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Hypothesis

Towards an understanding of the biological function of histone acetylation

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A model is presented which explains the biological function of posttranslational acetylation of core histones in chromatin. Along the lines of this model histone acetylation serves as a general mechanism to destabilize nucleosome core particles during various processes occurring in chromatin. Acetylation acts as a signal that modulates histone-protein and histone-DNA interactions and finally leads to the displacement of particular histones from nucleosome cores. The high specificity of the acetylation signal for different processes (DNA replication, transcription, differentiation-specific histone replacement) is achieved by site specificity and asymmetry of acetylation in nucleosomes. The essential features of this model are in accord with the more recent results on histone acetylation.

Chromatin; Histone; Acetylation; Nucleosome; Cell cycle

1. INTRODUCTION

Nucleosomes, the basic structure of eucaryotic chromatin, are composed of 2 molecules each of the core histones H2A, H2B, H3 and H4, with histone H1 located at the position where the DNA enters and leaves the nucleosome core [1]. The wrapping of DNA around histone octamers together with the formation of higher order structures make it possible to pack the total nuclear DNA (1.8 m length in a diploid human cell) into a nucleus of approx. 6 μm in diameter. Despite this extreme packing the DNA must be duplicated and distinct regions of the genetic information must be accessible to transcription and other nuclear processes in a highly ordered way. It is still one of the most challenging questions of molecular biology how this accessibility to various nuclear processes is regulated.

In 1964 Allfrey and co-workers [2] discovered the posttranslational acetylation of NH_2-terminal lysine residues of the core histones. It was proposed that postsynthetic acetylation of histones has a key function in the determination of active genes [3,4]. All four core histones, but not histone H1, are subject to reversible acetylation of NH_2-terminal lysine residues; H2A has one site for possible acetylation, H2B, H3 and H4 each having four acetylation sites [3]. The formation of ϵ-*N*-acetyllysine residues decreases the positive charge of the NH_2-terminal domains of the histones. Therefore, it was assumed that the decrease in charge weakens the binding of histones to the DNA, thus facilitating the accessibility of chromatin for transcription [3,4].

Considerable efforts have been undertaken to demonstrate changes of the physicochemical properties of highly acetylated nucleosomes in comparison to control chromatin. Hyperacetylated

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chromatin can be prepared after cultivation of cells in the presence of sodium *n*-butyrate, an inhibitor of histone deacetylase(s). Butyrate leads to the accumulation of hyperacetylated core histones [5]. In fact, the majority of physicochemical studies, comparing control and hyperacetylated chromatin or nucleosome core particles, failed to detect dramatic changes of the relevant physicochemical properties [6–9]. All together these studies do not favour the idea of a general 'opening' of nucleosomes or the loosening of higher order folding of chromatin as a consequence of histone hyperacetylation.

The main basis for the proposal now presented is provided by investigations of histone acetylation in the lower eucaryote *Physarum polycephalum*. This organism offers the advantage of a naturally synchronous cell cycle and the capability of entering various differentiation pathways. Using this experimental system it was shown that there is no specific coupling between acetylation and transcription during the cell cycle [10]. Furthermore, the template activity of chromatin for transcription with endogenous and exogenously supplied RNA polymerases did not change upon hyperacetylation of core histones [11]. Analysis of the acetylation pattern during a differentiation process of *Physarum* (spherulation) indicated that acetylation is somehow involved in the process of histone replacement [12]. In micronuclei of the protozoan *Tetrahymena thermophila* it was shown that acetylation is involved in the deposition of histones onto chromatin [13].

Strong evidence for the involvement of histone acetylation in histone replacement has come from detailed analysis of spermatogenesis, where hyperacetylation of preexisting H4 occurs in committed nonproliferating cells with declining transcriptional activity. During this differentiation H4 acetylation correlates with the selective replacement of histones by small basic proteins, the protamines, that pack the DNA in sperm cells. It was therefore postulated, that histone acetylation assists in the replacement process [14–16].

It was suggestive to test the general validity of this replacement hypothesis during the synchronous cell cycle of *Physarum*. It was shown that during DNA replication all preexisting core histones, and during transcription preexisting H2A and H2B, are acetylated in a highly specific manner and subsequently replaced by newly synthesized histone molecules [17,18]. Experiments with sodium *n*-butyrate as well as protamine competition studies showed that, similar to spermatogenesis [14] and to spherulation of *Physarum* [12], a distinct acetylation of histones serves as a signal for the transient displacement of histones from DNA. The results together with the more recent literature fit into a general concept of the function of histone acetylation.

2. THE MODEL

The central claim of the proposed model is the acetylation of preexisting histones in chromatin whenever these have to be removed from DNA during various processes occurring in the nucleus (fig.1). It is proposed that acetylation serves as a specific signal that modulates the interaction of histones with DNA and other proteins and also the stability of higher order folding of chromatin. A nucleosome contains 8 histone molecules and therefore has 26 possible acetylation sites. Asymmetry of acetylation within a nucleosome will further increase the number of possible acetylation states, for instance, once H4 molecule of a nucleosome may be tetraacetylated, while the second may be in some other acetylated state. Another increase in possible combinations is contributed by the site specificity of acetylation; this means that certain sites are acetylated for distinct nuclear functions. This huge number of possible acetylation states provides a fine modulation for introducing discrete levels of nucleosome destabilization. The destabilization is not achieved by charge decrease of the NH_2-terminal arms of the histones. Therefore, an unspecific hyperacetylation (e.g. with butyrate) cannot mimic this specific signal. A highly specific acetylation acts as a distinct signal for induction or maintenance of certain structural features of chromatin, although the molecular mechanism by which acetylation acts is still unknown.

This model attributes a general function to histone acetylation for the displacement of histones from chromatin. The signal for different processes, like DNA replication, transcription and differentiation-specific histone replacement (fig.1), is highly specific and distinct.

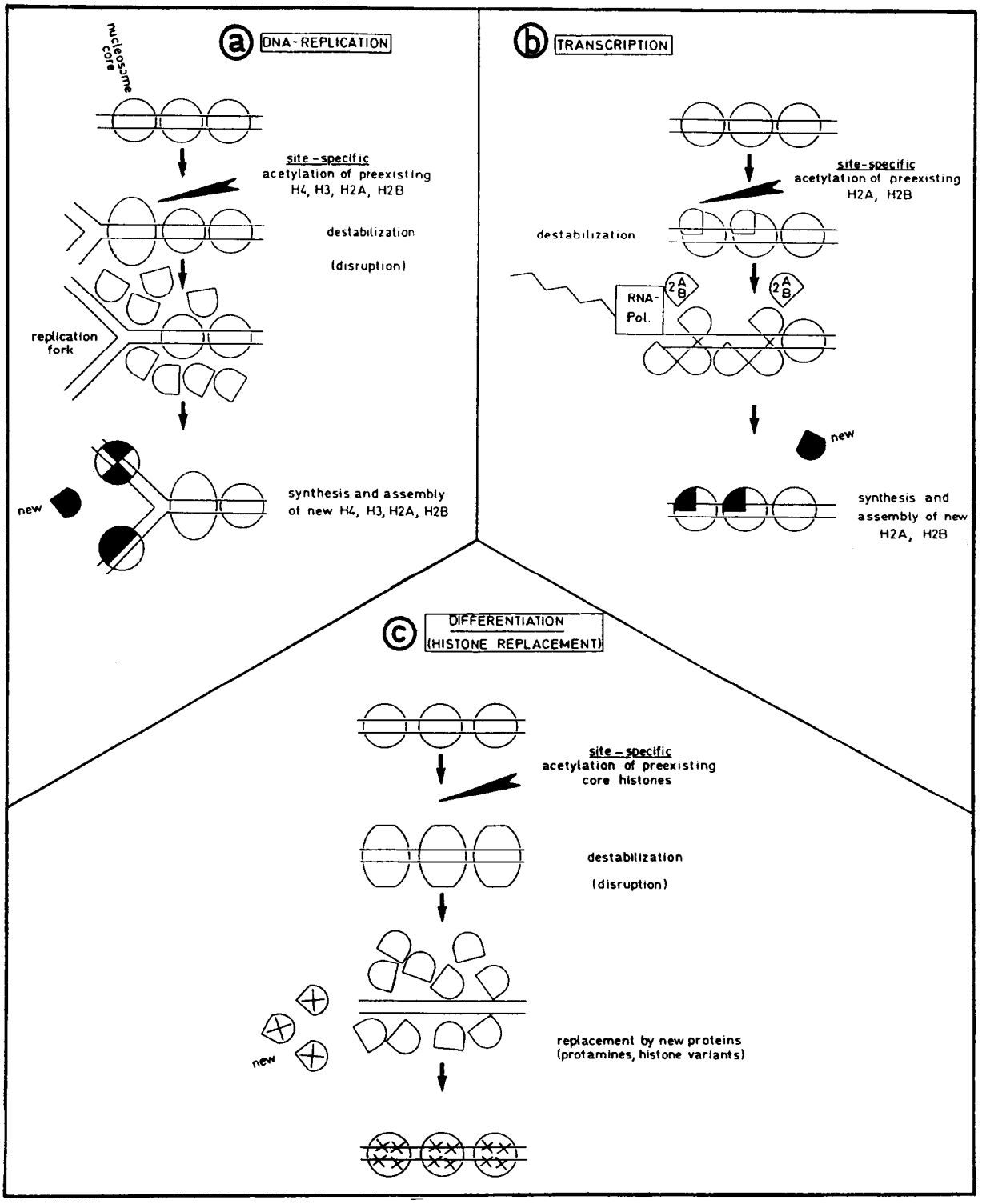

Fig.1. Proposed function of postsynthetic acetylation of core histones during various nuclear processes. (a) DNA replication; (b) transcription; (c) histone replacement during differentiation processes.

3. BIOCHEMICAL EVIDENCE

3.1. *Histone acetylation during the cell cycle and differentiation of* Physarum

It has been shown that a differentiation-specific histone synthesis occurs in the absence of DNA replication at a late stage of spherulation in *Physarum* [12]. This histone synthesis, which is essential for the subsequent germination of spherules, is preceded by a wave of acetylation into core histones. Thus, preexisting histones are acetylated and then replaced by newly synthesized molecules, probably histone variants (see fig.1c).

The same phenomenon was observed during growth of synchronous macroplasmodia. In S-phase all core histones are synthesized [17]. The assembly of newly synthesized histones into chromatin again is preceded by acetylation of all core histones. This acetylation is restricted to the preexisting histone molecules in chromatin [18]. Since nucleosomes have to be destabilized or even disrupted during DNA replication it is suggested that destabilization is mediated by acetylation of old core histones (fig.1a).

During the G_2 period of the *Physarum* cell cycle only histones H2A and H2B are synthesized [17]. It should be mentioned that during G_2 the bulk of transcription takes place (no DNA synthesis). In accord with the model (fig.1b) acetylation of only H2A and H2B is observed during the G_2 period [18]. There is good evidence that nucleosomes of transcribed sequences also have to be destabilized [19] and are most likely deficient in one H2A/H2B dimer [20,21]. The finding of acetylation of H2A and H2B and subsequent assembly of newly synthesized H2A and H2B into chromatin during transcription [18] are in line with these data.

3.2. *Diversity of acetylation, asymmetry of acetylation in nucleosomes and site specificity of acetylation*

26 lysine residues are accessible to posttranslational acetylation within a nucleosome. A tremendous heterogeneity of acetylation in a particular chromatin domain arises, if one considers that within such a region individual nucleosomes differ in their degree of acetylation. Moreover, there is evidence that nucleosomes are asymmetric with respect to the acetylation of a particular histone species [18]. This question of asymmetry (mosaic nucleosomes with respect to acetylation) was also addressed in a recent report on hyperacetylated H3 of nucleolar chromatin [22].

It is now well established that acetylation does not occur randomly among possible acetylation sites, but in a highly ordered way [23,24]. Therefore, different sites are acetylated for different biological functions. This is a strong indication that acetylation does not act via a simple neutralization of positive charges, but rather acts as a discrete signal for specific functions, as proposed in the model; protamine competition experiments reveal that butyrate-induced hyperacetylation cannot mimic such a signal, since the unique properties of S-phase chromatin (with a high acetylation state) are completely abolished upon hyperacetylation with butyrate [18]. Altogether this leads to an immense variety of possible acetylation states that allow the fine tuning of modulation of histone-protein or histone-DNA interactions.

3.3. *Acetylation during spermatogenesis; suppression of acetylation by cytostatic drugs*

As pointed out in section 1, histone acetylation is definitely involved in the process of histone replacement by protamines during spermatogenesis [14–16]. This histone exchange represents a special case which fits into panel c of the model (fig.1).

Another important support for the hypothesis comes from experiments with alkylating antitumor agents. It has been shown in Ehrlich ascites tumor cells that treatment with these agents causes a significant depression of histone acetylation that parallels the suppression of tumor cell growth [25]. All core histones are affected. Two agents suppress acetylation and proliferation without affecting histone biosynthesis. Along the lines of the model the suppressed acetylation of core histones would block the necessary displacement of core histones during DNA replication and thus lead to a perturbation of DNA synthesis with an inhibition of cell growth.

4. PERSPECTIVES

The hypothesis proposed in this paper attributes a general function to histone acetylation in the transient release of histones from nucleosomes.

Although the mechanism is the same for different nuclear processes, the acetylation signal itself is highly specific and distinct. Several nuclear processes that involve such a histone release could be tested in view of this hypothesis. Also the reevaluation of some of the old evidence on acetylation-transcription coupling seems warranted, since most of these previous results are themselves in line with the hypothesis, although the conclusions were not correct. The proposed modulation of nucleosome stability by histone acetylation and its influence on histone-protein and histone-DNA interactions raise the question of how other nuclear structures interact with nucleosomes and chromatin. Can histone acetylation affect the association of certain chromatin domains with the nuclear matrix [26]? Can histone acetylation interfere in the interaction of histones with hormone receptors [27] or other structural proteins, like tubulin [28]? What is the mechanism at the molecular level, by which acetylation modulates these interactions? Experimental answers to these questions could be also of importance for the understanding of other posttranslational modifications of histones occurring in chromatin.

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