

## Heterochromatin , from Chromosome to Protein

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### I THE CONCEPT OF HETEROCHROMATIN

#### Definition of Chromatin

In prokaryotes such as *Escherichia coli* there is no detectable heterochromatin, for the simple reason that there is no chromatin. The hereditary message is carried by a circular molecule of naked DNA, and there is no separate nuclear compartment.

In eukaryotes, however, the DNA is packaged in the form of a nucleoprotein complex called "chromatin". The hereditary message is, therefore, carried by the chromatin. It is located in a nucleus and is organised in several separate entities, the chromosomes.

#### The Concept of Heterochromatin

The concept of heterochromatin, as described by Emil HEITZ in 1928, was exclusively based on histological observations. He defined heterochromatin (HC) as being the chromosomal segments which appear extremely condensed and dark in colour in the interphase nucleus. The rest of the nucleus is occupied by euchromatin, or true chromatin, which appears diffuse and relatively light in colour. Heitz's observations highlighted the fact that, in the interphase nucleus, chromatin does not have a homogeneous appearance. Electron microscopy and X-ray diffraction have since confirmed the heterogeneous structure of chromatin: it 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. Euchromatin, in its active form, consists of a fibre with a diameter not exceeding 10-11nm. Its diameter corresponds to that of a nucleosome, which contains a 146 base pair double strand DNA segment, wound around 4 homodimers of the histones H2A, H2B, H3, and H4 .

The inactive euchromatin is enriched in linker histone H1. Histone H1 binds two consecutive nucleosomes, which causes the 10-11nm fibre to wind itself into a solenoid with a 30nm diameter. The 30nm fibre is further organised through interactions with non-histone proteins which fold the chromatin fibre in loops around an imaginary axis. These proteins include topoisomerase II, which is located, in particular, at the base of the loops, the scaffold protein 2 and lamins, in addition to other proteins. At this stage, the diameter of the chromatin fibre attains approximately 200nm. 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 (Table I).

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

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

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

### 1) Richness in satellite DNA

- *Constitutive HC* contains a particular type of DNA called satellite DNA, which consists of large numbers of short tandemly repeated sequences. There are various types of satellite DNA which can be separated by gradient density centrifugation. The best-known type, which is called *alpha-satellite DNA*, is rich in A-T and is located in the centromeric region of the chromosomes. *DNA satellite I*, which is also A-T rich, is located more specifically at the centromeres of chromosomes 3 and 4, the short arms of the acrocentrics, and the long arm of the Y chromosome. The *DNA satellites II* and *III* are both A-T rich, but they are also G-C rich. *DNA satellite II* is primarily located at the secondary constrictions of chromosomes 1 and 16. *Satellite III* is mostly present in the secondary constriction of chromosome 9, the short arms of the acrocentrics and the Y chromosome. The satellite DNA sequences have the distinctive feature of being able to fold on themselves and may have an important role in the formation of the highly compact structure of the constitutive HC.
- *Facultative HC* is not enriched in satellite DNA. It is characterised by the presence of G-bands, which are rich in *LINE*-type repeated sequences. These sequences, dispersed throughout the genome, could promote the propagation of a condensed chromatin structure.

### 2) Stability:

- *Constitutive HC* is stable and conserves its heterochromatic properties during all stages of development and in all tissues. This "heterochromatic" state is

linked to the satellite DNA it contains, and thus to the sequence of that DNA, which is clearly not reversible.

- *Facultative* HC is reversible, that is to say, it can change from the heterochromatic state to a euchromatic state, depending on the stage of development or the cell type examined. In this case, the reversibility of the heterochromatic state clearly demonstrates that the DNA sequence is not involved.

In females, one of the X chromosomes is inactive and has a heterochromatic state (Barr body) in the somatic cells. Before entering meiosis, it is reactivated and both X chromosomes form a normal bivalent, undistinguishable from the autosomal bivalents. The inactive X provides a classic example of facultative HC.

Another example of facultative HC is observed at the pachytene stage of male meiosis. At this stage, the X and Y chromosomes, joined by their telomeres, condense to form the inactive sex vesicle (SV). The heterochromatic state of the sex chromosomes at the pachytene stage of meiosis is transitory, and the SV can therefore be considered as being facultative HC.

### 3) Polymorphism

- *Constitutive* HC is highly polymorphic. This polymorphism can affect not only the size but also the localisation of the heterochromatin, and apparently has no phenotypic effect. Such variations are clearly observed on the secondary constriction of chromosome 9. The frequent polymorphisms that characterise constitutive HC are due to the instability of the satellite DNA.
- The *facultative* HC is not particularly rich in satellite DNA, and is therefore not polymorphic.

### 4) C-band Staining :

- *Constitutive* HC is strongly stained by the C-band technique. This staining could be the result of the very rapid renaturation of the satellite DNA following denaturation.
- *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.

### 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 the intensity of HC staining is directly proportional to its degree of condensation. The strongly condensed nature of heterochromatin has another consequence, in that it renders it inaccessible to DNase 1 and to other restriction enzymes in general.

### 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.

The DNA of the inactive X is very late replicating, as it replicates at the end of the late S phase. The incorporation of 5-Bromodeoxyuridine four hours before harvesting allows this to be clearly seen.

As regards the heterochromatic centromere regions, their replication precedes that of the inactive X and takes place at the beginning of the late S phase. The centromeric DNA must, therefore, replicate sufficiently early to allow the formation, with the centromeric proteins, of a nuclear-protein complex that will be functional during mitosis.

- The late replication of the HC 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.
- The late replication of HC also leads to a less efficient repair of its DNA, in the event of polymerase errors.

### **3) Heterochromatin DNA is methylated**

The DNA of *constitutive* HC is highly methylated, with the methylation occurring exclusively on the cytosines. An anti-5-methyl cytosine antibody therefore strongly labels all the regions of *constitutive* HC, thus showing both its richness in cytosines and their methylation.

As regards *facultative* HC, the methylation of the DNA is more discrete, and cannot be detected using an antibody directed against methylated cytosines. Nevertheless, restriction enzymes that are sensitive to methylation can be used to reveal strong methylation of the CpG islands, which are specifically located in the control regions of the genes.

### **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.

It has long been known that the hypo-acetylation of histone N-terminal tails is a modification that is associated with an inactive chromatin. In contrast, however, hyper-acetylated histones characterise the active chromatin. It is principally the lysines ("K" in single-letter code language) of the histones that are acetylated.

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 Acetyl Transférase** (HAT) in the case of co-activators, or **Histone De-ACétylases** (HDAC) in the case of co-repressors.

### **5) Histones from heterochromatin are methylated on lysine 9:**

This modification of the N-terminal tail of histones has only very recently been discovered and found to be involved in the process of heterochromatinisation of the genome. It characterises both *constitutive* HC and *facultative* HC.

Methylation of the H3 histone occurs on a very specific residue, lysine 9, hence its name, H3-K9. The H3-K9 lysine may be mono-methylated, di-methylated or tri-

methylated, and a high degree of methylation promotes the binding of the HP1 (Heterochromatin Protein 1) proteins.

#### **6) Heterochromatin is transcriptionally inactive:**

Incorporating tritiated uridine into a cell culture does not result in any labelling of the heterochromatin. Human *constitutive* HC does not contain any genes, which explains why transcription does not take place at these sites. In *Drosophila*, on the other hand, certain genes, such as the *rolled* and *light* genes, are usually located in and expressed from the *constitutive* HC. The *facultative* HC is relatively poor in genes, and its genes are not usually transcribed in a heterochromatic context.

#### **7) Heterochromatin does not participate in genetic recombination:**

It is generally accepted that *constitutive* HC does not participate in genetic recombination. The reason for this is that there is no preliminary pairing of the homologous heterochromatic regions, even though some aggregation of these regions is often observed. In any case, the polymorphism that characterises the heterochromatic regions would render such pairing difficult, if not impossible. Moreover, this is the reason why the pericentric inversion of the secondary constriction of chromosome 9 has no effect in terms of chromosome mechanics and can be considered as a normal variant.

Not only does the *constitutive* HC not participate in recombination, it 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.

#### **8) Heterochromatin has a gregarious instinct:**

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

Thus, in the interphase nuclei of the salivary glands of *Drosophila* larvae, the centromeres of polytene chromosomes, which are rich in heterochromatin, aggregate to form the chromocentres.

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. The relationship between the size and the number of HC blocks in interphase suggests a coalescence of the heterochromatic regions.

In the human, the short arms of the acrocentric chromosomes, which are mainly formed from heterochromatin, carry the nucleolar organiser regions. They are frequently associated in the interphase nucleus, and this does not appear to result solely from their common function. Indeed, numerous other chromosomes are involved in this association, and the participation of each of them is all the more marked because it carries a large HC block, as is the case for chromosomes 1, 9 or 16.

This tendency of the heterochromatin to aggregate appears to be strongly linked to the presence of satellite DNA sequences, but this is a property which may not be exclusive and may involve other additional sequences.

#### **IV FACTEURS INVOLVED in HETEROCHROMATINISATION**

There are probably various diverse ways of organising a genomic region into heterochromatin. Certain observations have, however, led to the identification of various elements that have an important role in the formation of heterochromatin, be it *constitutive* or *facultative*.

##### **1) Large arrays of tandemly repeated sequences.**

The fact that in the human genome, as well as in the genome of other organisms, the localisation of the satellite DNA visualised by FISH corresponds exactly to that of the *constitutive* heterochromatin stained by DAPI, highlights the potential role of satellite DNA in the formation of this type of heterochromatin. Indeed, this type of DNA sequence 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 DNA.

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. The presence of repetitive DNA, such as satellite DNA, appears to simply allow the chromatin to be compacted to a greater extent, as is the case for *constitutive* heterochromatin. The mechanism would appear to be as follows: the large arrays of tandemly repeated DNA sequences appear to be able to pair, thus forming characteristic structures. These structures would then appear to be recognised by specific proteins, such as the HP1 proteins, which in turn direct the formation of a higher-order chromatin.

##### **2) Methylation of DNA**

Although the presence of tandemly repeated sequences is important, it is not the only factor, as large repetitions of transgenes do not all lead to a transcriptional inactivation of the transgene. Most often, the *silencing* induced by the tandem repeats appears to be linked to the presence of prokaryotic DNA sequences that are rich in CpG and, therefore, likely to be methylated (for example, the *lacZ* gene). The base composition of the tandem repeat and, in particular, its ability to be methylated could therefore play an important role in the formation of heterochromatin.

Interestingly, it has recently been shown that there is a direct relationship between the *methylation* of DNA and the *de-acetylation* of histones, both of which characterise heterochromatic structures. The methyl binding protein MeCP2, which normally binds to DNA containing 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 heterochromatization.

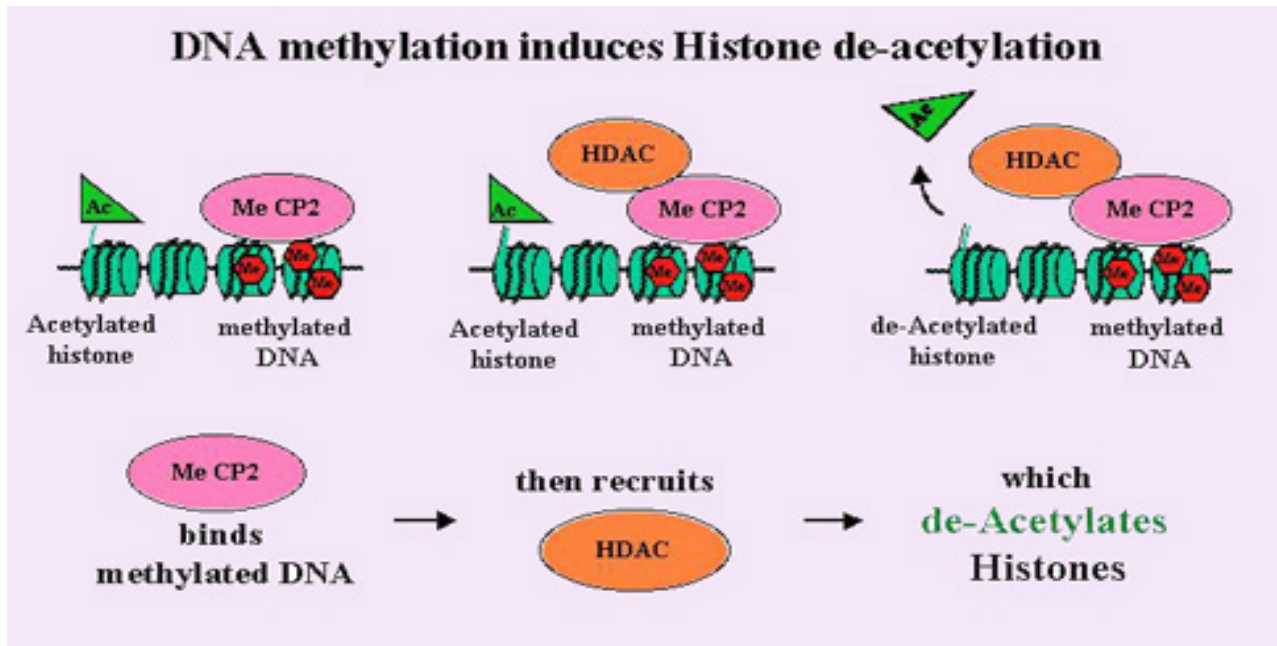


Figure 1: DNA methylation induces Histone de-acetylation, modification which characterizes histones in both heterochromatin and repressed euchromatin.

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).

However, the methylation of DNA is not indispensable for the formation of heterochromatin. It could be an element involved in stabilisation, as has been shown for the facultative HC of the inactive X. Indeed, in marsupials, the inactive X is not methylated and is much less stable than in eutherian mammals.

### 3) Regular organisation of nucleosomes

We have seen that sequences of prokaryotic DNA inserted into eukaryotes have the ability to be methylated and can induce the formation of heterochromatin. Heterochromatinisation may not, however, depend solely on the methylation of the CpGs contained in the DNA.

A study of the chromatin of different transgenes digested by a micrococcal nuclease provided interesting results. The chromatin of a transgene inserted into *constitutive* HC revealed a very regular organisation of the nucleosome that was much more regular than the organisation revealed by the same transgene inserted into an euchromatic region. It appears that this regularity of the structure is able either to take on a particular conformation or to be recognised by specific proteins, and, in this way, can promote the formation of heterochromatic structures.

### 4) Hypo-acetylation of Histones

We have seen that hypo-acetylation of histones is a characteristic of silent chromatin, whether it is heterochromatin or not. *In vitro*, the modification of the acetylation of the histones has a direct effect on the stability and compaction of the nucleosomes. Thus, blocking the de-acetylation of the histones by adding trichostatin A induces hyper-acetylation of the histones, which causes a more open chromatin structure.

The mechanism involved is simple: in the N-terminal tail of the histones, the basic amino-acids such as lysine are positively-charged at cellular pH and therefore interact with the negative charges of the DNA phosphates.

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

Hypo-acetylation of the histones is not the only modification of the N-terminal tails of the histones that characterises heterochromatin. Three other modifications have been shown to be more specifically linked to *silencing*: the phosphorylation of serine 10 of histone H3, the acetylation of lysine 12 of histone H4 and the methylation of lysine 9 of histone H3 (H3-K9). We will present the last of these modifications in more detail.

### 5) Methylation of H3-K9

Methylation of the histone H3 on lysine 9 is an epigenetic modification of histones that has recently been shown to be involved in the process of heterochromatinisation. This has been demonstrated not only in *constitutive* HC but also on the inactive X. The enzyme responsible for the methylation of H3-K9 in *constitutive* HC is the histone methyltransferase SUV39H1.

- On lysine 9 of H3, acetylation and methylation appear to be mutually exclusive. In *Drosophila*, therefore, the methyltransferase Suv39h is physically and functionally 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-immunoprecipitation of Suvar39h with HP1 suggests a heterochromatinisation mechanism based on the interaction of these two proteins and lysine 9. Nevertheless, the HP1 protein certainly acts in different ways in the formation of heterochromatin, since it is able to bind to histone 3 and histone H1, even when their N-terminal tails have been removed.
- Lastly, in *Neurospora crassa*, it has recently been shown that methylation of H3-K9 can cause methylation of DNA. The model proposed is as follows: a histone methyltransferase, characterised by a SET domain, such as Suvar39h, would methylate H3-K9 and induce the binding of a specific heterochromatin protein, such as HP1. The HP1 protein would then recruit a DNA methyltransferase (DNMT), which would methylate the DNA, thus stabilising the inactive state of the chromatin (Figure 2).

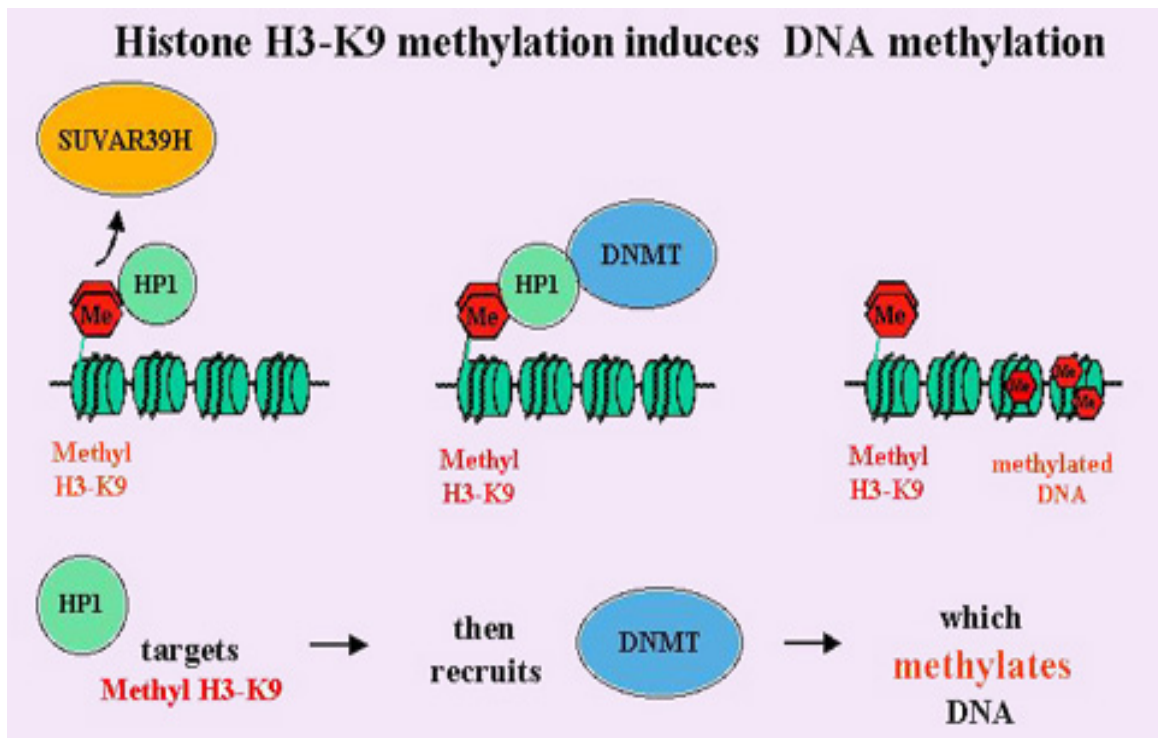


Figure 2: Histone H3-K9 methylation induces DNA methylation, modification which characterizes DNA in heterochromatin and repressive euchromatin.

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 Lysin 9 of Histone H3; HP1=Heterochromatin Protein 1; DNMT=DNA Methyl transferase).

## 6) HP1 proteins

Many different heterochromatin proteins are to be found in mammals, and at the present time little is known about them. The HP1 proteins do appear, however, 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 HP1 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 rearrangement of chromosomes, placed close to a centromeric region that is heterochromatic. This change in the position of the euchromatin has three consequences. The first is that the structure is modified to become much more compact. The second is the association of the newly translocated chromatin with HP1 proteins that are normally confined to centromeres. The third consequence is the repression of the genes contained in the translocated chromatin.

- In mouse, the insertion of a transgene close to the centromere may have similar consequences, with modification of the chromatin structure, the appearance of HP1 proteins and repression of the transgene. 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.

In all cases, HP1 proteins or their homologues appear to be an essential link in the formation of heterochromatin. These heterochromatin-specific proteins could have the role of *chromatin domain organisers*. The HP1 proteins thus appear to be able to recognise particular structures that are created by the pairing and/or the association of repeated DNA sequences. In addition, they appear to be able to establish secondary interactions with a large number of other proteins. The HP1 proteins are perfectly adapted for such interactions, as they have two domains of protein/protein interaction, the chromodomain (CD) and the chromoshadow domain (CSD).

## 7) 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 (**X** inactive **S**pecific **T**ranscripts) gene are nuclear RNAs that have an essential role in the facultative inactivation of one X chromosome, which occurs in the somatic cells in female mammals. This XIST RNA is necessary for the initiation of X inactivation, but not its maintenance. Other nuclear RNAs, such as H19, have been shown to be involved in the regulation of genes that are subject to genomic imprinting.

Some recent studies in mouse have suggested that nuclear transcripts may also be involved in the formation of constitutive HC. In mouse, as in most other species, the centromeric HC is particularly stable. It is characterised by the presence of a high concentration of methylated H3-K9 histone and heterochromatic HP1 proteins, which co-localise in the nuclei with regions strongly stained with DAPI. However, incubation of permeabilised mouse cells with RNase A causes rapid de-localisation of the HP1 and methylated H3-K9 signals, in relation to the heterochromatin foci. These data suggest that a nuclear RNA may be an essential *structural component* of constitutive HC. The RNA may either facilitate compaction of the centromeric HC or may serve as an additional binding site for the proteins that associate with the chromatin.

Surprisingly, the treatment with RNase A does not alter the methylated H3-K9 signals at the level of the Barr body. In fact, on the inactive X chromosome, the methylation of H3-K9 does not appear to lead to the binding of HP1 proteins. This suggests that the facultative heterochromatinisation of the inactive X may require a different mechanism to come into play from that 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.

### 1) Role of HC in the organisation of nuclear domains

- Studies of the organisation of the nucleus have shown that heterochromatin and euchromatin occupy different 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 to the periphery of the nucleus and, in particular, against the nuclear membrane, may be due to the characteristic properties of the protein HP1. This heterochromatin protein interacts specifically with the B lamin receptor, which is an integral component of the inner membrane of the nucleus.
- This organisation also has functional consequences. 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.
- The fact that HC is "gregarious", and that it tends to agglutinate, may give rise to similar functional consequences.

## 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.

- It is generally believed that the presence of centromeric HC is important for centromeric function. Thus, in certain organisms which have large blocks of centromeric HC, it has not been possible to identify a specific DNA sequence that defines the centromere itself. Moreover, in the yeast *Schizosaccharomyces pombe*, the homologue of the HP1 protein Swi6 is absolutely essential for efficient cohesion of sister chromatids during cell division.
- However, 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.
- In an attempt to synthesise all of the above observations, a hypothesis for the function of centromeric HC has been put forward; it may, *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.

## 3) Does centromeric HC act as a transporter?

Many proteins have been shown to be associated with centromeric HC, in particular on metaphase chromosomes. It can be assumed that certain proteins that must be present and functional at the very beginning of the G1 phase will not be able to be synthesised due to a lack of time. Following this hypothesis, binding to the centromere would be an ideal means for such proteins to traverse cell division unhindered, in order to be available at the beginning of G1.

#### 4) 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*. Transcription is directly controlled by the formation of local transcription complexes. This level involves relatively small DNA sequences (100 bp) linked to individual genes.
- Transcription may also be controlled at a more global level, in which case it is the *transcriptability* that is controlled. This level involves much larger sequences that represent a large chromatin domain. Such a domain can have one of two states: an active state, which is sensitive to endonucleases, and an inactive state, which is insensitive.

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 containing active genes 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. It is associated with a modification of the structure of the newly repressed euchromatin that involves the HP1 proteins, characteristic of heterochromatin.
- 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 target genes of which it controls the expression with blocks of centromeric heterochromatin. Thus, target genes could be repressed by a mechanism of inactivation in trans that propagates from the heterochromatin towards the adjacent euchromatin. Another hypothesis is that the protein IKAROS first inactivates the target gene by binding to its promoter and then transposes it into a heterochromatic nuclear domain in order to stabilise the inactivation.

## VI HETEROCHROMATIN DISEASES

### 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 and 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 localised on the long arm of chromosome 20 (Xu et al 1999).

The chromosomal anomalies mimic the anomalies obtained with 5-Azacytidine, which is a demethylating agent. Naturally, it is the satellite DNAs that are rich in G-C that are demethylated, that is to say, DNA satellites II and III, and, to a lesser extent, satellite I. Consequently, it is mainly the secondary constrictions of chromosomes 1 and 16 that present an instability.

Decondensation of the secondary constrictions alters the normal segregation of the sister chromatids, which explains the formation of multiradial figures, deletions, micronuclei, etc.

The centromeric HC that is rich in alpha-satellite DNA, then rich in A-T bases is not affected by this instability.

- They may be *acquired*, then associated with various types of cancer. 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. This observation strongly suggests that an anomaly of methylation could affect the satellite DNA in these acquired pathologies. Indeed, it has been shown that there is a global hypomethylation of the genome, associated, in particular, with a hypomethylation of DNA satellite II. The hypomethylation is generally correlated with a worsening of the phenotype. It may be that the tumour and oncogenesis progression are linked to an imbalance of genes that results from rearrangements involving the long arm of chromosome 1 or chromosome 16.
  - 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.

## 2) Diseases of the facultative heterochromatin

- They can result from a defect in the inactivation of an X chromosome in female somatic cells. Such a defect may, in particular, result from a mutation in the XIST gene that is essential for initiating the process of inactivation on the X chromosome. It 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 an hypofertility or 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, despite the presence of satellite DNA, 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.

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