HC are not fundamentally different from those of *facultative* HC. It therefore seems clear that the mechanisms involved in facultative heterochromatinisation, which are epigenetic mechanisms, are the same mechanisms that intervene in the repression of euchromatin in general.

Contributors : Marie-Geneviève Mattei, Judith Luciani *

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