REVIEW

Histone acetylation and control of gene expression

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Introduction

The post-translational acetylation of the core histones was first recognised more than a quarter of a century ago (Allfrey *et al.* 1964). Since then, considerable research effort has been expended in attempting to reveal the mechanisms by which acetylation is controlled and, most importantly, its functional significance. The reasons for the interest in this phenomenon are not hard to find. The intimate association of histones with cellular DNA and their extreme conservation through evolution both make it likely that any change in their structure, particularly one that involves a change in net charge, will have an effect on chromatin function. Thus, as frequently stated in introductory paragraphs, histone acetylation is potentially a major influence on events such as transcription, replication, DNA packaging through the cell cycle and DNA repair. However, despite the conceptual appeal of such a central role, definitive evidence is lacking and we have intriguing correlations rather than experimental data on which detailed molecular mechanisms can be based. Indeed, belief in the functional importance of histone acetylation has tended to rely on evolutionary arguments of the sort used to explain the popularity of sexual reproduction in the animal kingdom, namely: 'In evolutionary terms sexual reproduction must be important since so many species devote so much energy to it' (Leakey, 1976).

Very recently, novel genetic, biochemical and immunological approaches have been applied to the study of histone acetylation and have already provided valuable insights and highlighted exciting new directions for research. In this review I will attempt to describe and interpret these new findings and to speculate on the mechanisms by which histone acetylation may influence gene expression. I will not cover in any detail the early work on histone acetylation or the important involvement of histone acetylation in the deposition of newly synthesized histones on chromatin. These topics have recently been reviewed (Loidl, 1988; Csordas, 1990).

The histone acetylation–deacetylation cycle

Acetylation of the histones that organise the nucleosome core particle (H2A, H2B, H3 and H4) is a ubiquitous post-translational modification found in all animal and plant species so far examined (Csordas, 1990). Acetylation occurs at specific lysine residues, all of which occur in the amino-terminal domains of the core histones (Fig. 1, Table 1). Each acetate group added to a histone reduces its net positive charge by 1 and permits the resolution of the acetylated isoforms by electrophoresis (Alfageme *et al.* 1974).

Addition and removal of acetate groups is catalysed by specific enzymes (Matthews and Waterborg, 1985), the net level of acetylation at any particular site being the result of an equilibrium between these enzymes. The steady-state level of acetylation and the rates at which acetate groups are turned over vary both between and within different cell types, with half-lives that vary from a few minutes to several hours (Covault and Chalkley, 1980; Duncan *et al.* 1983; Zhang and Nelson, 1988*a*,*b*). It has been estimated that 30% of the histone in chicken erythrocytes is acetylated but 'frozen', i.e. does not turn over, while only about 2% turns over rapidly (Brotherton *et al.* 1981; Zhang and Nelson, 1986).

Individual lysine residues are selectively acetylated

Each electrophoretic isoform may itself be a mixture of molecules in which the total number of acetate groups is the same, but the sites acetylated are different. The sites that are acetylated may be just as significant, in a functional sense, as the change in net charge, particularly if the N-terminal regions of the core histones serve as recognition signals for protein binding (see below). Such considerations have encouraged attempts to establish whether the different acetylation sites are used in a controlled or a random fashion. This has been tested with histone H4 by isolation of the mono-, di, tri- and tetra-acetylated isoforms and sequencing of the amino-terminal region (Chicoine *et al.* 1986; Couppez *et al.* 1987; Thorne *et al.* 1990) or by the use of site-specific antibodies (Turner and Fellows, 1989; Turner *et al.* 1989). In each of the species tested so far, site usage has been shown to be non-random, though the frequency with which sites are used differs between species. Thus, in mono-acetylated H4 ($H4Ac_1$) from human or bovine cells lysine 16 is the predominant acetylated site, while in cuttlefish testis lysine 12 is used exclusively. In *Tetrahymena* $H4Ac_1$, acetylation is predominantly at lysine 7 (equivalent to lysine 8 in other species). In *Tetrahymena* and cuttlefish, sites are used in an almost invariant sequence through to the tetra-acetylated isoform (7, 5, 11, 15 and 12, 5, 16, 8, respectively), with the result that each charge isoform

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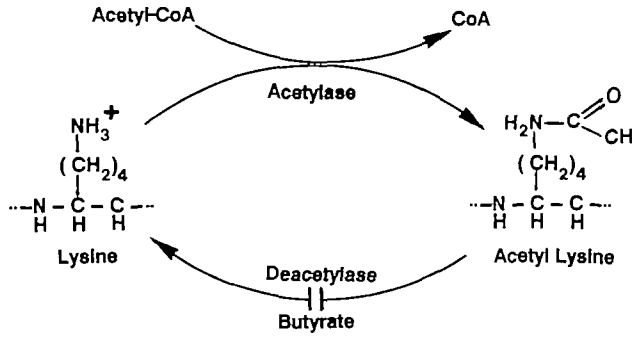

Fig. 1. The enzyme-catalysed acetylation and deacetylation of lysine residues.

Table 1. *Acetylation sites in mammalian histones*

Histone	Acetylation sites (in order of use)	Trypsin cleavage sites (in chromatin)
H2A	5, 9 (minor)	11
H2B	12/15*, 20, 5	20, 23
H3	14, 23, 18, 9	26
H4	16, 8/12*, 5	17, 19

*In H2B sites 12 and 15 are both frequently acetylated in the mono-acetylated isoform. In H4 either lysines 8 or 12 can be acetylated after 16.

The table is compiled from the data of Bohm *et al.* (1980, 1981, 1982), Turner *et al.* (1989) and Thorne *et al.* (1990).

consists essentially of a single 'positional' isoform. In human or bovine cells there is more flexibility in site usage, particularly in the more-acetylated isoforms.

The sequencing approach has recently been extended to include histones H3 and H2B from human and bovine cells (Thorne *et al.* 1990) and here too site usage is non-random but with a degree of flexibility reminiscent of that seen with H4. These results are summarised in Table 1. It should be emphasised that these results show only the steady-state level of acetylation at each site for each of the acetylated isoforms. For each isoform this level may be the net result of several different acetylation–deacetylation cycles, possibly occurring at different rates, in different parts of the genome and catalysed by different enzymes.

The antibody-based approach to estimating the usage of H4 acetylation sites requires antibodies specific for H4 molecules acetylated at each of the four different sites and involves quantitative Western blotting from acid–urea–Triton gels (Turner and Fellows, 1989; Turner *et al.* 1989). Despite the fact that three of the four H4 acetylation sites are very similar, all containing the Gly-Lys-Gly sequence motif, it has proved possible, by immunisation with synthetic peptides, to prepare antisera with the required specificities. The results obtained with these antisera are in good agreement with those given by sequencing. While this approach cannot provide the quantitative accuracy of sequencing, it has the advantage that it can be applied to small amounts of unpurified histone preparations and can therefore be used to explore site usage in various different cells, tissues and species. Recent experiments have shown that the pattern of site usage is essentially the same in all mammalian cells examined but changes over greater evolutionary distances (e.g. in *Drosophila* cultured cells lysines 8 and 12 are the predominant sites acetylated in $H4Ac_1$; R. Munks and B.M.T., unpublished).

The finding that the pattern of H4 acetylation differs between mammals, cuttlefish and *Drosophila*, while the sequence of the amino-terminal region of H4 and, presumably, the structure of the nucleosome are identical, suggests that the differences lie in the specificities and relative activities of the acetylating and deacetylating enzymes. Unfortunately, information on these fascinating and important enzymes is still limited (Matthews and Waterborg, 1985; Chan *et al.* 1988; Richman *et al.* 1988; Lopez-Rodes *et al.* 1989). The finding that histone acetylation is a controlled, non-random process, with a more or less defined order of site usage, strongly suggests the existence of enzymes whose ability to acetylate or deacetylate a given site is dependent on both the sequence of amino acids around the target lysine residue and the acetylation status of adjacent sites.

Histone amino-terminal domains are not essential for the assembly or structural integrity of the nucleosome core particle

Consideration of the role of histone acetylation in chromatin structure and function would ideally be based on a clear understanding of the roles of the core histone amino-terminal domains. Unfortunately, these have not yet been clearly defined, though some conclusions are well established.

(1) The amino-terminal domains of histones in core particles are both trypsin sensitive (reviewed by Bohm and Crane-Robinson, 1984) and, in general, accessible to monoclonal antibodies against amino-terminal epitopes (Muller *et al.* 1982, 1984; Whitfield *et al.* 1986). They are therefore unlikely to be buried in the core DNA.

(2) Removal of the amino-terminal tails exposes the core particle DNA at 20–35 and 60–80 bases from the 5′ end to attack by DNase I, suggesting that at least some tails interact with DNA in these regions (Whitlock and Simpson, 1977; Lilley and Tatchell, 1977).

(3) Only a few specific interactions between core DNA and histone amino-terminal regions have been detected. A methylation protection procedure (Lambert and Thomas, 1986; Hill and Thomas, 1990) has been used to identify lysine residues that are protected by interaction with DNA. The amino-terminal domain of H3 may bind weakly to region 7 of core particle DNA through residues 3–8 (Lambert and Thomas, 1986) and to the 10 bp (base pair) extensions that bind H1 in the intact nucleosome (Hill and Thomas, 1990). The amino-terminal domain of H4 is associated with core-particle DNA in sea-urchin sperm (Hill and Thomas, 1990) and a particularly close juxtaposition of histidine 18 and core DNA has been identified in chicken chromatin by cross-linking (Ebralidse *et al.* 1988). The amino-terminal domains of H2B and H2A bind weakly (H2B) or not at all (H2A), with core DNA (Lambert and Thomas, 1986; Hill and Thomas, 1990).

(4) High-resolution proton magnetic resonance (Cary *et al.* 1978) has shown that residues within the amino-terminal tails of histones H3 and H4 are associated with core DNA at low ionic strength, but released at salt concentrations above 0.3 M. Residues within the tails of H2A and H2B are mobile at all ionic strengths, suggesting that associations between these regions and core DNA can only be transient. (Such transient associations may well be detected by DNA–protein cross-linking.) Changes in ionic strength also alter the relative trypsin sensitivities of the amino-terminal regions (Harborne and Allan, 1983).

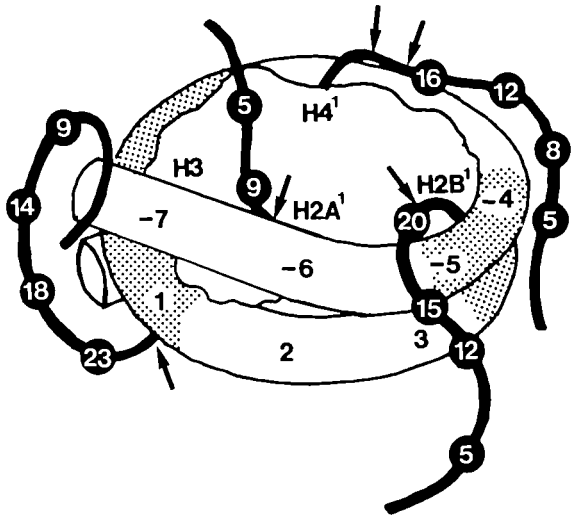

Fig. 2. A diagram of the nucleosome core particle showing the sizes and probable positions of the amino-terminal domains of the core histones. Lysine residues that can be acetylated *in vivo* are shown as numbered circles. Trypsin cutting sites listed in Table 1 are indicated by arrows (the site at residue 23 of H2B is not shown). For the sake of clarity the drawing shows only one amino-terminal domain for each pair of histones. Each domain is shown fully extended (i.e. 3.6 Å per amino acid residue), a situation that is unlikely to be realised *in vivo* for at least some of them (e.g. see Suzuki, 1989). The drawing of the nucleosome core particle is based on the model presented by Klug *et al.* (1980). Numbers along the core DNA indicate each complete helical turn either side of the dyad axis. Regions of core DNA that become more susceptible to DNase I cutting after removal of the histone amino-terminal tails are shaded. The experimental evidence from which the positions of the amino-terminal domains are derived is cited in the text.

Fig. 2 shows a core particle in which the amino-terminal domains are distributed in a way that is consistent with the data summarised above. The drawing should not be taken literally, but it does show accurately the relative sizes of the amino-terminal domains and the positions of the acetylation sites in relation to the relatively few known (or potential) sites of interaction with core DNA.

While knowledge of the interactions between the histone amino-terminal domains and core DNA is useful in formulating structural models of the nucleosome, it is of limited value in understanding their role *in vivo*. In isolated core particles the amino-terminal tails are deprived of the potential sites of interaction that are present in larger chromatin fragments, such as linker DNA, non-histone proteins, H1 etc. Such binding to core DNA as does occur may happen only through the absence of these other ligands. This interpretation is consistent with the evidence that, while histone amino-terminal tails are not necessary for either the assembly of the core particle *in vitro* or its structural integrity (Whitlock and Simpson, 1977), they are required for the assembly *in vitro* of higher-order chromatin structures (Allan *et al.* 1982).

Hyperacetylation of core histones causes subtle changes in chromatin structure

Extensive analysis by a variety of biophysical techniques has shown that control and hyperacetylated nucleosome core particles exhibit only subtle differences, suggesting, at most, a partial unwinding or loosening of the core DNA (Muller *et al.* 1982; Bode *et al.* 1983; Ausio and van Holde, 1986; Imai *et al.* 1986; Libertini *et al.* 1988). Acetylation does increase the DNase I sensitivity of the region about 60 bp from the ends of the core DNA, consistent with a relaxation of histone–DNA contacts at this site (Simpson, 1978). Norton *et al.* (1989) have quantified the effect of histone acetylation on the winding of DNA around the core octamer by assembling nucleosomes *in vitro* on circular plasmid DNA using histones with differing levels of acetylation. Each nucleosome assembled on circular DNA causes a change in linking number of about −1 (measured after relaxation and resealing of the supercoiled plasmid with topoisomerase). With highly acetylated nucleosomes, this change in linking number was reduced to about −0.8, which can be explained by the unwinding of about 20 bp of DNA from the histone core. The authors point out that this effect on DNA supercoiling means that the enzymes responsible for histone acetylation are functioning, in effect, as a eukaryotic gyrase.

The induction of histone hyperacetylation with the deacetylase inhibitor sodium butyrate has long been known to increase the DNase I sensitivity of chromatin (Vidali *et al.* 1978). This presumably reflects changes in higher-order chromatin structure. Butyrate also effects both expression of specific genes (Kruh, 1982) and cell cycle transit (e.g. see Fallon and Cox, 1979). It remains to be seen which, if any, of these effects are a direct result of increased histone acetylation. High levels of histone acetylation improve chromatin solubility (Alonso *et al.* 1987) and inhibit the ability of histone H1 to condense chromatin *in vitro* (Ridsdale *et al.* 1990). The latter finding is consistent with the positioning of the amino-terminal domain of H3 adjacent to the H1 binding site (Fig. 2) and also provides a mechanism by which acetylation may influence higher-order structure.

Transcriptionally active chromatin is enriched in acetylated histone

From the very earliest experiments on histone acetylation in stimulated lymphocytes it has often been proposed that increased levels of histone acetylation are associated with increased transcriptional activity (Allfrey *et al.* 1964). Various experimental approaches have shown that chromatin preparations that are enriched in transcribed genes are also enriched in acetylated histones and, conversely, that acetylated chromatin is enriched in transcribed genes (Allegra *et al.* 1987; Ridsdale and Davie, 1987; Johnson *et al.* 1987; Hebbes *et al.* 1988). Ip *et al.* (1988) have shown that chromatin fractions enriched in transcribing genes show a more rapid turnover of histone acetate groups than bulk chromatin. Finally, Tazi and Bird (1990) have shown that short chromatin fragments enriched in CpG-rich DNA contain highly acetylated core histones. CpG-rich DNA sequences are found upstream from many genes (Gardiner-Garden and Frommer, 1987).

Thus, the correspondence between transcriptional activity and increased acetylation is well established. However, the details remain to be filled in. For example, we do not know whether the acetylated histones are associated with coding DNA or regulatory elements or both. The immunoprecipitation experiments of Hebbes *et al.* (1988) suggest that coding DNA of the α-globin gene is associated with acetylated histone, whereas immunolabelling experiments indicate that the H4 that is associated with coding DNA in very rapidly transcribed (puffed)

genes in polytene chromosomes is not highly acetylated, though adjacent regions are (Turner *et al.* 1990). It is not known whether increased steady-state levels of acetylation precede gene expression or are simply a consequence of it. Answers to these questions will help us to decide whether histone acetylation is required for the initiation of transcription, is part of the mechanism of transcription or is simply a by-product of transcription-related chromatin decondensation. We also have little information on what aspects of histone acetylation are of functional significance. Is the change in net charge that results from acetylation the major factor or does acetylation at different lysine residues have specific functional consequences? A genetic approach using mutants of the yeast *Saccharomyces cerevisiae* is providing valuable information relevant to these questions.

Deletions of the amino-terminal domain of H4 prevent 'silencing' of the yeast mating type genes

It is an article of faith among students of histone acetylation that the amino-terminal domains of the core histones have a central role in chromatin structure and function. This belief relies heavily on the extreme evolutionary conservation of the histones and the view that sequences that have been retained for so long must have some use. It is therefore disconcerting for those of us who hold this view that mutants of the yeast *Saccharomyces cerevisiae*, with large deletions of the amino-terminal domains of either H2A, H2B or H4, are not only viable, but grow in culture just as well as wild-type cells (Wallis *et al.* 1983; Schuster *et al.* 1986; Kayne *et al.* 1988). The inescapable conclusion is that the amino-terminal domains of the core histones are not, individually, essential for the basic cellular functions necessary for growth and division of yeast cells, at least in the sheltered environment of the laboratory.

Yeast mutants carrying deletions of residues 4–14, 4–19, 4–23 and 4–28 in the amino-terminal domain of H4 are all viable (that with the next largest deletion, 4–34, is not), though the two largest deletions show an increase in doubling time and a lengthening of the G_2 period of the cell cycle (Kayne *et al.* 1988). There is evidence for unfolding of chromatin and/or repositioning of nucleosomes in these mutants. However, the most striking observation is that the mutants all show reduced mating efficiency, the effect being particularly dramatic in those in which all acetylatable amino-terminal lysines are absent, i.e. 4–19 and above. To appreciate the significance of this observation it is necessary to understand just a little of the mating behaviour of yeast cells.

To mate successfully, yeast cells must adopt one or other of two mating types, **a** or α, by producing the mating factor appropriate to that type and responding to the factor of the opposite type. Thus, an **a** type cell will produce the **a** type mating factor and respond to the α factor. Mating factors are produced by inserting a copy of either the **a**-determining or the α-determining gene at the mating locus, *MAT*. For this purpose yeast cells keep copies of both **a** and α genes at two normally silent loci, *HML*α and *HMR***a**. The cell 'chooses' the **a** or α mating type by inserting either into the *MAT* locus, where it is expressed.

In those H4 mutants in which mating efficiency was severely depressed the normally silent *HML*α and *HMR***a** genes were both constitutively transcribed, though these mutants neither produced nor responded to mating factors. The inappropriate expression of genes at these normally silent loci is presumably the cause of the severely reduced mating efficiency. It is important to note that this effect was not part of a general disruption of gene regulation. Thus, the genes *GAL10*, *PHO5* and *CUP1*, whose levels of transcription are determined by galactose and glucose, phosphate and copper, respectively, were all regulated normally.

These observations have been extended by substituting various amino acids for some or all of the amino-terminal lysine residues of H4 (Megee *et al.* 1990). Perhaps surprisingly, mutants in which all four amino-terminal lysines were substituted by arginine or asparagine were not viable whereas substitution by glutamine gave a phenotype very similar to the 4–28 deletion mutant (i.e. increased doubling time and severely reduced mating efficiency). A lysine–arginine substitution at position 16 had a slight effect on mating efficiency, as did histidine–tyrosine at position 18. Using a similar approach, Johnson *et al.* (1990) have shown that substitution of any of the residues 16–19 of H4 (Lys-Arg-His-Arg) with glycine results in derepression of the *HML*α and *HMR***a** genes.

How can this very specific effect on the mating type genes in H4 deletion or substitution mutants be explained? Repression or 'silencing' of the HMLα and HMR**a** genes requires both upstream DNA elements ('silencers') and a number of different proteins produced by defined genes, such as *RAP1*, *ABF1* and *SIR1–4* (Shore and Nasmyth, 1987; Diffley and Stillman, 1989; Rine and Herskowitz, 1987). A possible explanation for the properties of the H4 mutants is that the absence or alteration of the amino-terminal region prevents the assembly of the DNA–protein complex necessary for the long-term suppression (silencing) of the HMLα and HMR**a** genes. Johnson *et al.* (1990) have used a genetic approach to address this problem and have identified strong extragenic suppressors of the H4 16–19 point mutations in the *SIR3* gene, strongly suggesting that the protein encoded by this gene interacts with the amino-terminal region of H4 to silence the mating type genes, though this interaction is apparently not with the critical region 16–19 itself.

The yeast genome is less than 1/200th of the size of a typical mammalian genome and a large proportion is transcriptionally active. There is a high steady-state level of histone acetylation (Nelson, 1982). Thus, despite the fact that the amino-terminal domains of yeast and human H4 have identical amino acid sequences, the question arises of whether conclusions drawn from these experiments in yeast can be extended to higher eukaryotes.

No direct equivalent of the yeast mating type genes has been identified in higher eukaryotes and the long-term repression of genes in higher organisms may well involve quite different mechanisms from those used in yeast. However, in the next section I will describe experiments in a higher eukaryote, namely the fruit fly *Drosophila*, that suggest that here too histones may play a role in the long-term repression of genes, in this case by their incorporation into heterochromatin.

Heterochromatin formation and suppression of transcription may require interaction between core histones and non-histone proteins

If a gene is translocated from its normal locus to a position in or adjacent to a heterochromatic region (i.e. a region of

condensed, transcriptionally quiescent chromatin), then it may be inactivated. Sometimes this inactivation occurs relatively early in development and in some cells but not in others, giving rise to a mosaic or variegated phenotype. This phenomenon is known as position effect variegation. It has been observed in various species but has been particularly well studied in the fruit fly *Drosophila* (Spofford, 1976). In this species mutants have been isolated in which position effects are either enhanced (i.e. translocated genes are inactivated in a higher proportion of cells than in wild-type flies) or suppressed. Genetic analysis has led to the suggestion that some of these mutants encode proteins that participate in the formation of the DNA–protein complex that constitutes heterochromatin (Locke *et al.* 1988).

A gene that, when mutated, suppresses position effect variegation in *Drosophila*, *Suvar(3)7*, has recently been cloned and sequenced (Reuter *et al.* 1990). It encodes a novel zinc finger protein in which the DNA-binding fingers are separated by 40–107 amino acids. It is of particular interest for the present discussion that the regions between the fingers contain stretches of acidic amino acids. The authors speculate that the condensation of chromatin by *Suvar(3)7* involves binding of DNA by the widely separated zinc fingers and interaction of the intervening polyacidic stretches with positively charged chromatin proteins. These putative *Suvar*-binding proteins are most likely to be histones, particularly as the polyacidic stretches found in *Suvar(3)7* resemble those found in histone-binding proteins such as HMG 1 and 2 and nucleoplasmin (discussed by Csordas, 1990; Jantzen *et al.* 1990; Reuter *et al.* 1990). Evidence supporting a role for histones in heterochromatin formation comes from studies on *Drosophila* mutants in which histone gene multiplicity (normally 100–150 per haploid genome) is reduced (Moore *et al.* 1983). Although such mutants exhibit no visible, diagnostic phenotypic features, position effect variegation is suppressed, i.e. translocated genes are less frequently packaged into heterochromatin. Whether these putative interactions involve the core histones or H1 or both, remains to be established.

If heterochromatin-forming proteins do indeed interact with core histones, then the interaction is most likely to occur *via* the exposed, highly positive, amino-terminal domains and will therefore be sensitive to changes that alter the net charge of these regions, such as acetylation. In fact, evidence that histone acetylation may exert just such an effect is already available. Variegation of the eye colour gene *white* can be suppressed by exposure of mutant larvae to the deacetylase inhibitors butyrate or propionate (Mottus *et al.* 1980) while Dorn *et al.* (1986) have characterised a suppressor mutation *Su-var(2)1*^{01}, in which the suppressive phenotype is associated with an increase in the steady-state level of histone acetylation and an enhanced sensitivity of mutant larvae to butyrate.

Acetylated H4 is selectively distributed through the interphase genome

Knowledge of the distribution of acetylated histones in chromatin is central to the formulation of hypotheses on their role in chromatin function. It has recently become possible to examine this distribution directly by immunofluorescence microscopy using antisera specific for acetylated H4. This approach has been used to monitor developmental changes in the level and distribution of acetylated H4 in the macronuclei and micronuclei of *Tetrahymena* (Lin *et al.* 1989) and to show a striking increase in H4 acetylation in erythrocyte nuclei activated by fusion with transcriptionally active cultured cells (Pfeffer *et al.* 1988). Antibody labelling of metaphase chromosomes isolated from CHO cells and flow cytometry, has been used to show that H4 acetylation and deacetylation continue through metaphase and that the level of acetylated H4 in each chromosome is proportional to DNA content, i.e. there is no evidence of selective acetylation of particular chromosomes (Turner, 1989).

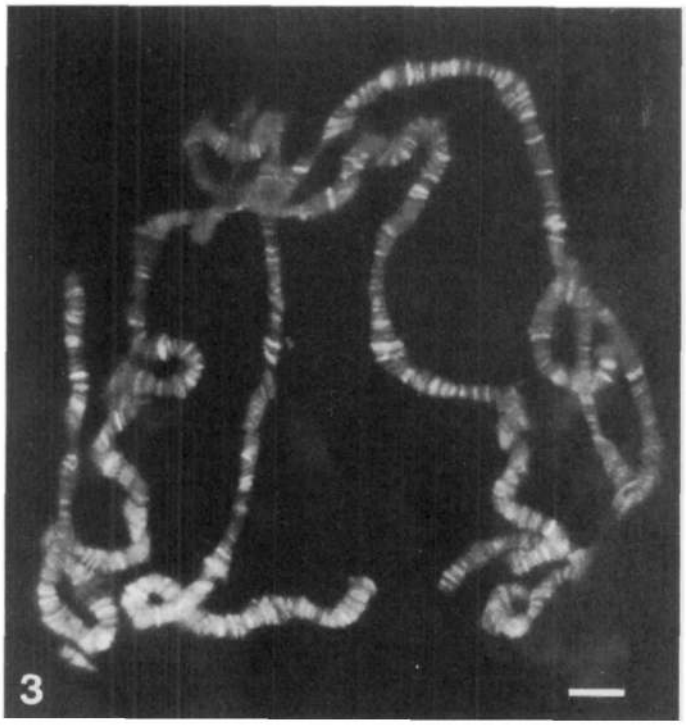

Fig. 3. Immunolabelling of polytene chromosomes from the salivary gland of *Drosophila melanogaster* with antibodies to acetylated histone H4 (R6/5; Turner *et al.* 1989). The antibodies reveal sharply defined islands of chromatin enriched in acetylated H4. Bar, 10 μm.

In an attempt to define the distribution of acetylated H4 through the interphase genome, the immunolabelling approach has been applied to polytene chromosomes from the insects *Chironomus thummi* (Turner *et al.* 1990) and *Drosophila melanogaster* (R. Simpson and B.M.T., unpublished). In both species there is a strikingly non-random distribution of the acetylated histone, with some regions highly enriched in acetylated H4 and others relatively depleted (Fig. 3). These islands of acetylated chromatin are quite distinct from the phase-dense bands that characterise these chromosomes and reflect differences in chromatin compaction. However, the size of the acetylated islands varies over approximately the same range as the phase bands and interbands, suggesting that they may incorporate sufficient DNA for one, or just a few, transcription units.

In so far as polytene chromosomes represent functionally normal interphase chromatin, with a level of histone acetylation not markedly different from that found in other cell types (B.M.T., unpublished), it is reasonable to conclude that the distribution of acetylated H4 seen in these chromosomes is representative of that present in

non-polytene insect cells. Whether it is also true of other species remains to be established, though the recent association between high levels of histone acetylation and specific regions of the mammalian genome suggests that it is (Tazi and Bird, 1990).

A model outlining possible roles of histone acetylation in control of gene expression

Fig. 4 outlines some of the structural transitions that occur as chromatin passes between inactive, transcribable and actively transcribed states, emphasising those events in which histone acetylation may play a role.

The chromatin shown in the upper part of the figure contains sharply defined domains that are, respectively, enriched and depleted in acetylated histones. The deacetylated region contains a DNA site (out of the figure) at which heterochromatin formation can be initiated in the presence of the appropriate silencing proteins (i.e. a silencer element). As shown down the left-hand side of the figure, binding of these proteins to DNA, histones and to one another, leads to the progressive condensation and long-term inactivation of chromatin (i.e. the formation of heterochromatin). The model proposes that heterochromatin formation is inhibited by increased histone acetylation, due to weakening of the charge-mediated interaction between core histones, particularly H4, and silencing proteins. The spread of the region of heterochromatin is halted at the boundary between the deacetylated and acetylated regions. It is quite possible that acetylation or deacetylation at only one or a small number of sites may prove to be critical, as suggested by recent experiments in yeast (Johnson *et al.* 1990). In this case, the boundary at which heterochromatin formation is halted is not necessarily between highly acetylated and underacetylated chromatin, but between chromatin acetylated or deacetylated at one or a few critical sites.

The large acetylated chromatin domain in the upper part of the figure contains a potentially active gene in a DNase I-sensitive form, organised as a 10 nm, beads-on-a-string fibre. Increased levels of histone acetylation may encourage this more extended conformation by inhibiting H1-mediated chromatin condensation (Ridsdale *et al.* 1990). The 5′ regions of many genes contain segments of CpG-rich DNA, CpG islands (Gardiner-Garden and Frommer, 1987) and one of these is shown in the figure. CpG islands are hyperacetylated, to the extent that H4 is predominantly in the tetra-acetylated form (Tazi and Bird, 1990). As tetra-acetylated H4 accounts for less than 2 % of total H4 in mammalian cells (it is below the limits of detectability with the usual gel loadings and staining methods) while CpG islands may account for about 1 % of the genome (i.e. 30 000 CpG islands with an average size of about 1 kb), the hyperacetylated region is unlikely to extend far beyond the CpG island itself (Bird *et al.* 1985; Gardiner-Garden and Frommer, 1987).

Gene activation typically involves loss of nucleosomes from specific regions, often those containing the promoter and upstream control elements, generating DNase I-hypersensitive sites (Gross and Garrard, 1988). Hyperacetylation of the core histones may facilitate this loss (displacement), by analogy with the hyperacetylation of sperm histones that precedes their displacement by protamine (Christensen and Dixon, 1982). Binding of transcription factors and polymerase generates the fully active transcription complex shown in the lower part of the figure. The histones in the actively transcribing gene are rapidly acetylated and deacetylated (Ip *et al.* 1988) in a reaction that may facilitate passage of the polymerase by controlling the level of DNA supercoiling (Norton *et al.* 1989), by mediating the transient release and reassociation of histones with DNA (Nacheva *et al.* 1989) and/or by permitting the structural changes in the nucleosome that are associated with transcription (Chen *et al.* 1990, and references therein). However, the viability of yeast

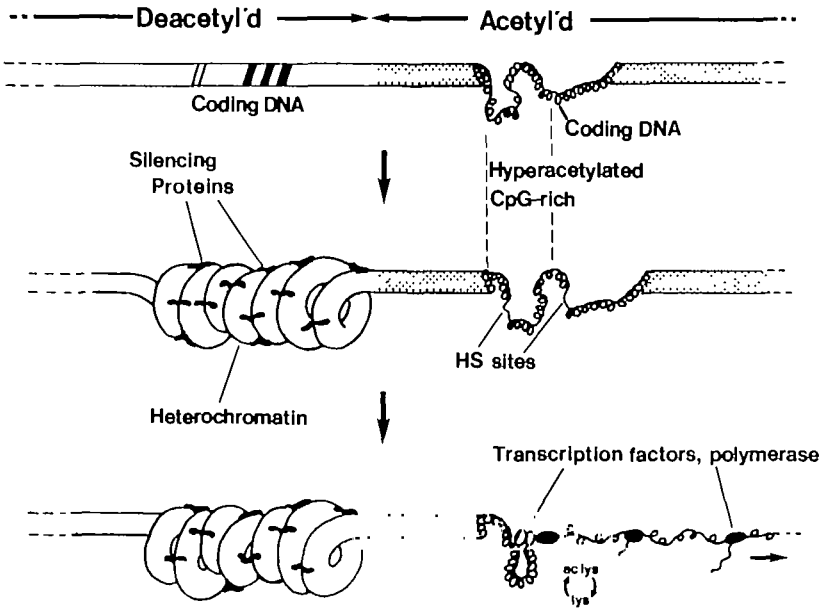

Fig. 4. A diagram showing some of the changes in higher-order chromatin structure that accompany gene activation and inactivation and in which histone acetylation may play a role. Chromatin is shown as the 30 nm solenoid or, in the case of the potentially active gene, the DNase I-sensitive, 10 nm nucleosomal fibre. The labels 'deacetyl'd' and 'acetyl'd' refer either to the overall level of acetylation, or to acetylation at one, or a small number of, sites. A hyperacetylated CpG-rich island is shown incorporating the upstream control elements and part of the coding region of the potentially active gene. Such islands have been found within or adjacent to many genes in animal species. Heterochromatin formation is shown diagrammatically in the middle and lower parts of the figure as further coiling of the 30 nm fibre, though the actual mechanism is unknown, as is the distribution of the silencing proteins responsible for mediating the condensation process. The model proposes that the spread of heterochromatin stops at the boundary between acetylated and deacetylated chromatin. Activation of the gene on the right of the figure involves the generation of DNase I-hypersensitive sites (HS sites) by loss of selected nucleosomes, possibly through displacement by transcription factors or other proteins. The fully active gene is shown in the lower part of the figure. Upstream chromatin has looped out to allow interaction between proteins bound to upstream control elements and the promotor region. The transcribed DNA contains nucleosomes, possibly structurally modified, which may undergo rapid turnover of their histone acetate groups. Experimental evidence for the various elements of the figure is discussed in the text.

mutants with large deletions of the amino-terminal domains of H2A, H2B and H4 shows that, in this species at least, these regions are not essential for transcription.

If the primary role of the amino-terminal regions of at least some core histones is in the long-term suppression of certain genes, then this goes some way to explaining the ability of yeast cells to survive major deletions of histone amino-terminal domains. A large proportion of the yeast genome is active or potentially active and a requirement for long-term suppression is likely to be confined to a small number of genes, possibly only the mating type genes. The model predicts that the overall steady-state level of histone acetylation in yeast will be high, which is indeed the case (Nelson, 1982), and that the histones associated with the silent mating-type genes will be under-acetylated or acetylated only at specific sites, which remain to be determined. Deletions of H4 may have a more profound effect on the viability of higher eukaryotes, in which the suppression of certain groups of genes by chromatin condensation may be an integral part of gene control during development (Reuter *et al.* 1990, and references therein). The model also predicts that heterochromatin will be depleted in acetylated histones, or at least show a distinctive pattern of site usage. Experiments to test this prediction are in progress.

Even the simple model presented in Fig. 4 contains speculative elements and unresolved questions. It does, however, provide a basis for experimentation and some elements are now readily testable. It will be particularly interesting to determine whether the putative interaction between the core histones and heterochromatin-forming, or silencing, proteins is mediated by simple net charge effects or by more specific interactions involving specific regions of the amino-terminal domains, in which case the individual sites that are acetylated may be of major functional significance.

We have it on good authority (Alberts and Sternglanz, 1990) that '...researchers throughout the world are thawing out their old preparations of histones...' and results relevant to the questions posed by this, and no doubt other, models should not be long in coming. Those of us who did not freeze our histones away in the first place await the results from these old, proteolysed samples with some trepidation.

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