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Hypothesis

Histone deacetylase

A key enzyme for the binding of regulatory proteins to chromatin

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Core histones can be modified by reversible, posttranslational acetylation of specific lysine residues within the N-terminal protein domains. The dynamic equilibrium of acetylation is maintained by two enzyme activities, histone acetyltransferase and histone deacetylase. Recent data on histone deacetylases and on anionic motifs in chromatin- or DNA-binding regulatory proteins (e.g. transcription factors, nuclear proto-oncogenes) are summarized and united into a hypothesis which attributes a key function to histone deacetylation for the binding of regulatory proteins to chromatin by a transient, specific local increase of the positive charge in the N-terminal domains of nucleosomal core histones. According to our model, the rapid deacetylation of distinct lysines in especially H2A and H2B would facilitate the association of anionic protein domains of regulatory proteins to specific nucleosomes. Therefore histone deacetylation (histone deacetylases) may represent a unique regulatory mechanism in the early steps of gene activation, in contrast to the more structural role of histone acetylation (histone acetyltransferases) for nucleosomal transitions during the actual transcription process.

Chromatin; Histones; Histone acetylation; Transcription factor; Oncogene protein; Histone deacetylase; Transcription

1. INTRODUCTION

The DNA of the eukaryotic cell nucleus is associated with histones to form nucleosomes, which represent the basic structural subunit of chromatin [1]. Histones can be reversibly modified by a number of posttranslational reactions, such as phosphorylation, acetylation, ADP-ribosylation and ubiquitination (see [2] for a recent review). The posttranslational acetylation of lysine residues within the N-terminal domains of core histones was first discovered by Allfrey and co-workers [3] and is assumed to play a critical role in the modulation of structural transitions of chromatin during different nuclear processes [2,4–8], although the precise mechanisms are still far from clear.

The dynamic state of histone acetylation is maintained by two enzyme activities, histone acetyltransferase and histone deacetylase. Histone acetyltransferases link the acetyl-moiety of acetyl-CoA to the ε-amino group of specific lysine residues. The presence of such an acetyl-group neutralizes a positive charge within the N-terminal part of the histone molecule (Fig. 1). This modification can be reversed by the action of deacetylases. The amino acid sequence of the N-terminal histone tails has been conserved during evolution; therefore the lysine positions which are accessible to this enzymatic acetylation are identical in a wide variety of organisms ranging from yeast to human. There are 26–28 possible acetylation sites within a nucleosome [4]; it has to be considered that these sites are concentrated on relatively small, but flexible protein domains of the nucleosome (Fig.1). The usage of these lysine residues for acetyltransferases has been shown to occur in a non-random fashion, thus generating a further level of complexity, although the extent of site specificity may be different among various organisms [9–14].

It was proposed that histone acetylation represents a general mechanism for the destabilization of nucleosomes [5,15]. Such a transient destabilization has to occur during nuclear processes that are accompanied by structural transitions of defined chromatin areas, i.e. DNA replication, transcription, DNA repair, recombination or differentiation specific exchange of histone variants. The site specificity, the high variability in the extent of acetylation, and the multiplicity of enzymes involved in this modification, would finally lead to highly specific acetylation patterns which serve as distinct signals for nucleosome destabilization in different

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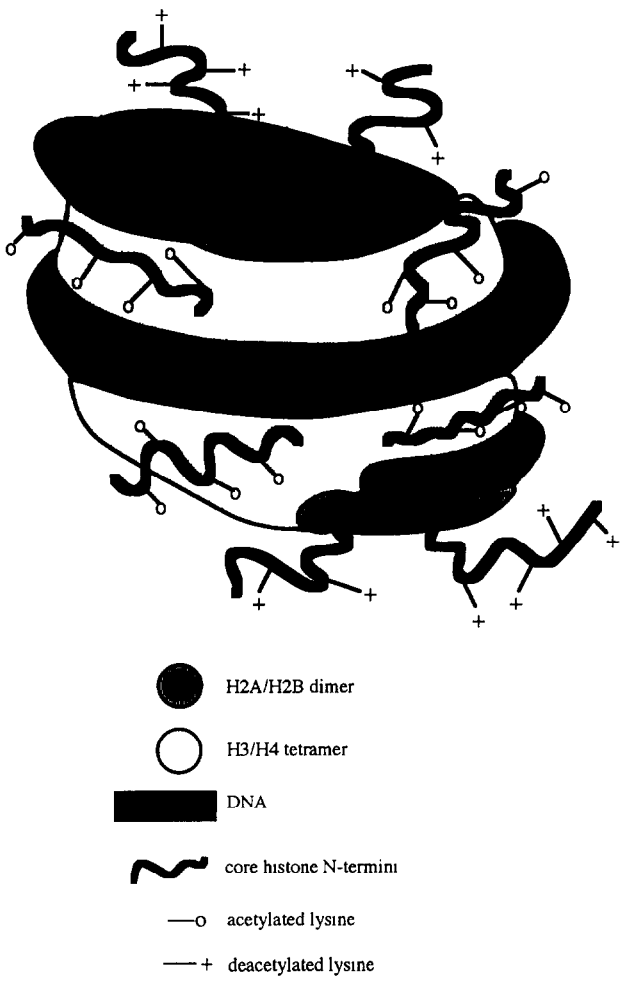

Fig. 1. Schematic drawing of the nucleosome core. The nucleosome core particle consists of two molecules of each of the core histones H2A, H2B, H3 and H4 and approx. 160 bp of DNA. H3 and H4 form a tetramer which binds two H2A/H2B dimers. The N-terminal histone domains contain a total of 28 lysine residues which undergo posttranslational acetylation and deacetylation. The diagram depicts the H3/H4 tetramer in its maximum acetylated form, the two H2A/H2B dimers in its deacetylated configuration.

nuclear processes. A central point of the previous proposal was that acetylation of all histones, but especially H3 and H4, would be a prerequisite for the nucleosomal structural transitions concomitant with DNA replication and exchange of histone primary structure variants in differentiation, whereas specific acetylation of H2A and H2B would be required for the destabilization of nucleosomes during transcription [5]. Since the original proposal, sound experimental evidence has accumulated, indicating that transient H2A/H2B deficiency accompanies transcriptional initiation and elongation [16–21]. For the transcription of the ribosomal genes of *Physarum* it has been recently shown in vivo, that actively transcribed gene fragments are devoid of H2A and H2B, whereas non-transcribed regions have the normal histone complement [22]. In accord with the proposed role of histone acetylation during transcription it could be demonstrated that the acetylation of H3 and H4 did not differ between actively transcribed and non-transcribed ribosomal gene chromatin [22]. However, contradictory results have been published indicating an involvement of H3 and H4 acetylation in the active transcription of genes [23]. Moreover, a direct link between the extent of H4 acetylation and transcription was shown, using an immunological approach [24].

The reason for the apparent discrepancies is still not understood, although we suppose that histone acetylation may contribute at different levels during the activation and transcription of genes. Recent data [25] argue against the involvement of H3 acetylation in transcriptional activation, since both, deletions and acetylation site substitutions within the N-terminus of H3 allowed hyperactivation of the *GAL*1 promotor as well as a number of *GAL*4-regulated genes in yeast. In contrast, the N-terminal sequence of H4 is required for promotor activation in vivo; mutations in H4 acetylation sites reduced the activation of the *GAL*1 and *PHO*5 promotor in yeast [26]. However, *GAL*1 and *PHO*5 contain positioned nucleosomes within their promotors, whereas *GAL*4 as a constitutive gene, is probably not tightly packed in nucleosomes [26]. The different dispensibility of acetylated sites among the N-termini of H3 and H4 indicates that the functional impact of histone acetylation is complex and maybe entirely different in the individual histone species.

An alternative approach to a better understanding of the function of histone acetylation is the investigation of the enzymes involved in the dynamic equilibrium of this modification, histone acetyltransferases and deacetylases. Acetyltransferases can be classified with respect to their intracellular location and substrate specificity into nuclear A-type and cytoplasmic B-type enzymes. Extensive investigations of the substrate specificity of histone acetyltransferases in yeast, pea, maize and *Physarum* have revealed that H3 and H4 are the predominant substrate molecules for these enzymes [27–31], whereas H2A and H2B only represent minor substrates, although multiple enzymes or enzyme forms exist. On the other hand, analysis of the substrate specificity of histone deacetylases, using HPLC purified, radioactively pre-labelled, individual core histone species, revealed an overproportional affinity of these enzymes for H2A and H2B [30,32,33]. This indicates that the regulation of H2A and H2B acetylation could preferentially occur on the level of deacetylation. Moreover, the occurance of multiple deacetylases suggests a key role for histone deacetylases in establishing and maintaining certain properties of eukaryotic chromatin.

2. HISTONE DEACETYLASES

Histone deacetylases have been most extensively studied in plant cells [29,33,34]. In maize embryos three

distinct enzymes are present; HD1A, HD1B and HD2, which differ in their substrate specificity. The substrate specificity of HD1A is furthermore regulated by reversible phosphorylation, giving rise to a number of phosphorylated enzyme forms [32]. HD2 is differentiation specific, since it represents the main deacetylase activity in dormant embryos, but disappears during progression of germination [35]. It was suggested that HD2 has an essential function in DNA repair, since its activity pattern closely resembles the course of repair synthesis during the early germination of maize embryos.

The fact that histone deacetylase HD1A is phosphorylated and as a consequence exhibits a dramatic shift in its substrate specificity [32], provides evidence that this enzyme could be involved in cellular signal transduction pathways. Together with the finding that the enzyme has a high substrate preference for H2B, and phosphorylation causes a dramatic increase of the affinity for H2A, we propose that histone deacetylases can be specifically involved in the early transcriptional activation of genes.

3. HYPOTHESIS

One of the most challenging problems of molecular biology is the understanding of how genes are activated from a repressed into an actively transcribed state. A great number of proteins, that are either localized in the nucleus or exert their regulatory function in the nucleus by DNA- or chromatin binding, possess stretches of acidic amino acids (anionic domains), apart from other distinct structural motifs. Such anionic regions are much more common in nuclear than cytoplasmic proteins. Among these nuclear proteins are enzymes (e.g. DNA polymerase, topoisomerase [36,37]), nucleoplasmin [38], HMG-proteins [39], chromosomal and nuclear matrix proteins [40,41], nuclear proto-oncogene proteins and tumor suppressor gene products (e.g. c-*myc*, c-*jun*, p53 [42–44]) and transcription factors [45–48]. As already pointed out by Earnshaw [49] it is conceivable that the binding onto chromatin of regulatory proteins or enzymes with anionic regions, or the association of distinct DNA regions with anionic motifs of structural proteins could be modulated by the acetylation of N-terminal core histone domains. If such proteins come into contact with the protein part of nucleosomes, it is unlikely that contact sites are located within the globular histone domains, which are mostly inaccessible due to the DNA wrapped around this globular core. The much more probable contact location are the short, but flexible N-terminal parts, especially of H2A and H2B. Since these N-terminal tails contain a cluster of acetylated lysine residues, a transient, yet highly specific deacetylation would facilitate the molecular contact with anionic protein domains (Fig. 2).

The action of a specific histone deacetylase will rapidly increase the net positive charge of the N-terminal domains. The specific deacetylation of these sites within a very small chromatin area would enable the association of anionic regions of regulatory proteins or transcription factors. This binding could occur concomitant with a sequence specific recognition of DNA-sites by regulatory proteins and therefore represent one of the first steps in the gene activation cascade. Since regulatory proteins may activate chromatin from a silent, rather condensed structural state, the model allows the binding or stabilization of regulatory proteins via chromatin binding domains at positions upstream or downstream of a sequence specific DNA recognition site, due to higher order structures. Analysis of histone acetylation in chicken globin gene switching revealed highly acetylated histones in both, the transcriptionally active and inactive, but 'poised' gene [50]. Both transcriptionally active and inactive parts of the ribosomal gene chromatin of *Physarum* contained highly acetylated core histones [22]. These data demonstrate that 'poised', but even transcriptionally inactive genes, may at least partially contain acetylated histone subspecies. Therefore, a specific histone deacetylase would be able to generate additional positive charge in specific areas of such chromatin.

According to our proposal, H2A and H2B, apart from being minor substrates for histone acetyltransferases, have to be rapidly deacetylated by a specific histone deacetylase when early transcriptional activa-

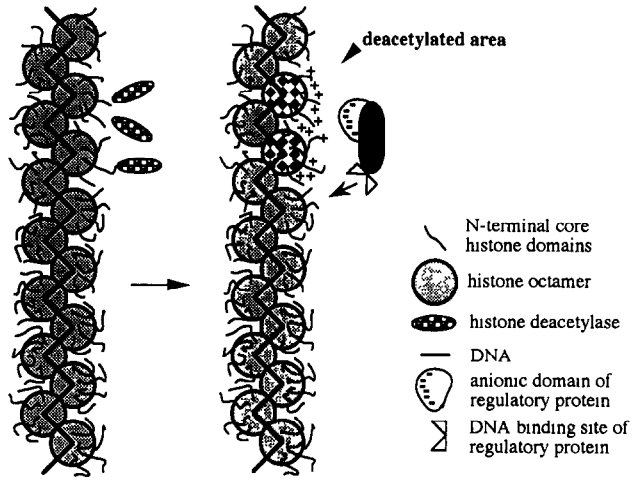

Fig. 2. Hypothetical role of histone deacetylase for the binding of regulatory proteins to chromatin. A specific histone deacetylase transiently deacetylates N-terminal lysine residues of especially H2A and H2B in a distinct chromatin area. This leads to a hotspot of positive charge in only few nucleosomes without pronounced structural changes of the overall chromatin structure. The anionic region of regulatory proteins (transcription factors, proto-oncogene proteins), enzymes, but also structural proteins (nuclear matrix components) can then establish a contact that enables the DNA recognition site to bind. Due to the higher-order folding of chromatin, the anionic contact site and the DNA recognition site may be several nucleosomes apart.

tion occurs. Our recent finding of HD1A phosphorylation with the resulting increase in H2A specificity suggests that identical pathways of intracellular signal transduction, which are generally involved in the activation of genes, could regulate the substrate specificity of the deacetylase via phosphorylation/dephosphorylation mechanisms. We postulate that a transient H2A and H2B deacetylation is required for the binding of regulatory proteins, e.g. transcription factors, to nucleosomal structures at an early step of gene activation. Our proposal is compatible with the site-specific acetylation of H2A and H2B necessary for transcription [5].

The time course from activation of a repressed gene to the active transcription by RNA polymerase involves a cascade of different events. At least two distant steps can be defined: (i) The binding of regulatory proteins to a chromatin domain, as the initial step of activation which would require the rapid, local deacetylation of core histones, in particular H2A and H2B. (ii) The destabilization of the nucleosome and the removal of H2A/ H2B dimers in a progressive displacement mode [5,17,18,51] as the final step of activation. This final displacement can be dependent on the site specific acetylation of H2A and H2B. We propose that chromatin structural transitions necessary for transcription also occur on a different level, e.g. on the level of acetylation of H3 and H4. However, acetylation of core histones by the action of histone acetyltransferases, leading to actual changes in the structural stability of nucleosomes [5], is distinguished from the specific role of transient deacetylation at the initial phase of gene activation. For this reason, our model attributes a regulatory function to histone deacetylation (= histone deacetylases) in the early transcriptional activation of genes, but a more structural role to histone acetylation (=histone acetyltransferases) in actual changes of the nucleosome structure at the final stage of the transcriptional activation (e.g. the removal of H2A/H2B dimers).

4. EVIDENCES, PERSPECTIVES

A number of recent observations on transcriptional activation and the intranuclear localization of regulatory proteins can be explained in terms of our model. It has been shown that the glucocorticoid receptor-mediated activation of mouse mammary tumor virus long terminal repeat fusion genes is strongly inhibited by sodium butyrate, a non-competitive inhibitor of animal and fungal histone deacetylases. Inhibition of the deacetylation reaction in this case caused the inhibition of the activation of transcription [52,53].

We have recently demonstrated that the nuclear localization of the c-*myc* protein, a protein with anionic domains, is dramatically affected by in vivo inhibition of histone deacetylase by butyrate (1 mM) in the myxomycete *Physarum polycephalum* [54,55]; the c-*myc* protein is associated with the nuclear matrix in a cell cycle dependent way [54]. In contrast to a number of other compounds tested, only butyrate completely abolished this specific association [55] without affecting the amount of this protein in the nuclei. In parallel butyrate leads to a distinct change of the acetylation pattern of the nuclear matrix bound core histones (Loidl, unpublished results). This finding presents circumstantial evidence that inhibition of histone deacetylation interferes with the correct binding of a DNA binding protein to a nuclear substructure.

For almost two decades n-butyrate was used as an experimental tool in the investigation of histone acetylation and its effects on chromatin structure and function. Apart from its inhibitory effect on histone deacetylases, this compound has been used as an inducer of cellular differentiation [56]. Recently, it has been shown that another substance, the antibiotic Trichostatin A, is a highly specific inhibitor (K_i 3.4 nM) of mammalian histone deacetylase [57]; strikingly Trichostatin is also a potent inducer of Friend cell differentiation [58]. These results again indicate the possibility that the specific inhibition of deacetylation may contribute to cellular reorganization.

It has been recently demonstrated that butyrate and Trichostatin inhibit the transcriptional activation function of *Myo*D and myogenin in proliferating myoblasts and differentiated myotubes [59], although the in vitro DNA binding ability remained unaffected. The authors explained this loss of transcriptional activation with the inhibition of histone deacetylase and the resulting change in the acetylation pattern of nucleosomes. In human breast cancer cells low concentrations of butyrate lead to reduced prolactin receptor gene expression; this inhibition was independent of ongoing protein synthesis and not due to an alteration of prolactin receptor mRNA half-life [60]. Our model would account for such an effect.

For the regulatory gene *are*A, which mediates nitrogen metabolite repression in *Aspergillus nidulans*, it has been shown that only mutations in the zinc finger domain and in the acidic region resulted in a loss of gene activation, thus confirming that anionic domains are indispensible for gene regulation [61].

Our current view of chromatin organization in general attributes an inhibitory function to nucleosomes for processes like DNA replication [62] and transcription [51,63,64]. For this reason one favours mechanistic ideas to explain the disintegration or transient removal of nucleosomes which results in the most accessible form of genetic information-free DNA. However, recently a first example of the opposite mechanism has been presented: nucleosomes and the correct chromatin assembly act as positive elements for retroviral integration site selection [65,66]. It was shown that the retroviral integration machinery prefers distinct target sites within nucleosomes, but not at all nucleosome-free DNA. Since nucleosomes are a uniform and invariable

structural unit, the best candidates for modulating their properties are changes in the N-terminal histone domains. It is widely accepted that the acetylation of the N-terminal lysines of core histones plays an important role for chromatin structure and function. However, we propose that the specific deacetylation and therefore the focused increase of the net positive charge of the nucleosome could play an equally powerful role in determining the properties of nucleosomes by a specific modification of contact sites for the binding of regulatory proteins, enzymes and structural elements, especially in the gene activation mechanism.

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