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Reviews

The role of histones and their modifications in the informative content of chromatin

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Abstract. It is traditionally accepted that the DNA sequence cannot by itself explain all the mechanisms necessary for the development of living beings, especially in eukaryotes. Indeed part of the information used in these processes is stored in other ways, generally called 'epigenetic', whose molecular mechanisms are mostly unknown. The ultimate explanation for them might reside in the non-DNA moiety of chromatin which may play an active role in heredity ('chromatin information'). Histones are the universal structural component of chromatin. However, recent studies strongly suggest that histones, and their modifications – especially the reversible acetylation of lysines – may act as a recognition signal for regulatory proteins and they may participate, for this reason, in gene regulation. This type of information could be maintained through its replication and, ultimately, it could form the molecular basis of certain processes related to the development of the eukaryotic organisms.
Key words. Chromatin; epigenetic mechanism; histone acetylation; imprinting; nucleosome.

Introduction

Most of the known mechanisms which govern the fundamental transactions in molecular biology: replication, transcription, recombination, transposition, etc., are governed by protein-DNA interactions. Because proteins are coded by the DNA sequence, it can be said that all the information necessary for the living cell comes ultimately from the DNA sequence. However, it is widely accepted that the DNA sequence itself cannot account for all the molecular mechanisms required for the development of living beings, and than part of the information used in these processes is stored in some form other than as classical genetic information. This other information has been called 'epigenetic', and most of the mechanisms it uses are unknown to date.

The situation is more complex in eukaryotes because processes such as differentiation, genome compartmentalization, sexual reproduction, etc., use epigenetic information to a larger extent than in prokaryotes. Perhaps the key to understanding the differences between prokaryotes and eukaryotes in the use of epigenetic mechanisms is that in eukaryotes the genetic material is packed in a complex structure, chromatin, in which DNA is intimately associated with a series of proteins whose main recognized function is to accompany it from one generation to another.

The non-DNA moiety of chromatin has traditionally been considered as a structural component that lacks other more active functions, and that takes part only indirectly in the process of gene regulation. However, chromatin is not uniformly structured; it presents regions with very different levels of packing. Moreover, this packing may change to other more or less compact structures which may facilitate, impede or regulate the access of other proteins necessary for the processes mentioned above[53]. The current point of view thus assigns a passive role to the chromatin proteins.

It is, on the contrary, very improbable that all the eukaryotes have solved all these structural or passive functions in the same way, with the same chromatin organization and an invariable structural unit, the nucleosome. The high degree of conservation of the nucleosomal structure from yeast to mammals is probably an indication of the central role of this structure in the life of the eukaryotes. It is most probable that in eukaryotes chromatin organization carries out more functions than those usually recognized.

Most of the protein mass in chromatin consists of histones which, by means of the nucleosomal structure, are the main organizers of chromatin. Because histones are the major protein constituents of chromatin they must play a central role in the epigenetic mechanisms associated with chromatin. These small proteins, present in most eukaryotes, have a highly conserved amino acid sequence. For example, calf histone H4 contains 102 residues and only differs in one, two and eight amino acids, respectively, from the same histone in sea urchin, pea and yeast[53]. Not all the histones are equally conserved: H4 and H3 are very similar in all the species, H2A and H2B are more variable, and the outer histone H1 shows an evolutionary rate similar to that of other less well-conserved proteins[24].

The strong conservation of the inner histones suggests that they perform other functions than the mere packing of chromatin and that those functions are in-

separably associated with their amino acid sequence and structure.

Histones are modified very specifically in vivo

Surprisingly, in spite of the close relationship between structure and function, histones undergo multiple post-translational modifications, most of them after their incorporation into chromatin. Thus, different amino acid residues can be phosphorylated, acetylated, methylated, poly-ADP-ribosylated or ubiquitinated[53]. Given that histones, as opposed to the minor chromosomal proteins, are identical throughout the chromatin, their modification may represent a good opportunity to store structural information. Histone H1 and H3 phosphorylation only correlates with the process of metaphase chromosome condensation[8]. On the other hand, the acetylation of lysine residues seems to be more suitable for the purpose of storing information, for several reasons: 1) all the organisms studied possess acetylated histones; 2) it is, quantitatively, the most important modification; 3) it decreases the positive net charge of the histone; 4) it is reversible, and 5) it affects the most conserved inner histones of the nucleosomal core. Acetylation is, moreover, extremely specific, since it occurs in particular ε-amino groups of the lysines in the N-terminal region of the four histones. The position of these lysines within the histone sequences is, again, highly conserved in all the eukaryotes.

Histones H4, H3 and H2B can be acetylated in four lysines, whereas histone H2A can be acetylated only in one lysine (fig. 1) (reviewed in refs 11 and 53). Therefore the nucleosomal core can possess between zero and 26 acetyl groups. The number of theoretically different acetylation patterns of a single nucleosome is, thus, over 67 million, much higher than the number of nucleosomes in an eukaryotic cell. This complex nucleosomal acetylation pattern could act as a signal system or code if there

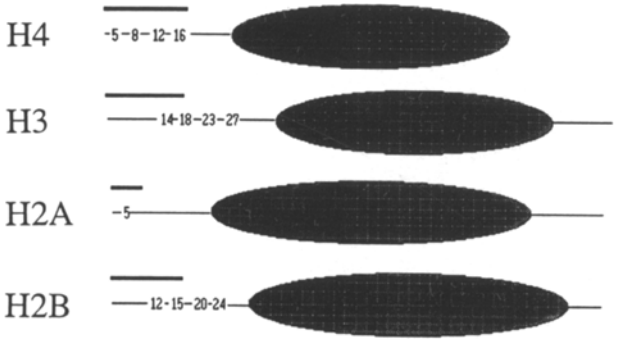

Figure 1. Position of the acetylable lysines within the core histone sequence. The positions are marked by the number of the amino acids. The ellipse represents the globular domain of the histone. The thick bar marks the disordered domain which is unable to adopt a secondary structure.

exist enzymes capable of maintaining some histone molecules, or acetylation sites within the same histone, acetylated, and some others deacetylated.

In vivo there are two enzymatic activities which can alter the acetylation state of the histones: histone acetyltransferases (HAT) and histone deacetylases (HD). Multiple HAT activities have been detected in different organisms. In rat liver[57] or in yeast[33] four HATs, with different substrate specificities, have been found. In yeast, three HAT enzymes are nuclear; these are able to acetylate histones assembled in nucleosomes, and each of them has a preferred histone as substrate. The fourth enzyme is cytoplasmic and only acts on free H4 histone[34]. Nuclear HAT activities specific for individual histones[48], or with a marked preference[18, 27, 28, 50, 57], have also been detected in other eukaryotes.

HD enzymes, less studied than HAT, seem to play a minor role in relation to the specificity of acetylation, since they are frequently able to deacetylate efficiently all the four core histones[3, 47], although there are examples of partial specificity[47, 54].

HATs appear to be complex, highly specific enzymes that are mainly responsible for the acetylation pattern of histones. Their specificity may extend even to the target lysines of a single histone. For instance, an H4 histone-specific HAT from pea acetylates only lysines 5, 12 and 16, without affecting lysine 8[37]. The in vivo determination of modified positions in mono-, di-, tri-, and tetra-acetylated H4 histone in several eukaryotes has revealed a fairly strict order for the acetylation of lysines. In cuttlefish, only lysine 12 is acetylated in the monoacetylated form; the diacetylated form contains acetyl groups in lysines 12 and 5, the triacetylated in lysines 12, 5 and 16 and the tetraacetylated in 12, 5, 16 and 8[10]. The order is not the same in all the species studied, i.e., in *Tetrahymena* the order is 7, 4, 11, 15. In this organism, in the transcriptionally inactive micronucleus the acetylated lysines in the diacetylated form are 4 and 11 and in the active macronucleus 7 and 4[9]. This feature is not exclusive to H4; similar results have been obtained for other histones (see ref. 51). If lysines were acetylated following a rigorous order the enzymatic machinery would be very unsophisticated, and the number of different acetylated forms of nucleosomes drastically reduced. However, the fact that the order of acetylating lysines is different for different organisms or within the same organism (e.g. *Tetrahymena*) leads us to propose that all HAT enzymes, in vivo, have to be very selective with regard to single lysines of single histones.

The selection of acetylation sites could be performed either by a set of HAT isoenzymes with different specificities, or by a reduced number of enzymes which discriminate between the lysines by means of fine tuning exercised by protein factors analogous to those that lead RNA polymerase II to specific promoters.

Histone acetylation can play several roles

Histone acetylation has traditionally been related to the transcriptional activity of chromatin[2]. Different experimental approaches have shown that active chromatin is more acetylated than inactive chromatin (reviewed in ref. 51).

The usually accepted explanation for this relationship is that the reduction in positive charges of the lysines by acetylation provokes a weakening of the DNA-histone interactions and, therefore, a relaxation in chromatin structure, opening it up to the transcriptional apparatus[4] (fig. 2). However, several studies show that histone acetylation or hyperacetylation do not have major effects on nucleosome structure[29]. Moreover, it has recently been shown that the histone N-terminal tails do not contribute to core particle stabilization[5], and that these tails face outwards. Since these unstructured histone tails contain the acetylation sites, we can conclude that they have no major effect on the nucleosome core particle. On the other hand, acetylation seems to destabilize the higher order structures of chromatin, such as the solenoid, and it has been shown that histone acetylation is involved in the maintenance of DNA topology, acting as a DNA gyrase (for review see refs 4 and 20). Following this reasoning, Grunstein[21] has proposed that histone acetylation may unfold chromatin domains, producing an inducible state. Only when chromatin is in this inducible state can the activator proteins find their target sequences leading to transcription initiation.

However, it is difficult to think that a phenomenon so specific, selective and ordered as histone acetylation has the single function of disentangling chromatin. Moreover, it is well established that histone acetylation is involved in other processes[11] such as assembly of nucleosomes during DNA replication, the replacement of histones by protamines during spermatogenesis, and DNA repair, and more recently, even the ability to repress or activate several genes has been attributed to it (see below). Loidl[30] has suggested that the acetylation of selected histones could serve as a specific signal for the launching of these processes, although he proposed that histone acetylation causes the removal of acetylated H2A and H2B histones. It has been possible to establish a relationship between the acetylation of a specific histone, or even a specific site within a histone, and a particular function. For instance, only H2A and

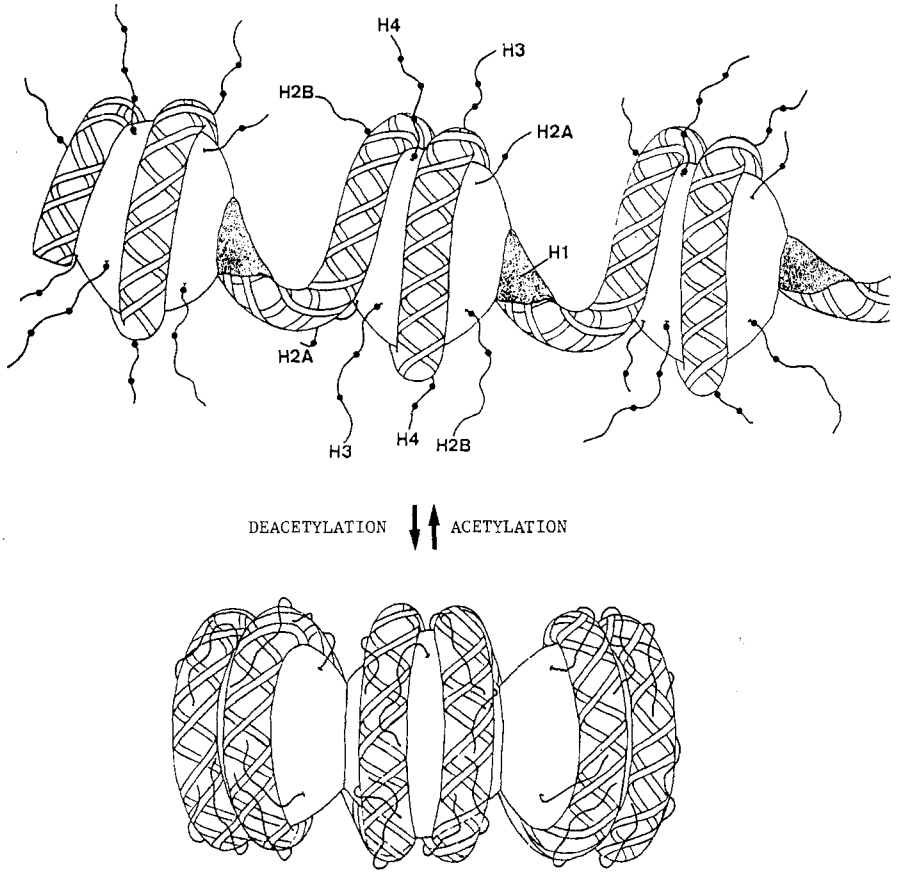

Figure 2. Scheme of the possible role of core histone acetylation/deacetylation in nucleosome packing. Wavy lines represent, on an approximate scale, the N-terminal region of histones unable to adopt secondary structure. Black circles stand for acetylated lysines. Top: the N-terminal ends do not have a positive net charge and could be oriented outwards. Bottom: the N-terminal ends have a positive net charge and would be folded towards the DNA negative charges.

H2B are acetylated during the transcriptionally active G2 period of the *Physarum* cell cycle[31], and the acetylation of lysine 5 is related to histone deposition[42]. More recently, using indirect immunofluorescence with antisera that can distinguish H4 molecules acetylated at each of the four lysine residues, it has been shown that the distribution of the different H4 species in interphase chromosomes from *Drosophila* larvae is not random but that each acetylated species has its own unique distribution pattern. Thus, lysine 16 acetylated H4 is found almost exclusively in the hyperactive X chromosome in male larvae, but not in male autosomes or in female chromosomes[52]. This makes it possible that this specific acetylation was involved in the dosage effect seen for X-linked genes. Therefore, there must be a direct relationship between the acetylation of a specific histone residue, the enzymes responsible for its acetylation, and a specific functional state of chromatin. It has recently been proposed that HD may have a key role for the binding of acidic regulatory proteins to chromatin by means of the selective and local increasing of positive charges within the core histone N-terminal domains[32]. Also, in a less specific manner, histone acetylation has been related to transmissible 'imprinting' effects on gene activity (see below) which means that both structural and informative functions are compatible and that the living organisms have probably been able to exploit the vast possibilities offered by histone acetylation to develop new functions during evolution.

Directed mutations in the yeast H4 histone gene

A new approach to the study of the biological function of histone acetylation has been made by obtaining and analyzing yeast strains with histone H4 mutant alleles[12, 25, 26, 36, 40].

The first of these interesting investigations in Grunstein's lab[26] showed that deletion of the DNA sequence coding for amino acids 4 to 28, which includes the four acetylable lysines, does not affect cell viability but does produce an inability to mate. This result was a setback for the theory of the relationship between histone acetylation and gene regulation. However, subsequent studies of the molecular mechanisms leading to loss of mating ability were very informative.

The mating capacity of two haploid cells, *MATa* and *MATα*, to produce a diploid *MATa/MATα*, which is unable to mate, depends, among other things, on three unlinked DNA sequences: *MAT*(α or *a*), *HMLa* and *HMRα*. The only transcribable locus is *MAT*, whereas *HMLa* and *HMRα* are permanently silent, in spite of the sequences of the *HMLa* and *HMRα* promoters being identical, to those of *MATa* and *MATα*, respectively. If the yeast strain possesses the *MATa* allele, the cell mating type is *a*, which is able to mate with α cells, while if the strain possesses the *MATα* allele, the cell mating type is α, which is able to mate with *a* cells. It is essential for the haploid cell to keep *HMLa* and *HMRα* genes repressed because their transcription would give rise to the presence, in the same cell, of the *a* and α functions, as in the diploid, making it unable to mate. This is, in fact, what occurs with the histone H4 N-terminal mutants mentioned above. They are unable to mate because the *HMLa* and *HMRα* loci are not repressed[21]. The molecular reason proposed to explain this is the incapacity of the Sir3p repressor to interact with the mutated H4 which might weaken the H4-DNA binding in the silent loci[25]. The biological function of the N-terminal tail of H4 has been extensively studied by means of limited deletions and amino acid substitutions. Figure 3A shows a scheme of the most significant results obtained in this field with regard to mating ability. The repression of the silent mating loci requires the presence of amino acids 15–19, given that the 4–14 deletion does not affect mating capacity (mutant 1), whereas the 4–19 deletion (mutant 2) abolishes it drastically. Moreover, the lysines in positions 5, 8 and 12 are irrelevant for repression, since the substitution by either non-charged amino acids (mutants 3 and 4) or the non-acetylable charged Arg (mutant 15) does not significantly reduce the mating capacity.

On the contrary, when Lys 16 is replaced by a neutral amino acid, such as Ala, Gly or Gln (mutants 6, 7 and 8), the mating ability is lost. Surprisingly, the change of Lys 16 for Arg, simulating the non-acetylated form of lysine (mutant 9), has no important consequences. Therefore the presence of a positive charge on Lys 16 is indispensable to repress the mating-type cassette. When amino acids 17, 18 and 19 are substituted by positively charged or neutral amino acids similar effects are produced, but in these cases directed mutagenesis is not so informative about the in vivo function of these amino acids because they cannot be charged and discharged by HAT and HD like the ε-amino group of Lys. However, Lys 16 of H4 in the nucleosome placed over the silent cassette is never acetylated to keep the loci repressed. This means that the deacetylated state of a particular amino acid of a particular nucleosome carries out an informative function as a signal for permanent repression.

Using the same approach, Durrin et al.[12] have shown that the N-terminal tail of H4 also participates in vivo in the activation of several inducible genes in yeast (fig. 3B). Thus, the deletion of amino acids 4–23 (mutant 10) reduces the activation of *GAL1* and *PHO5* genes by a factor of 20 and 5 respectively. In this case artificial 'deacetylation', by means of the substitution of Arg for each of the four acetylable Lys (mutants 11, 12, 13 and 14), does not produce inhibition of the activation. However, when the change simultaneously affects three or four Lys (mutants 15 and 16) a significant

A)

MUTANT	SEQUENCE	% MATING
wt	Ser Gly Arg Gly ***Lys*** Gly Gly ***Lys*** Gly Leu Gly ***Lys*** Gly Gly Ala ***Lys*** Arg His Arg Lys --	100
1	[····· Δ4-14 ·····] --	80 and 100
2	[····· Δ4-19 ·····] --	0.001 and 0.01
3	Ala Ala Ala --	20 and 40
4	Gly Gly Gly --	50
5	Arg Arg Arg --	100
6	Ala --	0.04 and 96
7	Gly --	<0.01
8	Gln --	<0.01
9	Arg --	50

B)

MUTANT	SEQUENCE	% ACTIVITY *GAL1*	% ACTIVITY *PHO5*
wt	Ser Gly Arg Gly ***Lys*** Gly Gly ***Lys*** Gly Leu Gly ***Lys*** Gly Gly Ala ***Lys*** Arg His Arg Lys --	100	100
10	[····· Δ4-28 ·····]--	5	21
11	Arg --	156	139
12	Arg --	138	124
13	Arg --	87	125
14	Arg --	131	126
15	Arg Arg Arg --	26	89
16	Arg Arg Arg --	19	48
17	Arg Arg Arg Arg --	2	1

Figure 3. Effect of the amino acid changes in H4 histone in the activity of several genes.
A Effect on the mating capacity of yeast cells. The sequence of the N-terminal part of the histone in the wild type (wt) is shown at the top. The acetylable lysines are indicated in italics and boldface. Only the changed residues are marked in the mutants. The right column contains the percentage of mating ability with regard to the wild type (100%). Two different amounts refer to *a* and α mating types respectively.
B Similar to part *A*, except that the right column contains the percentage of mRNA production of the *GAL1* and *PHO5* genes with regard to the wild type strain (100%).
Mutants 1, 2, 3 and 6 from ref. 40; 4, 5, 7, 8 from ref. 25; 9 from ref. 36; 10 to 17 from ref. 12.

reduction in the activation is obtained. This result seems to indicate that several Lys need to be acetylated for the activation to be produced. It is necessary to point out that the substitution of all four Lys by Arg (mutant 17) appears to produce more general alterations, because those mutants show a longer doubling time than the wild type and, moreover, not only the inducible genes *GAL1* and *PHO5* but also the constitutive *PRC1* are affected. This result is not obtained with the rest of the mutants. On the other hand, activation is also partially inhibited by the substitution of the four Lys by Gln, which mimics the extreme situation of the four lysines being acetylated. Unfortunately, however, the substitution of individual Lys by Gln or Gly, which could help to clarify the individual contribution of each Lys to the activation of these inducible genes, has not yet been done.

All these results lead to several important reflections.

1) Mutations on a typically structural protein of chromatin, histone H4, do not lead to phenotypes with chromatin structural abnormalities with the expression of many genes affected, but to the repression or loss of the activation capability of certain loci. These effects are specific, given that deletions in N-terminal tails of histones H3, H2A and H2B do not affect those processes[12].

2) It has been established that *trans* repressor (Sir3p)[25] or activator (Pho2p)[17] factors interact with the nucleoprotein complex of chromatin and not with DNA sequences – and the complexes formed are probably heritable as suggested by the results on the *HML* locus discussed in the last section of this article.

3) The acetylated or deacetylated state of certain lysines is essential, at least in some cases, for the interaction with the *trans* factor.

It is important to point out that the directed mutagenesis results discussed above are apparently specific for histone H4, since similar recent experiments of Mann and Grunstein[35] on histone H3 gave completely different results. In this histone, the deletion of amino acids 4–40 results in a minor effect on the mating ability but leads to the hyperactivation of Ga14p-regulated genes (*GAL1*, *GAL2*, *GAL7*, and *GAL10*). The substitution of the four potentially acetylable Lys by Arg or neutral amino acids leads to the same hyperactivating effect.

The *PRC1* gene, however, is not affected by these substitutions. All these results mean that the function of histones H3 and H4 is different and that the events observed in H3 mutants cannot be attributed to the acetylation state of lysines.

Histones may act, then, as regulators of genetic expression. Selective acetylation of certain lysines, controlled by HAT and HD, may be one of the ways to accomplish this. How can the information contained in the pattern of lysine acetylation be propagated through successive replications? In eukaryotes it is not only the DNA that is replicated but the whole chromatin structure must be replicated as well. Although this is still a matter of controversy[16,22], it has been recently proposed that in a well-characterized in vitro replication system the replication of chromatin is carried out without the complete dissociation of histones[7]. At any rate, it is currently accepted that parental histones are immediately incorporated in one or both daughter strands[7,22,53,56]. Parental histones – especially H3–H4 tetramers[56] – and their acetylation pattern, are directly involved in the reconstruction of chromatin structures on daughter cells, which can maintain cellular functions dictated and acquired by these modifications. Could there be a HAT enzyme which, during replication, modifies the newly added histone octamers using the old ones as a template? This seems to be highly speculative, but this kind of HAT-replicase would explain how the acetylation state of some of the lysines is finely tuned, as is the case of Lys 16 of the histone H4 in the promoters of the silent cassettes. In fact a similar process is already known: the pattern of DNA methylation in prokaryotes is maintained through successive generations by the selective methylation of hemimethylated DNA. This example of epigenetic information preservation has at least two biological functions: to identify the maternal strand for the repair of mispaired bases (*dam* methylase) and to protect the cell against alien DNAs (restriction-modification systems).

Another example of conservation of the epigenetic information is the perpetuation of the genomic imprinting that we describe below. This implies a chromatin replication, including the preservation of the sexual-specific methylation pattern of the alleles and, perhaps, of other specific patterns, such as histone acetylation, that can take part in the molecular mechanism of cell memory.

Do histones play any role in cell memory?

The involvement of chromatin structure in the preservation of a specific gene expression pattern is widely accepted. It has been shown that during the cellular differentiation of higher eukaryotes, chromatin structure plays an important role in a faithful transmission of the gene activity pattern in each cellular line. There are several observations indicating that changes in the location of a given gene lead to changes in its expression as a consequence of a variation in its chromatin structure. The activity level of a translocated gene is dictated by the surrounding chromatin structure, and this structure is propagated through successive cell generations, maintaining the identity of the cell lineage[13,14,23,41,46,49]. One example is the observation in several metazoans that chromosomal rearrangements can cause a gene to be translocated to a new position close to heterochromatin regions – the chromatin which is condensed even in interphase nuclei – and this sometimes produces variegated phenotypes. This effect is due to the variegated expression of that gene within the cell population. This phenomenon has been called 'position-effect variegation' (PEV) (reviewed in 13, 23, 49). The random repression of the translocated gene is decided in the first cellular divisions in the embryo, and it is stably inherited in subsequent divisions. This event does not occur exclusively in metazoans. In yeast cells, the mating ability of certain mutants of the HML locus[43], and the expression of telomere proximal genes[19], can persist clonally within a genetically identical population, suggesting that *trans*-acting regulators may be responsible for this epigenetic phenomenon.

Gene repression in PEV seems to be caused by transcriptionally active euchromatin regions being packaged into inactive heterochromatin structures (fig. 4A). In some unknown way the inhibitory effect of the heterochromatin, with regard to the transcriptional activity, is extended to the neighboring translocated euchromatin[55]. The new inactive state of the translocated gene or genes is maintained through successive cellular divisions, and thus constitutes a heritable form of gene inactivation.

If the inactive state of the genes caused by chromatin structural alterations is heritable, we can imagine that active genes will be transmitted to the daughter cells with a defined chromatin structure – depending on their position in the chromosome, or even on which chromosome they are in – which is able to maintain the active condition required for transcription. In both cases the active or inactive state should be 'imprinted' in the chromatin structure (fig. 4). For this reason, this imprinting should be stably maintained, suggesting that the most general structural components of chromatin, the histones, should have an important role in it, perhaps through their interaction with other, more specific, proteins such as the products of *Drosophila Suvar(3)7* and *Su(var)205* genes[13,15,44,45]. In support of this idea, there are several examples in which the histones or even their modifications may affect the extent of PEV. Moore et al.[38] found that a reduced dosage of histone genes suppresses PEV. The degree of histone acetylation is strongly correlated with PEV. Factors that increase acetylation suppress PEV. For instance, it has been

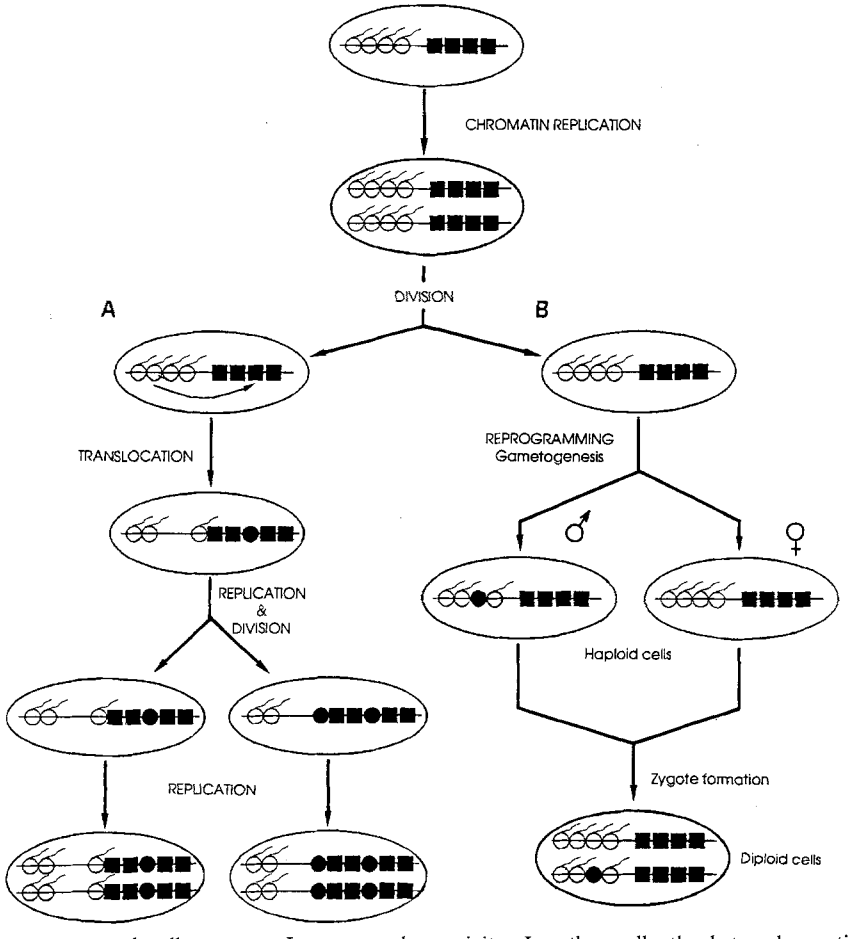

Figure 4. Chromatin structure and cell memory. In a general model (top part of the figure) the transcriptional state of the genes has to be imprinted in their chromatin structure. Active, or potentially active, genes (open circles) are placed in euchromatin regions (left in the cell) and inactive genes (filled squares) are placed in heterochromatin regions (right in the cell). The chromatin structure is replicated (copied) to obtain two daughter cells with identical patterns of gene expression (cell memory).
A The PEV phenomenon demonstrates that chromatin structure plays a role in cell memory. When a chromosomal rearrangement places an active euchromatic gene close to the heterochromatic regions during the first divisions of development, the active or inactive state of the gene is decided. In some cells the gene maintains its euchromatic structure, and thus its transcriptional activity. In other cells the heterochromatin region extends to it and the gene is permanently inactivated (filled circles). The chromatin structure is then replicated, generating a variegated phenotype. This model predicts that if a gene is translocated to a profound heterochromatin region it will be inactivated in all cases. *B* During the maturation of germinal cells, the imprinting of the whole genome is reprogrammed, mainly through changes in the DNA methylation pattern, to produce sex-specific imprinting in the haploid gametes. When the zygote is formed, some genes conserve the imprinting of their original parental gamete, 'genomic imprinting', through successive cell generations. In every chromatin replication the imprinting is copied. The cases of selective inactivation of whole chromosomes in insects and mammals may respond to a similar process.

shown that butyrate, an inhibitor of animal HD, suppresses PEV[39]. Mutants of *Drosophilia Suvar(2)1*, *Suvar(2)10* and *Suvar(3)3* loci are sensitive to butyrate, and the level of histone H4 acetylation is significantly reduced in *Suvar(2)10* mutants (reviewed in 45). Because histone deacetylation seems to be required for chromatin condensation (see for instance ref. 4) the hyperacetylated state of histones may cause the defects in heterochromatinization related to PEV suppression.
It is reasonable to think that the signal code stored in the protein moiety of chromatin has been subjected to the same selective pressures as the DNA sequence. In this case, this information must be transmitted not only during the replication of somatic cells but also during sexual reproduction through the gametes. Male gametes lose most of their original chromatin proteins during spermatogenesis, and these are replaced by other proteins. In female gametes, however, the chromatin organization is preserved; this fact could explain the sexual transmission of this kind of information.
In contrast to what normally occurs in somatic cells from higher eukaryotes, or to unicellular organisms, the pattern of gene expression is reprogrammed during gamete maturation. This is a consequence of the particular function of those cells, which will give rise to all the types of cells in the new organism. The reprogramming is different for male and female gametes and, strikingly, the difference persists even after successive divisions of the zygote. This seems to be a new type of imprinting, in which the two alleles of a gene are differently expressed,

in spite of being placed in identical chromosomal positions, and retain the original imprinting of their parental gametes (fig. 4B) (for a review see ref. 49).

The molecular bases for both types of imprinting, positional and genomic, and their mode of inheritance, are poorly known. Alberts and Sternglaz[1] have proposed that some proteins of the heterochromatin remain bound to DNA during replication and are inherited by both daughter cells. Later those proteins might cooperatively initiate the reassembly of heterochromatin on each new chromosome. Other models to explain cell memory have been proposed: different microenvironments for different regions of chromatin, DNA methylation, and temporal separation of replication[46]. In all these cases, differences in some components apart from the DNA sequence are involved. The system of chromatin heredity may be, at least in part, responsible for the cell memory of the gene state and may explain that the change in the chromosomal situation of a gene may influence its expression.

In the particular case of genomic imprinting, cytosine methylation is the most probable cause[49]. However, it cannot be discounted that other mechanisms may also take part in genomic imprinting. For instance, several mutations of the *Suvar(2)1* locus, which affect H4 histone deacetylation, display imprinting-like consequences indicating that the acetylation of the histones might also play a role in paternal effects[45]. It thus seems reasonable to suggest that the inheritance of chromatin structure mentioned above may contribute, either directly or mediated by the pattern of DNA methylation, to both types of imprinting.

Because imprinting is probably maintained and modified in all cases by specific proteins and enzymes it is, ultimately, controlled by genetic rules. However, as a preliminary approach, we can speak about an 'imprinting code' which controls the phenotype over genetic and environmental influences. It is, then, epigenetic and subjected to potential mutations. Probably histones, and their modifications – especially the acetylation of lysines – play a major role in that code.

Conclusions

We have seen that chromatin contains, besides the DNA sequence, a complex scheme of signals which are able to activate or inactivate regions of the genome or even particular genes. Histone acetylation seems to play a central role in all these cases. In fact even the tertiary structure of DNA in chromatin contains information, because the precise positioning of the DNA double helix on the surface of the histone octamer is itself a system to permit or prevent the access of regulatory proteins to specific DNA sequences. This has been called 'conformational information'[6]. Also, the binding of regulatory proteins – regulatory information – is

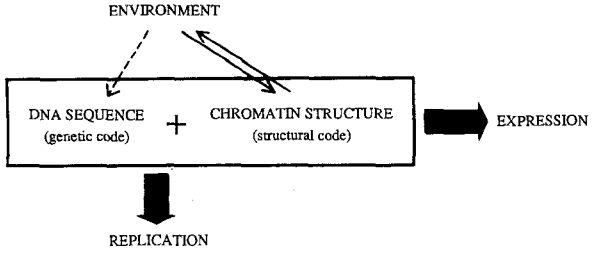

Figure 5. Two components of the chromatin information. The perpetuation of the gene pattern expression in eukaryotic cells requires two informational components, the DNA sequence and the chromatin structure, in which that sequence is contained. Both components are important because whichever is altered, the phenotype is changed. Just as there is a genetic code for the DNA sequence, a structural code (histone modifications, specific proteins, DNA methylation,...) must exist. This structural information is wider and more flexible and interacts continuously with the environment, while the DNA sequence can only receive influences from the environment in the form of aleatory mutations. Finally, given that daughter cells usually have the same phenotypic requirements as the mother cell, the structural information has to be copied during DNA replication.

transmissible. We might consider that all these components are parts of a 'chromatin information' which is transmitted simultaneously with the DNA sequence to the daughter cells (see fig. 5). This kind of information, which continuously interacts with the environment, is more flexible and can enable the cells to adapt to new environmental conditions. In the final analysis, it could support the molecular bases of certain eukaryotic processes such as differentiation or sexual reproduction. Obviously, a system like this must have its transmission guaranteed through generation after generation of cells and, in this sense, it is necessary to remember than, in eukaryotes, DNA replication is accompanied by an accurate duplication of the rest of the components and of their precise locations. During this process, the code of signals stored in chromatin, the 'chromatin information', is copied.

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1 Alberts, B., and Sternglanz, R., Chromatin contract to silence. Nature *334* (1990) 193–194.
2 Allfrey, V. G., Faulkner, R., and Mirsky, A. E., Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc. natl Acad. Sci. USA 51* (1964) 786–794.
3 Alonso, W. R., and Nelson, D. A., A novel yeast histone deacetylase: partial characterization and development of an activity assay. Biochim. biophys. Acta *866* (1986) 161–169.
4 Ausió, J., Structure and dynamics of transcriptionally active chromatin. J. Cell Sci. *102* (1992) 1–5.
5 Ausió, J., Dong, F., and van Holde, K. E., Use of selectively trypsinized nucleosome core particle to analyze the role of the histone "tails" in the stabilization of the nucleosome. J. molec. Biol. *206* (1989) 451–463.
6 Beato, M., Transcriptional control by nuclear receptors. FASEB J. *5* (1991) 2044–2051.

7 Bonne-Andrea, C., Wong, M. L., and Alberts, B. M., *In vitro* replication through nucleosomes without histone displacement. Nature *343* (1990) 719–726.

8 Bradbury, E. M., Reversible histone modifications and the chromosome cell cycle. Bioessays *14* (1992) 9–16.

9 Chicoine, L. G., Schulman, I. G., Richman, R., Cook, R. G., and Allis, C. D., Nonrandom utilization of acetylation sites in histones isolated from *Tetrahymena*. Evidence for functionally distinct H4 acetylation sites. J. biol. Chem. *261* (1986) 1071–1076.

10 Couppez, M., Martin-Ponthieu, A., and Sautière, P., Histone H4 from cuttlefish testis is sequentially acetylated. Comparison with acetylation of calf thymus histone H4, J. biol. Chem. *262* (1987) 2854–2860.

11 Csordas, A., On the biological role of histone acetylation. Biochem. J. *265* (1990) 23–38.

12 Durrin, L. K., Mann, R. K., Kayne, P. S., and Grunstein, M., Yeast histone H4 N-terminal sequence is required for promoter activation *in vivo*. Cell *65* (1991) 1023–1031.

13 Eissenberg, J. C., Position effect veriegation in *Drosphilia*: towards a genetics of chromatin assembly. Bioessays *11* (1989) 14–17.

14 Eissenberg, J. C., and Elgin, S. C. R., Boundary functions in the control of gene expression. Trends Genet. *7* (1991) 335–340.

15 Eissenberg, J. C., James, T. C., Foster-Hartnett. D. M., Hartnett, T., Ngan, V., and Elgin, S. C. R., Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effect variegation in *Drosophilia melanogaster*. Proc. natl Acad. Sci. USA *87* (1990) 9923–9927.

16 Fairman, M. P., Nucleosome segregation-divided opinions. Bioessays *12* (1990) 237–239.

17 Fascher, K.-D., Schnitz, J., and Hörz, W., Role of trans-activating proteins in the generation of active chromatin at the *PHO5* promoter in *S. cerevsiae*. EMBO J. *9* (1990) 2523–2528.

18 Garcea, R. L., and Alberts, B. M., Comparative studies of histone acetylation in nucleosomes, nuclei, and intact cells. Evidence for special factors which modify acetylase action. J. biol. Chem. *255* (1980) 11454–11463.

19 Gottschling, D. E., Aparicio, O. M., Billington, B. L., and Zakian, V. A., Position effect at *S. cerevisiae* telomers: reversible repression of pol II transcription. Cell *63* (1990) 751–762.

20 Grunstein, M., Histone function in transcription. A. Rev. Cell Biol. *6* (1990) 643–678.

21 Grunstein, M., Nucleosomes: regulators of transcription. Trends Genet. *6* (1990) 395–400.

22 Gruss, C., and Sogo, J. M., Chromatin replication. Bioessays *14* (1992) 1–8.

23 Henikoff, S., Position-effect variegation after 60 years. Trends Genet. *6* (1990) 422–426.

24 Isenberg, I., Histones. A. Rev. Biochem. *48* (1979) 159–191.

25 Johnson, L. M., Kayne, P. S., Kahn, E. S., and Grunstein, M., Genetic evidence for an interaction between SIR3 and histone H4 in the repression of the silent mating loci in *Saccharomyces cerevisiae*. Proc. natl Acad. Sci. USA *87* (1990) 6286–6290.

26 Kayne, P. S., Kim, U.-J., Han, M., Muller, L. R., Yoshizaki, F., and Grunstein M., Extremely conserved histone H4 N-terminus is dispensable for growth but essential for repressing the silent mating loci in yeast. Cell *55* (1988) 27–39.

27 Kelner, D. N., and McCarty, K. S., Porcine liver nuclear histone acetyltransferase. Partial purification and basic properties. J. biol. Chem. *259* (1984) 3413–3419.

28 Libby, P. R., Rat liver nuclear N-acetyltransferases: separation of two enzymes with both histone and spermidine acetyltransferase activity. Archs Biochem. Biophys. *203* (1980) 384–389.

29 Libertini, L. J., Ausió, J., van Holde, K. E., and Small, E. W., Histone hyperacetylation. Its effects on nucleosome core particle transitions. Biophys. J. *53* (1988) 477–487.

30 Loidl, P., Towards an understanding of the biological function of histone acetylation. FEBS lett. *227* (1988) 91–95.

31 Loidl, P., and Gröbner, P., Postsynthetic acetylation of histones during the cell cycle: a general function for the displacement of histones during chromatin rearrangements. Nucleic Acids Res. *15* (1987) 8351–8366.

32 López-Rodas, G., Brosch, G., Georgieva, E. I., Sendra, R., Franco, L., and Loidl, P., Histone deacetylase. A key enzyme for the binding of regulatory proteins to chromatin. FEBS lett. *317* (1993) 175–180.

33 López-Rodas, G., Tordera, V., Sánchez del Pino, M. M., and Franco, L., Yeast contains multiple forms of histone acetyltransferase. J. biol. Chem. *264* (1989) 19028–19033.

34 López-Rodas, G., Tordera, V., Sánchez del Pino, M. M., and Franco, L., Subcellular localization and nucleosome specificity of yeast histone acetyltransferase. Biochemistry *30* (1991) 3728–3732.

35 Mann, R. K., and Grunstein, M., Histone H3 N-terminal mutations allow hyperactivation of the yeast *GAL1* gene *in vivo*. EMBO J. *11* (1992) 3297–3306.

36 Megee, P. C., Morgan, B. A., Mittman, B. A., and Smith, M. M., Genetic analysis of histone H4: essential role of lysines subject to reversible acetylation. Science *247* (1990) 841–845.

37 Mingarro, I., Sendra, R., Salvador, M. L., and Franco, L., Site-specificity of pea histone acetyltransferase B *in vitro*. J. biol. Chem. *268* (1993) 13248–13252.

38 Moore, G. D., Sinclair, D. A., and Grigliatti, T. A., Histone gene multiplicity and position effect variegation in *Drosophilia melanogaster*. Genetics *105* (1983) 327–344.

39 Mottus, R., Reeves, R., and Grigliatti, T. A., Butyrate suppression of position-effect variegation in *Drosphila melanogaster*. Molec gen. Genet. *178* (1980) 465–469.

40 Park, E.-C., and Szostak, J. W., Point mutations in the yeast histone H4 gene prevent silencing of the silent mating type locus *HML*. Molec. cell. Biol. *10* (1990) 4932–4934.

41 Paro, R., Imprinting a determined state into the chromatin of *Drosophilia*. Trends Genet. *6* (1990) 416–421.

42 Pesis, K. H., and Matthews, H. R., Histone acetylation in replication and transcription: turnover at specific acetylation sites in histone H4 from *Physarum polycephalum*. Arch. Biochem. Biophys. *251* (1986) 665–673.

43 Pillus, L., Any which way but loose – determining a transcription state in yeast. Bioessays *13* (1991) 303–304.

44 Reuter, G., Giarre, M., Farah, J., Gausz, J., Spierer, A., and Spierer, P., Dependence of position-effect variegation in *Drosophilia* on a dose of a gene encoding an unusual zinc-finger protein. Nature *344* (1990) 219–223.

45 Reuter, G., and Spierer, P., Position effect variegation and chromatin proteins. Bioessays *14* (1992) 605–612.

46 Riggs, A. D., and Pfeifer, G. P., X-Chromosome inactivation and cell memory. Trends Genet. *8* (1992) 169–174.

47 Sendra, R., Rodrigo, I., Salvador, M. L., and Franco, L., Characterization of pea histone deacetylases. Plant molec. Biol. *11* (1988) 857–866.

48 Sendra, R., Salvador, M. L., López-Rodas, G., Tordera, V., and Franco, L., A plant histone acetyltransferase specific for H3 in nucleosomes. Plant Sci. *46* (1986) 189–194.

49 Solter, D., Differential imprinting and expression of maternal and paternal genomes. A. Rev. Genet. *22* (1988) 127–146.

50 Sures, I., and Gallwitz, D., Histone-specific acetyltransferases from calf thymus. Isolation, properties and substrate specificity of three different enzymes. Biochemistry *19* (1980) 943–951.

51 Turner, B. M., Histone acetylation and control of gene expression. J. Cell Sci. *99* (1991) 13–20.

52 Turner, B. M., Birley, A. J., and Lavender, J., Histone H4 isomorfs acetylated at specific lysine residues define individual chromosomes and chromatin domains in *Drosophila* polytene nuclei. Cell *69* (1992) 375–384.

53 van Holde, K. E., Chromatin. Springer-Verlag, New York (1988).

54 Vidali, G., Boffa, L. C., and Allfrey, V. G., Properties of an acidic histone-binding protein fraction from cell nuclei. Selective precipitation and deacetylation of histone f2a1 and f3. J. biol. Chem. *247* (1972) 7365–7373.

55 Wilson, C., Bellen, H. J., and Gehring, W. J., Position effects on eukaryotic gene expression. A. Rev. Cell Biol. *6* (1990) 679–714.

56 Yamasu, K., and Senshu, T., Conservative segregation of tetrameric units of H3 and H4 histones during nucleosome replication. J. Biochem. *107* (1990) 15–20.

57 Yukioka, M., Sasaki, S., QI, S.-L., and Inoue, A., Two species of histone aceyltransferase of rat liver nuclei. J. biol. Chem. *259* (1984) 8372–8377.