Hypothesis

Histone deacetylase complexes: functional entities or molecular reservoirs

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Abstract Recent investigations have allowed the identification of an increasing number of distinct nuclear multi-component complexes containing several types of enzymatic activity. Many of the complexes containing histone deacetylases are believed to control transcription and chromatin remodeling. We suggest here that at least some of these abundant complexes are likely to be 'molecular reservoirs' of dynamic composition that exchange factors with other less abundant and functional complexes, according to specific required activities. **© 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

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1. Introduction

Multiple histone acetyltransferases (HATs) control the state of chromatin acetylation and hence play a key regulatory role in modulating the structure and the function of the chromatin [1]. These acetyl-mediated signals are reversed by multiple histone deacetylases (HDACs) counteracting the effect of specific HATs [2]. In higher eukaryotes, HDACs can be subdivided into three distinct groups known as class I, class II and class III, according to similarities of their sequences to those of yeast founding members: RPD3, HDA1 and SIR2 for classes I, II and III, respectively [3]. Interestingly, it has recently been shown in yeast that these deacetylases may have overlapping functions but could also influence distinct cellular processes [4]. In higher eukaryotes, HDACs belonging to the three classes mentioned above also appear to have overlapping as well as distinct regulatory roles [3]. These HDACs are found within complexes composed of proteins believed to regulate the activity of their catalytic subunits. At least three types of class I HDAC-containing complexes have been described in higher eukaryotes. Two of these complexes, NuRD/Mi2/NRD and Sin3/HDAC, contain both HDAC1 and HDAC2 [5]. The third type contains essentially HDAC3 and appears to be specifically associated with the repressive activity of the nuclear receptor co-repressor [3]. Moreover, the over-expression of class II HDACs has been shown to give rise to specific nuclear cores named matrix-associated deacetylase (MAD) bodies [6]. These nuclear cores contain several members of class I deacetylases in addition to the class II HDACs. However, all the cellular HDACs are probably not in these complexes, since individual HDACs were found associated with various transcriptional regulators [2]. Here we briefly review the composition of known HDAC-containing complexes and discuss the possibility that some might constitute molecular reservoirs containing HDACs and other regulatory molecules. These reservoirs would have a dynamic composition. They would serve as privileged nuclear sites where functionally related molecules would accumulate and from which they would be, individually or as complexes, carried to the sites of action.

2. Class I HDAC-containing complexes

The very first attempt leading to the identification of HDAC1 showed that the enzyme may function as a multiprotein complex. Indeed, the use of a trapoxin-based affinity matrix allowed the simultaneous purification of HDAC1 and an associated protein, RbAp48 [7], and HDAC2 was first cloned in a two hybrid screen using the transcription factor YY1 as a bait [8]. These early investigations suggested that these two HDACs could be part of multi-component complexes in cells. Subsequent isolation of mammalian mSin3 complex confirmed this hypothesis and showed the simultaneous presence of these two enzymes in a complex [9–11]. Further investigations demonstrated the existence of another HDAC-containing complex formed of endogenous HDAC1/2 associated with an ATP-dependent nucleosome remodeling activity, called nucleosome remodeling histone deacetylase complex or NuRD (or NuRD/Mi2/NRD) [12–15]. These independently isolated complexes share common proteins such as HDAC1/HDAC2, MTA1/2, RbAp46/48, and Mi2/CHD3/4. The dermatomyositis-specific autoantigen Mi2/CHD3/4 family proteins contain an ATPase/DNA helicase domain that is likely to be responsible for the ATPase activity of the NuRD/Mi2/NRD complex. These works also showed that NuRD/Mi2/NRD complexes were distinct from Sin3/HDAC since neither Mi2/CHD3/4 nor MTA1/2 was found in this later. The fact that HDAC1/2 almost always co-purifies with RbAp46/48 suggests the existence of a core deacetylase

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complex capable of participating in both NuRD/Mi2/NRD and Sin3/HDAC complexes. Surprisingly, however, the purified baculovirus-expressed deacetylase core complex, formed of HDAC1/2 and RbAp46/48, shows little or no HDAC activity. These observations suggest that the deacetylase activity of HDAC1/2 in the complex is regulated by partner proteins other than RbAp46/48. In agreement with this hypothesis, a stable HDAC1/2 complex has recently been identified which does not contain Rbp46/48 [16]. Indeed, these proteins have not been found in many transcription factor-containing HDAC1/2 complexes (for instance see [17]). Finally, the deacetylase activity of RbAp46/48–HDAC1/2 core complex was found to be dependent on the presence of MTA2, recruited through its direct interaction with MBD3 [18]. These findings firmly established the fact that the HDAC activity of a complex is regulated by its components.

The third class I HDAC, HDAC3, is also part of a complex. Indeed, immuno-purified HDAC3 associates with different sets of proteins including nuclear receptor co-repressors, SMRT and N-CoR [19]. Reciprocally, purified SMRT complex contains HDAC3 [20–22]. Furthermore, of the two multiprotein N-CoR complexes which were recently isolated, one contained HDAC3 [23].

In summary, HDAC1/2 and HDAC3 participate in distinct nuclear complexes. However, one should keep in mind that these are major abundant and stable cellular HDAC complexes. Cells would also contain many other minor ones involving various transcription factors as well as some of the components of the major complexes described above.

3. Class II HDAC-containing complexes

Endogenous class II HDAC complexes have not yet been isolated. However, ectopic expression of HDAC5 and 7 in CV-1 cells led to the identification of novel nuclear domains, namely the MAD bodies whose formation largely depends on the integrity of associated deacetylase activity [6]. Co-localization studies demonstrated that the MAD bodies are distinct from other well-characterized nuclear bodies such as the POD (or promyelocytic leukemia oncogenic domains), or the SC-35 RNA-processing domains [6]. Analysis of HDAC7 complex components revealed that the MAD bodies contain factors such as RbAp46/48, HDAC1, MTA2, and MBD3, also components of the NuRD/Mi2/NRD and Sin3/HDAC complexes. In addition, HDAC7 immunoprecipitates with mSin3A/3B and the nuclear receptor co-repressors SMRT and N-CoR. Interestingly, HDAC3 was also shown to associate with ectopically expressed HDAC4/5 and this association is likely to be tightly regulated (see below).

One of the unusual features of class II HDACs is their ability to shuttle between the nucleus and the cytoplasm. This property is believed to be cell type-specific and signal-dependent. With the exception of HDAC6, all the ectopically expressed class II HDACs have been shown to predominantly localize in the nucleus of CV-1 and HeLa cells [24–26]. In other cell lines, however, these HDACs localize primarily in the cytoplasm [27–30]. Interestingly, HDAC4/5 have been shown to immunoprecipitate with 14-3-3 family proteins, this association being dependent on the phosphorylation of HDAC4/5 [27,28]. Indeed, mutation of the serine residues, potential sites of phosphorylation, in HDAC4/5/7 inhibited their interaction with 14-3-3 ([27,28,30], Kao et al., unpublished data). All these data strongly suggest that the phosphorylation-dependent interaction between HDAC4/5 and 14-3-3 modulates the intracellular localization of these HDACs and hence their function. This hypothesis was further supported by the demonstration that the activation of calcium/calmodulin-dependent protein kinase, specifically phosphorylating HDAC5, induces myogenesis by disrupting the repressive MEF2–HDAC complexes and stimulating the HDAC nuclear export [29,30]. Conversely, the activation of the Ras-MAPK pathway results in the accumulation of HDAC4 in the nucleus of C2C12 myoblasts [31].

4. Concluding remarks

Analysis of the two major class I HDAC complexes, Sin3/HDAC and NuRD/Mi2/NRD, showed that HDAC1/2 and RbAp46/48 form the core of both complexes. Depending on the nature of its associated components, this core may participate to either one of these two complexes. Moreover, the nucleocytoplasmic shuttling of the class II HDACs strongly suggests the dynamic nature of nuclear or cytoplasmic complexes containing these HDACs.

Here we propose that certain HDAC complexes, rather than being functional entities, would actually be sites of accumulation for proteins collectively involved in one or several specific functions. These reservoirs would play a logistic role in permanently providing regulatory and structural proteins to regions of intense nuclear activity. For example, sites of DNA replication could be such regions, constantly in need for various enzymes involved in both DNA replication and chromatin assembly. Major HDAC complexes might serve as reservoirs capable of delivering some of its subunits, such as RbAp46 [32] as well as HDAC1 and/or HDAC2, to the active chromatin assembly machinery, so that they deacetylate histones after the assembly of nucleosomes. Although there is no direct evidence for the association of NuRD/Mi2/NRD and/or Sin3/HDAC complexes with the replicating DNA, it was recently shown that BCL6 cores, formed after the expression of BCL6 oncogene, were associated with replication foci and that replication progressed around these cores [33]. These BCL6 bodies, which are distinct from the well-characterized PML cores, could actually have the same constitution as the recently identified MAD bodies. Indeed, many of the components of the MAD bodies are known to interact with BCL6, including the three related class II deacetylases HDAC4, HDAC5, HDAC7, nuclear co-repressor SMRT/N-CoR, as well as class I deacetylases (Lemercier, Albagli, Kao, Evans and Khochbin, unpublished results). Interestingly, these BCL6 cores are dynamic structures. In G1 phase cells, BCL6 shows a diffuse nuclear distribution, whereas the S phase of the cell cycle is associated with the formation of BCL6 cores surrounded by replicating DNA [33]. These observations suggest that BCL6 interaction with partner proteins is also a dynamic process and support the idea of the BCL6 cores being sites of molecular trafficking. These BCL6 cores could therefore be considered as molecular reservoirs supporting chromatin maturation and remodeling, after DNA replication.

Besides the HDAC-containing nuclear complexes, an increasing number of individual transcription factors are found associated with HDACs. For instance HDAC1 or HDAC2 were found associated with Rb, YY1, Sp1, BRCA1, etc. [2], none of which was found in the two well-characterized

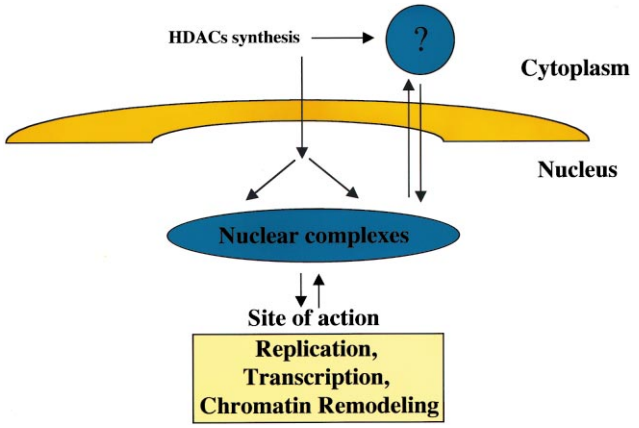

Fig. 1. Schematic representation of the 'molecular reservoir' hypothesis. Nuclear complexes serve as molecular reservoirs supporting several activities. After translation, HDACs enter the nucleus to participate in nuclear complexes or remain in the cytoplasm. Nuclear HDAC-containing complexes have a dynamic constitution and are sites of molecular trafficking, receiving proteins from the cytoplasm and sending them to sites of action, such as zones of DNA replication, transcription and chromatin remodeling. Some of the HDACs are also submitted to a regulated nucleocytoplasmic shuttling and are therefore sent to yet unknown cytoplasmic complexes.

NuRD/Mi2/NRD and Sin3/HDAC complexes. Also, other transcription factors, such as p53, c-Ski, Ikaros, etc., were shown to interact with HDAC1/2 associated with several components of the two deacetylase complexes [2], but again, neither of them was associated with the 'holo' NuRD/Mi2/NRD and Sin3/HDAC complexes. These observations argue in favor of our hypothesis and suggest that NuRD/Mi2/NRD, Sin3/HDAC or other abundant HDAC-containing complexes may be reservoirs that would send, upon request, HDACs and/or other associated proteins to their sites of action.

Finally, characterization of *Xenopus* deacetylase-containing complexes has provided strong arguments in favor of the 'reservoir hypothesis' (Fig. 1). Indeed, the endogenous pool of oocyte RPD3 (a *Xenopus* class I HDAC) was found to be equally distributed between the nucleus and cytoplasm but RbAp48 involved in the core of both *Xenopus* Sin3/HDAC [34] and NuRD/Mi2/NRD [12] was found only in the nucleus [35]. These findings strongly suggest that the cytoplasmic pool of RPD3 can supply the nuclear complexes which in turn may provide the components of various nuclear repressor complexes such as those involved in methylated DNA transcriptional repression [34].

This 'reservoir' hypothesis also helps to explain why a single complex contains distinct HDACs. Indeed, it is not understandable why a single functional entity would contain both HDAC1 and HDAC2. These two deacetylases are almost identical in their 2/3 N-terminal region but contain divergent sequences in their C-terminal portions suggesting that they could have the same substrate specificity with the potential to be recruited by different partners [36]. In this context, it is difficult to explain why a single complex, instead of increasing the number of one enzyme, would recruit two different enzymes with the same substrate specificity. According to our hypothesis these two HDACs would be stored in 'reservoir' complexes and would individually be delivered to their specific sites of recruitment.

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