Review

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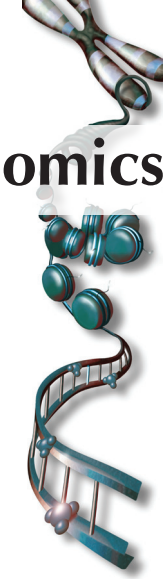
Epigenomics



Regulation of class IIa HDAC activities: it is not only matter of subcellular localization

In response to environmental cues, enzymes that influence the functions of proteins, through reversible post-translational modifications supervise the coordination of cell behavior like orchestral conductors. Class IIa histone deacetylases (HDACs) belong to this category. Even though in vertebrates these deacetylases have discarded the core enzymatic activity, class IIa HDACs can assemble into multiprotein complexes devoted to transcriptional reprogramming, including but not limited to epigenetic changes. Class IIa HDACs are subjected to variegated and interconnected layers of regulation, which reflect the wide range of biological responses under the scrutiny of this gene family. Here, we discuss about the key mechanisms that fine tune class IIa HDACs activities.

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The class IIa HDACs

Lysine acetylation is a post-translational modification (PTM) that, when exercised on histones, plays a key role in marking transcriptionally active genomic regions. Similarly to other PTMs, acetylation is not restricted to chromatin remodeling but it can modulate different molecular machineries, which control cell cycle progression, actin nucleation, splicing and nuclear transport [1].

Lysine-acetylation homeostasis is under the supervision of two families of enzymes, with antagonistic activities: the histone acetyl transferases and the histone deacetylases (HDACs). HDACs repertoire in mammals comprises 18 genes that can be grouped into five subfamilies on the basis of their sequence homology and phylogenetic criteria [2]. To the subclass IIa belong *HDAC4*, *HDAC5*, *HDAC7* and *HDAC9* (for an excellent review on HDACs see [2]). These proteins share specific and characteristic features, which are highlighted in Box 1.

Class IIa HDACs as part of multiprotein complexes are involved in the regulation of assorted cellular responses. They generally act at the apex of specific genetic programs, by influencing the landscape of gene expressed in a specific context. Although principally investigated as regulators of transcription and in particular of myocyte enhancer factors (MEF2) transcription factors, alternative partners and functions, in particular when localized in the cytoplasm, cannot be excluded (for a discussion on some class IIa HDACs partners see [3]).

Similarly to other epigenetic modifiers, class IIa HDACs do not recognize the DNA, instead by interacting with a selected number of transcription factors, they are recruited on specific genomic regions in a sequence-dependent manner [2]. During embryonic development, class IIa HDACs are actively involved in controlling specific differentiation pathways and tissue morphogenesis. In adult tissue they are part of several adaptive responses (for recent reviews see [4,5]).

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Box 1. Class IIa histone deacetylases hallmarks.

- An extended amino-terminal region involved in the interaction with several partners and different transcription factors, which recruits these repressors on specific genomic regions
- A carboxy-terminal deacetylase domain that, in vertebrates, is enzymatically 'inactive' (or at least inactive vs acetyl-lysine)
- The co-presence of nuclear localization sequences and of nuclear export sequences, which confers environmental signaling regulated nuclear/cytoplasmic shuttling
- Interactions with several partners, comprising multiprotein complexes recruiting class I histone deacetylases, which confer the deacetylase activity

Not surprisingly, these arenas of copious interventions find correspondence in the multiple options available to oversee class IIa HDACs activities. In this review, we will discuss about these different options and how cells and the microenvironment govern class IIa HDACs activities.

Transcription is the first decision: when & where

Paradoxically, although the tissue-specific expression of class IIa HDACs was immediately considered a distinctive trait of this subfamily [4], a limited number of studies have addressed the control operated at the level of transcription on these genes. Apparently, class IIa HDACs transcription is under the control of many signaling pathways.

Genomic organization

The complexity of such regulation is mirrored by the existence of several transcriptional variants. A summary of the genomic organization of the different class IIa HDACs is shown in Table 1. Concerning *HDAC4* only a single isoform has been described up to now, composed by 26 coding exons and 1 noncoding exon (mRNA 8980 bp and ORF/open reading frame, 3252 bp-long) [6]. The reference sequence of the longer RNA encoded by *HDAC5* locus is 5324 bp and produces a 3369 bp ORF that comprises all coding exons of its paralog *HDAC4* [6]. A second described transcription variant of *HDAC5* lacks exons 14 and 15 and produces a protein of 75 amino acids (aa) shorter, characterized by a deficient catalytic domain [6]. Two different splicing isoforms of *HDAC7* have been characterized [6,7]: the most common spliced isoform is made up of 25 exons and an unspliced isoform that retains the first intron after the first ATG [7]. This unspliced mRNA could in principle produce two products. A small peptide of 7 aa and a protein of 22 aa shorter compared with the common form [7]. In this case a second ATG proficient in translation is used [7]. The unspliced and spliced isoforms differentially influence cell proliferation and their generation is regulated during vascular smooth muscle cells (VSMCs) differentiation [7,8]. Curiously, the last 612 bp of HDAC7 are antisense respect to the *SLC48A1* gene, raising the possibility of an involvement in controlling the translation of this gene [6].

The canonical isoform of *HDAC9* is made up of 23 coding exons, which are translated into a 1011 aa polypeptide [6,9]. A well-known variant of *HDAC9* is called *MITR* or *HDAC9ΔCD* (*HDRP*/*MITR*) [10]. It generates a shorter protein (593 aa) lacking the deacetylase domain. This variant is not a simple truncation of full-length *HDAC9*, because it retains 16 unique residues codified by intron 12 which are 3′ to the canonical splice donor site [9]. Despite the truncation, MITR represses MEF2-dependent transcription [10].

Table 1. Class IIa histone deacetylases genomic organization.

Histone deacetylases	Localization	Extension	TSSs	SV	mRNA	Exons	Longer ORF
HDAC4	Chr 2q37.3 minus strand	353479	7	16 s 6 uns	1	27 (26c/1nc)	3256
HDAC5	Chr 17q21 minus strand	46893	8	17 s 6 uns	2	27 (26c/1nc)	3369
HDAC7	Chr 12q13.1 minus strand	37890	17	31 s 5 uns	2	25	2856
HDAC9	Chr 7p21.1 plus strand	912579	9	17 s 6 uns	6	23	3033

Extension: Genomic bp; mRNA: Described mature mRNAs; ORF: Open reading frame; S: Spliced; SV: Different splicing variant; TSS: Different transcription start site; Uns: Unspliced.
Data were taken from [6].

Removal of exon 7 during splicing generates a protein (*HDAC9*Δexon7) which is 37 aa shorter and that lacks two serines (Ser223 and Ser253) involved in the nuclear export and a portion of the nuclear localization signal. Another exon that may be removed is the twelfth. The corresponding protein is 981 aa long and, since the exon 12 contains the SUMOylation site, this splice variant cannot be SUMOylated [9,11]. Additional *HDAC9* splicing variants include an isoform lacking both the seventh and the twelfth exons [9] and a deletion of a portion of the 15th exon, which generates similarly to *MITR*, a protein deprived of the deacetylase domain [9].

Transcriptional control

Experimentally, the expression of HDAC4 can be repressed by mithramycin [12], which after binding to GC-rich sequences displaces the Sp transcription factors [13]. Binding of Sp1 and Sp3 to the *HDAC4* promoter was confirmed by EMSA and ChIP experiments and the manipulation of Sp1 and Sp3 expression was coupled to a parallel variation of *HDAC4* expression [12]. Furthermore, the antineoplastic properties of the turmeric root derivative curcumin seem to partially depend on the inhibition of the Sp1 action on *HDAC4* promoter [14].

Also *HDAC7* transcription seems to be under the control of Sp1. In particular, it was reported that Sp1 stimulates the transcription of *HDAC7* mRNAs during the PDGF-BB-induced differentiation of murine embryonic stem cells into smooth-muscle cells (SMCs) [15]. *HDAC7* expression is similarly induced by PDGF-BB also in VSMCs [16].

In addition to Sps, class IIa HDACs transcription must depend on additional circuits, operating in a tissue-specific manner. For example, *HDAC4* expression is induced after denervation in atrophic muscles, where it influences a metabolic shift [17]. A feed-forward mechanism is involved in such regulation. The initial nuclear relocalization of HDAC4 in atrophic muscles causes the activation of myogenin, which in turn induces *HDAC4* transcription, thus alimenting the expression of the deacetylase [17].

In bones, *HDAC4* is the highest expressed class IIa HDACs and it plays an irreplaceable role in the process of chondrocyte hypertrophy [18]. During skeletogenesis, the levels of HDAC4 increase in murine prehypertrophic chondrocytes in the growth plate at E18.5, while HDAC4 is not expressed at detectable levels in proliferating chondrocytes, in bones and osteoblasts [18]. The contribution of the transcriptional machinery in this switch is unknown.

Similarly, *HDAC7* is highly expressed in pre-B cells but dramatically downregulated, both at RNA and protein levels, during lineage conversion to macrophages [19]. Although HDAC7 repression is necessary for the transcription of MEF2-target genes, which are important for macrophage functions, the mechanisms that control HDAC7 levels still need to be investigated [19].

In embryonic stem cells Oct3/4 prevent HDAC4-mediated repression of stemness, by binding the first intron of the deacetylase and thus interfering with splicing maturation [20]. This regulation was hypothesized to limit the negative influence of class IIa HDACs on stemness, possibly through the repression of *Oct3/4* and *Klf4* genes.

Upregulation of class IIa HDACs transcription is observed during muscle differentiation, as part of a negative-feedback loop, to fine tuning the rate of differentiation. In this case MEF2A, MEF2C and MEF2D are able to bind the promoter of *Hdac9* and to induce its expression [21]. It is not clear at the moment whether MEF2s regulate directly also the transcription of other class IIa HDACs (see below).

The control operated by MEF2s on class IIa transcription could also be involved in the compensatory responses observed in different experimental settings. In certain cell lineages when a class IIa HDAC member is depleted another member is upregulated [22–24]. Once MEF2 get rid of a class IIa HDACs member, they might transcribe other members of the family, by binding to the proximal promoter (in case of *HDAC5* and *HDAC9*) or to the enhancer (for *HDAC4* and *HDAC7;* see below).

Although few information are available on the transcriptional control operated on class IIa HDACs, the ENCODE project disposes of the ChIP-Seq data for several transcription factors (TFs) and other elements controlling transcription [25]. Numerous elements including TFs, epigenetic modifiers and architectural proteins bind the proximal promoter of *HDAC4* and *HDAC7*, thus suggesting that transcription of these two deacetylases is under intense supervision (Figure 1). Less covered are the proximal promoters of *HDAC5* and of *HDAC9*. Binding of MEF2 TFs was confirmed in the proximal promoter of *HDAC9* (Figure 2). MEF2s bind also the *HDAC5* proximal promoter, hence also this deacetylase can be under control of MEF2s as part of a negative feedback loop [24]. In general, the complexity of TFs and epigenetic regulators assembling onto the promoters of class IIa HDACs testifies the importance of the transcriptional control in the homeostasis of this gene family.

Different proto-oncogenes and cell cycle supervisors bind the promoters of class IIa HDACs (Figures 1 & 2). Interestingly, in support of an involvement of *HDAC4* and HDAC7 in the processes of oncogenic transformation [26], some proto-oncogenes such as JUN, FOS, MYC, which control the G0/G1 transition, bind the proximal promoters of these deacetylases [25]. These

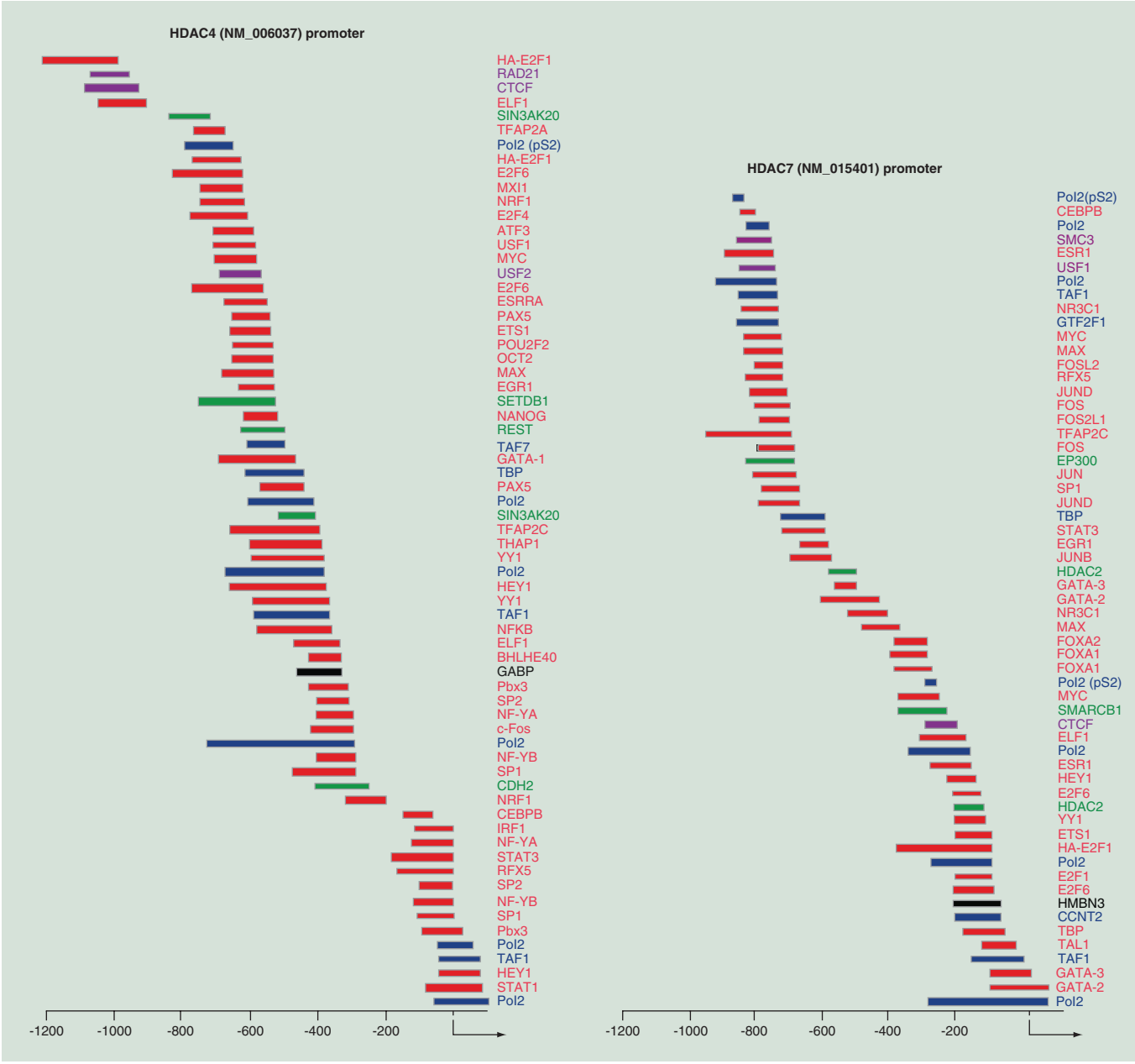

Figure 1. Available ChIP-Seq data from eight different cells lines were analyzed for the binding to the proximal promoters (-1/-1000) of HDAC4 and HDAC7. Transcription factors (red), epigenetic modifiers (green), basic transcriptional machinery (blue), architectural proteins (violet) and their relative region of binding are indicated.

proto-oncogenes could be responsible for the upregulation of *HDAC7* mRNA observed in response to PDGF or serum stimulation [15] and of *HDAC4* during differentiation of osteoblasts exposed to EGF-like ligands [27]. The ENCODE project finds at least six regulators, which bind the immediate early promoter of all the four class IIa HDACs: Pol2, TAF1, TBP, SP1, YY1, MAX. Not surprisingly, the first three belong to the basal transcription machinery. The presence in this list of Sp1 confirms the early studies above described. Regarding YY1, it usually binds the chromatin in close proximity of the transcription start site of highly expressed genes [28]. The contemporary presence of MAX and YY1 on the promoters of these deacetylases suggests that class IIa HDACs could be part of the Myc transcription network.

Control of the mRNA stability: miRNAs targeting class IIa HDACs in physiological contexts

The regulation of the mRNA stability offers another strategy to impact on class IIa HDACs. microRNAs

targeting these enzymes were initially discovered by studying muscular and chondrocyte differentiation. During myogenesis MEF2, in addition to the negative feedback loop involving *HDAC9*, controls also the expression of miR-1, which targets *HDAC4* mRNA, thus fueling a positive feedback loop [29,30]. However, this model is complicated by the fact that miR-1 can also target the 3′UTR of *MEF2A* [31]. A similar positive feedback loop is operative during neurogenesis. In this case MEF2C promotes the transcription of miR-9, which targets the 3′UTR of *HDAC4* [32].

In myoblasts the relative abundance of miR-1 and miR-133 contributes to the equilibrium between proliferation and terminal differentiation [29]. In particular, miR-1 targets the 3′UTR of *HDAC4* and promotes myogenesis, while miR-133 destabilizes serum response factor and sustains proliferation. Curiously, the two miRNAs derive from the same polycistronic pre-miRNA and are transcribed together [29]. Importantly, in embryonic hearth and in the somite, MEF2 together with MyoD positively regulate the transcription of both these miRNAs, by binding to an miR-1/-133 intragenic enhancer [30].

miR-1 is required for the correct fusion of myoblasts but it is also induced by the mTOR pathway and has prohypertrophic properties [33]. miR-1 stimulates also chondrocyte hypertrophy again through HDAC4 inhibition [34]. Another miRNA upregulated during chondrogenesis is miR-365. In particular, miR-365 is mechanosensitive and determines the induction of Hh and collagen X expression, the latter through the direct targeting of HDAC4 [35]. During osteoblast differentiation, HDAC4 is also regulated by miR-29b, which promotes osteogenesis [36].

TGF-beta represses myogenesis and muscle differentiation. In C2C12 cells, TGF-beta inhibits muscle differentiation, through the repression of miR-206 and miR-29 and thereby by augmenting HDAC4 expression [37]. Importantly, miR-206 represses hypertrophy of myocytes *in vitro* but has no effects in the regulation of muscle hypertrophy *in vivo*, although in both cases it causes a massive drop in HDAC4 levels [37]. Among the plethora of responses regulated by miR-206, it was described that the repression of HDAC4 delays amyotrophic lateral sclerosis progression and promotes regeneration of neuromuscular synapses [38,39]. Finally, miR-206 displays strong tumor-suppressive properties in gastric cancers, again partially due to the repression of HDAC4 [40]. Recently, a cross-talk between the NRF2, HDAC4 and miR-1/miR-206 was described in cancer cells and *in vivo* tumor models [41]. In KEAP-1-deficient lung cancer cells NRF2 is hyperactive and promotes the reduction of cysteines 667 and 669 in HDAC4 (see below). As a consequence, HDAC4 accumulates in the nucleus [41,42] and represses the transcription of miR-1 and miR-206 [41]. Among miR-1 and miR-206 targets there are key-genes involved in the control of pentose phosphate pathway and tricarboxylic acid cycle [41]. Therefore the activation of NRF2 in cancer cells, at least in part through the HDAC4-mediated repression of miR-1/miR-206, reprograms glucose metabolism toward the pentose phosphate pathway thus providing the substrates needed to support cell growth [41].

Another pathway controlling *HDAC4* mRNA stability, via the same miRNAs (MEF2/miR-1/miR-206/NOTCH3) is active in myoblasts, where it ensures that differentiation takes place with the right timing [43,44]. NOTCH3 delays muscle differentiation at least in part by promoting the dephosphorylation of MEF2. An increase in MEF2 levels is sufficient to overcome this inhibition and to directly transcribe miR-1 and miR-206. In turn, these miRNAs have a double prodifferentiative effect since they target both HDAC4 [29] and NOTCH3 [43].

Certain studies have proposed a role of HDAC4 in the pathogenesis of Huntington's disease [45,46]. In agreement, targeting of HDAC4 by miR-22 shows neuroprotective effects [47]. miR-22 is upregulated also during myocyte differentiation and cardiomyocyte hypertrophy [47]. Its overexpression is sufficient to induce cardiomyocyte hypertrophy *in vivo,* while miR-22-null hearts are resistant to stress-induced cardiac hypertrophy [48]. These phenotypes at least in part might be explained by the suppression of HDAC4 [48].

miR-22-mediated silencing of *HDAC4* is also involved in the pathogenesis of emphysema [49]. In particular, high levels of miR-22 are present in antigen presenting cells, derived from a murine model of lung emphysema. This upregulation causes a strong decrease in HDAC4 levels and the subsequent increase in the release of IL-6. IL-6 stimulates the activation of the TH17 subset of helper T cells that are responsible for the establishment of a state of chronic inflammation [49].

Finally, some miRNAs regulated during embryogenesis recognize *HDAC4* mRNA, thus abolishing its repressive activities on certain loci. For example in the fetal brain, levels of the epigenetic regulators methyl CpG-binding protein 2 and HDAC4 are kept low by presence of miR-483-5p [50].

While in literature the stability of the 3′UTR of *HDAC4* has been studied in details, little information are currently available on the regulation of the other class IIa HDACs by miRNAs. Few miRNAs target the 3′UTR of *HDAC5* in an exclusive manner. One of them is miR-2861 [51,52]. Similarly and redundantly to miR-1, miR-2861 is a positive regulator of chondrocyte hypertrophy and osteoblast differentiation, through

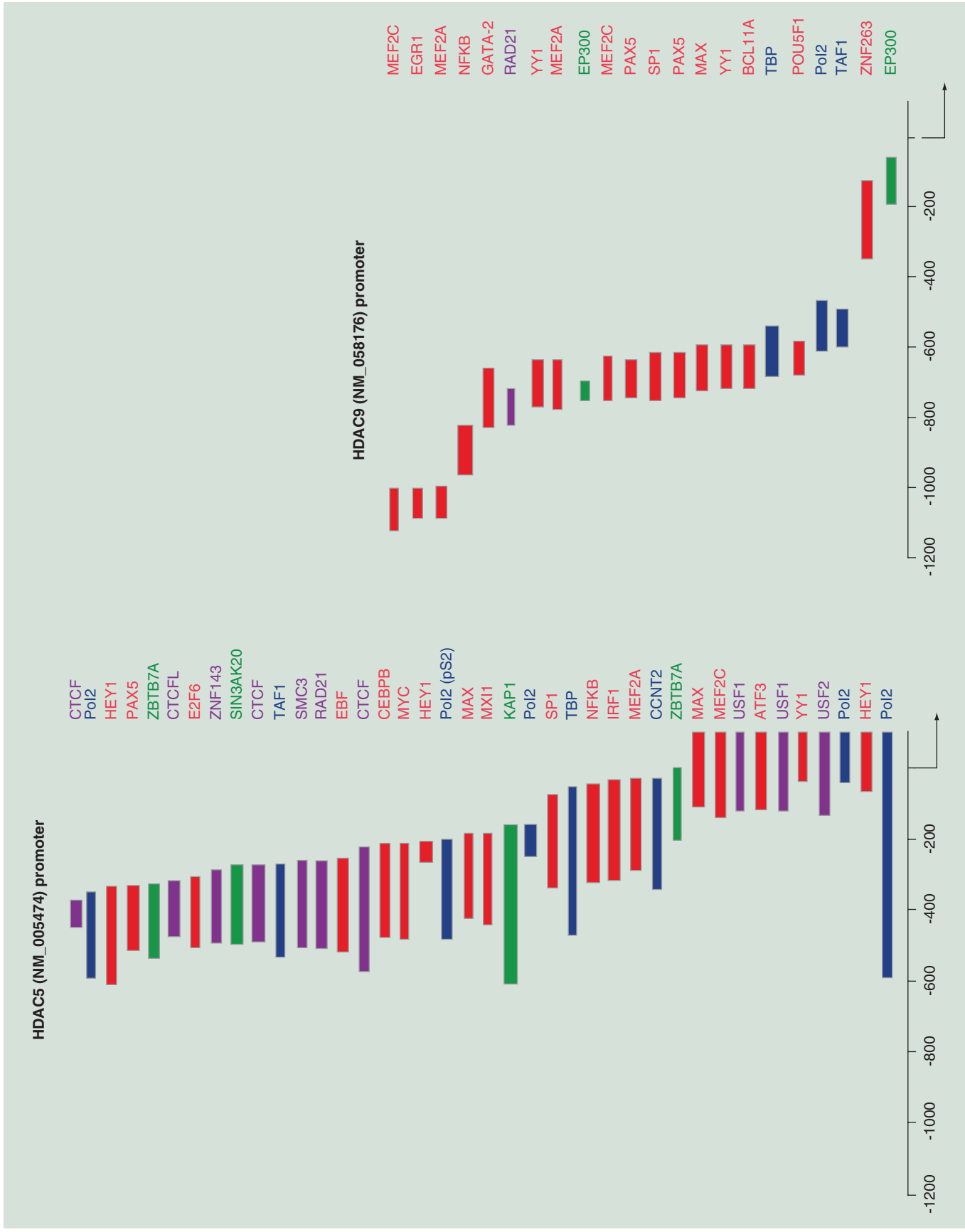
HDAC5 (NM_005474) promoter
CTCF
Pol2
HEY1
PAX5
ZBTB7A
CTCFL
E2F6
ZNF143
SIN3AK20
CTCF
TAF1
SMC3
RAD21
EBF
CTCF
CEBPB
MYC
HEY1
Pol2 (pS2)
MAX
MXI1
KAP1
Pol2
SP1
TBP
NFKB
IRF1
MEF2A
CCNT2
ZBTB7A
MAX
MEF2C
USF1
ATF3
USF1
YY1
USF2
Pol2
HEY1
Pol2
-200
-400
-600
-800
-1000
-1200
HDAC9 (NM_058176) promoter
MEF2C
EGR1
MEF2A
NFKB
GATA-2
RAD21
YY1
MEF2A
EP300
MEF2C
PAX5
SP1
PAX5
MAX
YY1
BCL11A
TBP
POU5F1
Pol2
TAF1
ZNF263
EP300
-200
-400
-600
-800
-1000
-1200

Figure 2. Available ChIP-Seq data from eight different cells lines were analyzed for the binding to the proximal promoters (-1/-1000) of HDAC5 and HDAC9 (see facing page). Transcription factors (red), epigenetic modifiers (green), basic transcriptional machinery (blue), architectural proteins (violet) and their relative region of binding are indicated.

the targeting of *HDAC5* and the subsequent activation of RUNX2 [51]. miR-2861 also targets the 3′UTR of *HDAC5* in hamster ovary CHO cells [52]; by decreasing HDAC5 levels, miR-2861 enhances the productivity of these cells that are frequently used as cell factories for the production of recombinant proteins, feeding the industrial and economic interest around this miRNA.

Very recent is the finding that *HDAC9* dysregulation may be involved in the age-related bone loss [53]. In particular, old mice are characterized by an increase of miR-188 levels in bone marrow stromal cells. miR-188 targets *HDAC9* and *RICTOR* expression and the silencing of these two genes seems to be sufficient to regulate the switch between osteogenesis and adipogenesis in bone marrow stromal cells [53].

Control of the mRNA stability: miRNAs targeting class IIa HDACs in cancer

Interestingly, most of the miRNAs that target the 3′UTR of class IIa HDACs are repressed in certain cancers and display tumor suppressive traits. This observation is in agreement with the reported oncogenic properties of class IIa HDACs [23,26]. miR-1 is repressed in several cancers, such as lung cancer [54], hepatocellular carcinoma (HCC) [55] and chordoma [56]. In these contexts its repression is associated to the upregulation of FoxP1, MET and HDAC4 [54,55]. A similar upregulation of HDAC4 was correlated, in several HCC samples, with a reduced expression of miR-22 [57]. HDAC4 is targeted also by miR-140. In osteosarcoma and colon cancer cells this miRNA is associated with chemosensitivity [58].

Waldenström macroglobulinemia, a rare B cell low-grade lymphoma, is characterized by a reduced expression of miR-9* [59]. This microRNA directly affects the levels of HDAC4 and HDAC5. Restoring of miR-9* levels provokes the downregulation of class IIa HDACs and increases p21/CDKN1A levels [60,61], thus limiting Waldenström macroglobulinemia cells proliferation [59]. An autoregulatory loop, as above described for miR-1, involves miR-200a and it is deregulated in HCC. miR-200a affects the stability of HDAC4 and, in a feedback manner, the deacetylase decreases miR-200a transcription, by disturbing the binding of Sp1 to its promoter. Downregulation of miR-200a enhances the proliferation and migration of HCC cells, whereas its upregulation inhibits both the responses [62].

Similar correlations between HDAC4, microRNAs and tumors have been observed in breast cancer, where low levels of miR-125a-5p are bad prognostic markers and the majority of the tumor suppressive properties of miR-125a-5p depends on HDAC4 suppression [63].

In B cells, unlike the aforementioned cases, the upregulation of a miRNA (miR-155) that targets HDAC4 has proliferative effects [64]. These differences could reflect cell lineages specificities or the contribution of additional genes under the influence of miR-155.

In tongue squamous cell carcinoma, miR-140-5p can influence the expression of a gene cluster that includes ADAM10, LAMC1, PAX6 and HDAC7. These genes affect cell motility and could be responsible for the metastatic phenotype [65]. miR-34 can repress HDAC7 as well as HDAC1 expression. In breast cancer miR-34 is significantly downregulated, HDAC1 and HDAC7 levels are augmented and as a consequence HSP70 is deacetylated. The authors proposed that such modification confers resistance to chemotherapy-induced autophagic cell death [66].

A summary of the different microRNAs targeting class IIa HDACs is reported in Table 2.

Proteolytic processing or massive degradation

Selective proteolysis can influence class IIa HDACs activities. During apoptosis, HDAC4 and HDAC7 are cleaved, respectively, by caspase-3 [67] and caspase-8 [68]. In both cases the cleavage products increase the apoptotic rate [67–69], but only for HDAC4 the amino-terminus generated-fragment is competent for MEF2 repression [67].

Another selective proteolytic processing was observed to modulate the hypertrophic response. In cardiomyocytes, protein kinase A (PKA) activation causes the cleavage of HDAC4 between residues 201 and 202, operated by an unidentified protease [70]. The generated amino-terminal fragment accumulates in the nucleus and it is competent for MEF2 repression but it is incompetent for SRF repression [70]. The binding site for PKA is present only in HDAC4 (aa 638–651), among all class IIa HDACs. The anti-hypertrophic effect of PKA is sufficient to antagonize the pro-hypertrophic actions of CaMKII, without affecting cardiomyocyte survival [70].

The ubiquitin-proteasome system (UPS) can also affect class IIa HDACs levels. Treatment of HEK293 cells with ALLN and MG132, two inhibitors not entirely proteasome-specific, provokes an increase in the levels HDAC4, HDAC5 and HDAC7 [71]. In the case of HDAC7, the authors suggested that

Table 2. miRNAs targeting class IIa histone deacetylase.

miRNA	Target	Biological process regulated	Pathway/feed-back	Ref.
miR-1	HDAC4	Myogenesis, chondrocyte hypertrophy, some cancers	Induced by MEF2; induced by myod after mtor activation	[29–31,33–34,54–56]
miR-9	HDAC4	Neurogenesis	Induced by MEF2C	[32]
miR-2861	HDAC4, HDAC5	Chondrocyte hypertrophy, productivity of recombinant CHO cell lines		[51,52]
miR-365	HDAC4	Chondrocyte hypertrophy		[35]
miR-29a	HDAC4	Myogenesis		[37]
miR-206	HDAC4	Myogenesis, muscle hypertrophy, amyotrophic lateral sclerosis, gastric cancer	Represses by HDAC4 in KEAP-1 deficient lung cancer cells	[37–41]
miR-22	HDAC4	Neurodegeneration, myogenesis, cardiac hypertrophy, hepatocellular carcinoma		[47–49,57]
miR-483-5p	HDAC4	Neurogenesis		[50]
miR-140	HDAC4	Chemosensitivity		[58]
miR-9*	HDAC4, HDAC5	Waldenström macroglobulinemia pathogenesis		[59]
miR-200a	HDAC4	HCC proliferation and migration	Repressed by HDAC4	[62]
miR-125a-5p	HDAC4	Breast cancer aggressiveness		[63]
miR-155	HDAC4	B-cells proliferation		[64]
miR-140-5p	HDAC7	Metastatization of tongue squamous cell carcinoma		[65]
miR-34	HDAC7	Breast cancer refractoriness to treatment		[66]
miR-188	HDAC9	Osteogenesis		[53]

When available, a pathway of regulation is provided.
CHO: Chinese hamster ovary; HCC: Hepatocellular carcinoma.

HDAC7 is degraded mainly in the cytoplasm after its phosphorylation-mediated export from the nucleus [71].

The UPS degradation of HDAC7 in the cytoplasm was recently confirmed during endochondral ossification [72]. Class IIa HDACs are negative modulators of endochondral ossification, at the stage of chondrocyte hypertrophy, by repressing the activity of RUNX2 and MEF2s [18,73]. HDAC7 is highly expressed in proliferating cells within the growth plate and its postnatal deletion increases the proliferation rate, because of β-catenin activation. During chondrocytes maturation, HDAC7 is exported into the cytoplasm where it is degraded by the UPS and liberates β-catenin [72].

The degradation of HDAC4 and HDAC5 was observed *in vivo* in muscles during fiber type conversion [74]. Contrary to HDAC7, the degradation of these class IIa HDACs takes place in the nucleus [74]. Nuclear degradation of class IIa HDACs was confirmed in untransformed cells exposed to serum starvation [75]. In this case GSK3β phosphorylates HDAC4 on serine 298 and this phosphorylation acts as a priming event required for its ubiquitylation and nuclear degradation (Figure 3 and Table 3) [75].

Degradation of class IIa HDACs as an environment driven process was observed also in a rat osteoblastic cell line. Here, the stimulation of differentiation with parathyroid hormone (PTH) causes PKA-dependent phosphorylation of HDAC4 on serine 740, its export in the cytoplasm and the degradation through a system that is lysosomal dependent [76]. Another set of studies using mice knock-out for *HDAC4* and *HDAC5* indicates that during osteoclast differentiation, PTH signaling favors MEF2C-dependent transcription by inducing HDAC4 poly-ubiquitylation and degradation via the E3 ubiquitin ligase SMURF2 [77]. Whether these differences reflect a specific cellular context still needs to be investigated.

Class IIa HDACs can also be SUMOylated [11]. In particular, HDAC4 becomes SUMOylated on lysine 559 by the SUMO E3-ligase RanBP2 on the nucleopore complex, during nuclear import [11]. The SUMOylation increases the interaction of HDAC4 with HDAC3 and therefore its repressive capability [11]. Also HDAC5 and HDAC9, but not HDAC7, are SUMOylated respectively on lysines 605 and 549 [11]. The lack of HDAC7 SUMOylation is probably due to the absence of the glutamine rich region [78].

Class IIa HDACs are not merely targets of SUMO E3-ligases, but several evidences indicate that they could promote the SUMOylation of some partners. They are involved in the activation of Ubc9, the SUMO E2-ligase [79]. In this manner, class IIa HDACs promote the SUMOylation of MEF2s [79], of promyelocytic leukemia protein [80] and of the nuclear receptors LXRα/NR1H3 and LXRβ/NR1H2 [81]. In the last example, SUMOylation stimulates the binding of the nuclear receptors to STAT1 and thus the inhibition of an inflammatory response [81].

In & out from the nucleus

The control of the nuclear/cytoplasm shuttling is a widespread strategy to influence class IIa HDACs activities. This regulation provides evident advantages in terms of reaction time. A redistribution of class IIa between the two compartments is a quick response that allows an immediate and reversible adaptation of the cells to the new environmental conditions [82–84]. Once dephosphorylated and nuclear, class IIa HDACs may associate with HDAC3 and N-CoR/SMRT, forming the enzymatically active multiprotein complex capable of driving epigenetic changes [85].

Since class IIa HDACs exert their function mainly in the nucleus, a cytoplasmic accumulation is generally considered as a negative regulation [23,86]. For example, the nuclear localization prevails in undifferentiated cells, whereas differentiated cells prevalently accumulate class IIa in the cytosol [87].

By controlling the availability of the NLS to bind importin-α and of the NES to bind CRM1, cells modulate class IIa HDACs protein localization. Phosphorylation is the PTM used to modulate these bindings and hence, to control class IIa subcellular localization (Figure 3 & Table 3). CaMKII was initially discovered as the kinase that elicits the nuclear export [86]. Today we know that various kinases are involved in such task. The supervisors of the opposite action (dephosphorylation and nuclear import) were discovered much later, associated to the activity of the phosphatases PP1 and PP2A [84,88–89].

The control is operated through the phosphorylation of at least three (four in HDAC7) serine residues conserved among HDAC4, 5, 7 and 9 (HDAC4: Ser 246, 467, 632; HDAC5: Ser 259, 497, 661; HDAC7: Ser 155, 181, 321, 446; HDAC9: Ser 220, 451, 611); these phosphorylation events facilitate the binding by dimers of 14-3-3 chaperones [90,91].

Binding of 14-3-3 proteins could either mask the NLS, thus preventing the nuclear import [82,83], or unmask the NES and thus promoting the direct interaction with CRM1 [89], or both conditions (although direct evidences of an interaction between HDAC4 and CRM1 are not available). It is still an open question whether the interaction between 14-3-3 proteins and class IIa HDACs occurs in the nucleus, in the cytoplasm or in both compartments. The interaction with 14-3-3 proteins would induce a conformational change in the deacetylases, which would make them able to expose the carboxy-terminal fragment containing the NES to CRM1 [86]. A work by Nishino *et al.* proposed that instead, the 14-3-3 proteins act primarily by slowing down the nuclear import of the deacetylases, in particular of HDAC4 [83].

In cardiomyocytes, HDAC4 is phosphorylated by the splicing isoforms, b and c, of CaMKIIδ and this finding demonstrates that the phosphorylation of HDAC4 could occur both in the nucleus (isoform b) and in the cytoplasm (isoform c). In the first case the export is favored whereas, in the second, the nuclear import is prevented [92].

Different class IIa HDACs members evidence different propensity to accumulate into the nucleus. For example, in transformed fibroblasts HDAC5 is almost nuclear, while HDAC4 is largely cytoplasmic or present in both compartments [93,94]. This observation indicates that within the same cell the two HDACs undergo different cycle of phosphorylation/dephosphorylation.

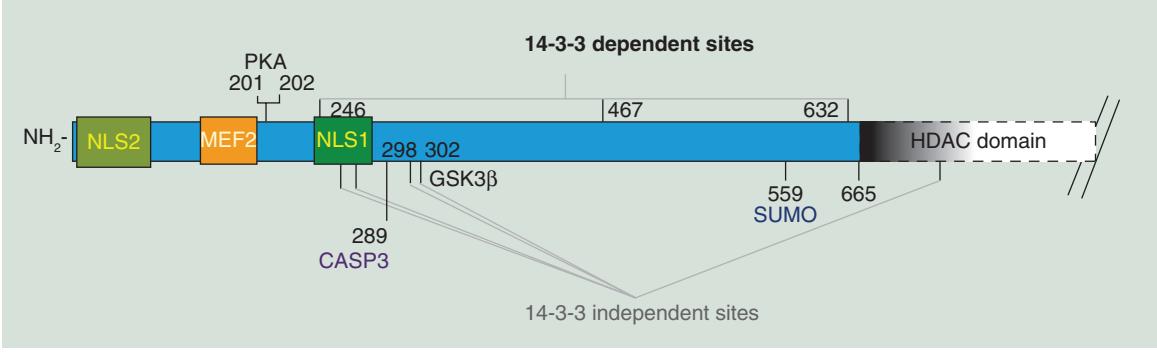

Figure 3. Schematic representation of class IIa histone deacetylases amino-terminal region highlighting the principal domains and the 'canonical' and 'not canonical' 14-3-3 phosphorylation sites see also Table 3. As prototype of class IIa we selected HDAC4.

Table 3. Main kinases involved in class IIa histone deacetylases phosphorylation.

Kinase	HDAC4	HDAC5	HDAC7	HDAC9
CaMKI, IV	246, 467	259, 497	155, 181, 321	220, 451
CaMKII	467, 632	–	–	–
PKD1	246, 467, 632	259, 497, 661	155, 181, 321, 446	220, 451, 611 (?)
C-TAK1, EMK	246	259	155	220 (?)
AMPK	246, 467 (?)	259, 497	?	?
SIK1	246, 467	259, 497	155, 321 (?)	220, 451 (?)
SIK2	246, 467, 632	259, 497, 661	155, 321, 446	220, 451, 611 (?)
PKA	265, 266, 740	278, 279	–	243
DIRK1B	266	276		240
GSK3β	298, 302			
AURKB	265	278		242
CDK5	–	279	–	–

Phosphorylation together with the residues targeted.
?: Not defined.

In this review the discussion is focused on three families of kinases.

Calcium-regulated kinases

The kinases responsive to calcium are historically associated to HDACs nuclear export. CaMKI and IV phosphorylate all family members and show preference for residues 246 and 467 (in HDAC4 and the corresponding aa in other deacetylases), while CaMKII preferentially phosphorylates serines 467 and 632 of HDAC4 [87,95]. CaMKII phosphorylates and exports directly only HDAC4, because only HDAC4 has a CaMKII-specific docking site, centered on Arg601 [95]. However, since HDAC4 can form heterodimers with HDAC5 and 9, but not with HDAC7 [96], the association between HDAC4 and HDAC5/9 renders such proteins responsive to CaMKII. In particular HDAC4 interacts strongly with HDAC5 through the glutamine-rich region [96]. The lack of this region in HDAC7 explains its inability to interact with HDAC4 [78,96].

The calcium-mediated export of class IIa HDACs is involved in the regulation of many physiological processes, such as myogenesis, hypertrophy and neuronal survival [97–99]. In general, the prosurvival effect associated to HDAC4 nuclear export depends on the activation of a MEF2-transcriptional response [98].

In cardiomyocytes, the pro-hypertrophic stimuli can be transduced by cAMP via the exchange protein directly activated by cAMP sensor, which activates PLC, H-Ras and CAMKII. This pathway culminates in the cytoplasmic accumulation of HDAC4 [100]. Exchange protein directly activated by cAMP is a guanosine nucleotide exchange factor for the Rap small GTPase. In particular PLC, and the subsequent signaling cascade of the inositol 1,3,5 triphosphate causes a release of calcium in the cytoplasm that determines the export of HDAC4 via CaMKII, followed by the activation of MEF2-dependant transcription [100].

For further and more detailed reviews on calcium-mediated class IIa regulation see [5,90,91].

PKD

PKD, a serine/threonine kinase activated by PKC, was associated to class IIa HDACs export during lymphocyte maturation and thymic selection [101,102]. B-lymphocyte activation, following BCR (B cell receptor) engagement is accompanied by the stimulation of PKD1 and PKD3, which in turn phosphorylate HDAC5 and HDAC7 on classical 14-3-3 sites. As a consequence, HDAC5 and HDAC7 accumulate in the cytoplasm and chromatin relaxation can occur [102], thus switching on the MEF2 transcriptional program [103]. Since the two kinases are redundant, in order to abrogate the export of HDAC5, a double knock-out of PDK1 and PDK3 is required.

PKD1 is also a regulator of T-lymphocyte thymic selection [104]. In 'resting' double positive $CD4^+CD8^+$ thymocytes, nuclear HDAC7 maintains switched off the MEF2 genetic program and in particular the transcription of the proapoptotic nuclear receptor *Nur77*|*NR4A1*, the main responsible for the negative selection. In response to T-cell receptor engagement, PKD1 becomes active and phosphorylates HDAC7, which is exported into the cytoplasm. This export determines the activation of MEF2s and the derepression of *NR4A1* that promotes cell death [101,104].

PKD is also an important inducer of cardiac hypertrophy via phosphorylation and export of HDAC5 and

the relative derepression of MEF2 [105]. Mice with a cardiac-specific deletion of PKD1 show diminished hypertrophy in response to pressure overload or chronic adrenergic and angiotensin II signaling. Several selective inhibitors of PKD are now under clinical studies for the treatment of malignant cardiac hypertrophy [105]. PKD1 seems to be an inducer of hypertrophy also in VSMCs. Here, treatment with the hypertrophic inducer angiotensin II stimulates the phosphorylation of HDAC5 via PKD1 [106]. Similarly, angiotensin II through PKD1 triggers HDAC4 (serines 246/632), HDAC5 (serines 259/498) and HDAC7 (serine 155) phosphorylation in intestinal epithelium [107].

Finally, exogenous expression of PKD1 in type II skeletal muscle fibers promotes the phosphorylation and the nuclear export of HDAC4 and HDAC5, the subsequent activation of MEF2 TFs and the fibers-switch from fast/glycolytic into red oxidative (slow-twitch type I) [108], in accordance to the phenotype observed in transgenic MEF2C-VP16 mice [74].

LKB1-ARK family

The tumor suppressor kinase LKB1 regulates different downstream kinases, which belong to the ARK family (AMP-related kinases) and includes AMP-activated protein kinase (AMPK), microtubule affinity regulating kinases (MARKs), SNF-related kinases (SNRKs), NUAKs (NUAK family, SNF1-like kinases), BR serine/threonine kinases and salt inducible kinases (SIKs) [109].

Early studies reported that MARK kinases phosphorylate class IIa HDACs constitutively on a conserved residue (S159 in HDAC7, S246 in HDAC4, S259 in HDAC5). This base-line phosphorylation facilitates the subsequent signal-dependent phosphorylation by other kinases of the remaining residues required for 14-3-3 binding [110]. MARK/PAR-1 kinases are involved in the determination of polarity. During early embryogenesis they control gastrula polarization in *Drosophila* [111] and the first asymmetric division in *Caenorhabditis elegans* [112]. Scenarios where the nuclear or cytoplasmic activities of class IIa HDACs are still unexplored.

The AMPK

The AMPK is activated under conditions of metabolic stress and ATP depletion [109]. Some evidences correlate AMPK activity to HDAC4 and HDAC5 cytosolic accumulation [113,114]. In skeletal muscle, the stress induced by physical exercise is sufficient to trigger the export of HDAC4 and 5 and this relocalization correlates with the activation of AMPK and CaMKII [115]. In myotubes the AMPK agonist AICAR (5-amino-imidazole-4-carboxamide-1-beta-D-ribofuranoside) triggers HDAC5 phosphorylation at S259 and S498 and association with 14-3-3 isoforms. HDAC5 phosphorylation determines its detachment from the *GLUT4* promoter and the concomitant increase in *GLUT4* expression [116]. This metabolic regulation can be supervised also by other kinases such as PKDs [117].

The SIK subfamily

The SIK-subfamily is composed of three isoforms: SIK1, SIK2 and SIK3, conserved from *C. elegans* to humans. SIK1 expression is highly induced in adrenal glands of high-salt diet-fed rats, whereas SIK2 is highly expressed in adipose tissue. By contrast, SIK3 is ubiquitously expressed [118]. Several studies have reported correlations between SIKs activities, class IIa HDACs relocalization and transcriptional changes [119–121].

The SIK-class IIa HDAC axis is a critical component of the adaptation to fasting in liver [22]. After feeding, the release of insulin stimulates in liver and skeletal muscle, the synthesis of glycogen and in adipocytes the storage of energy reserves. These responses involve the engagement of Akt and culminate in the phosphorylation-mediated inactivation of PGC-1α and FOXO1/3 [122]. The FOXO TFs are also negatively regulated by acetylation, which promotes their export into the cytoplasm. Here, the SIK-class IIa HDACs axis becomes protagonist.

In mouse hepatocytes, in response to insulin SIK2 mediates phosphorylation, 14-3-3 binding and cytosolic accumulation of HDAC4. Accordingly, the fasting hormone glucagon, through the PKA-mediated inhibition of SIK2 favors dephosphorylation and nuclear accumulation of HDAC4 [123].

Inhibition of LKB1/AR kinases is followed by the nuclear relocalization of class IIa HDACs, a key-event in order to promote the activation of the gluconeogenesis in the liver. Nuclear class IIa HDACs associated with HDAC3 in order to deacetylate and activate FOXO1/3. Then, FOXO1/3 stimulate the transcription of key enzymes for the gluconeogenesis [22].

In the adipose tissue, modulation of SIK2 influences HDAC4 phosphorylation through a multiprotein complex, which comprises also CREB-regulated transcription co-activator 2 and 3, as well as PP2A. This complex is under the supervision of PKA and is involved in the regulation of *GLUT4* transcription and glucose uptake [124].

Recently, HDAC4 has been described as an immune-metabolic sensor. In particular under overnutrition, leptin reduces inflammatory gene expression via HDAC4 nuclear accumulation [125]. PKA-dependant inhibition of SIKs (following AMPc increase) represents the operative arm that allows the HDAC4-dependent repression of NF-κB activity and of proin-

flammatory genes in M2 macrophages [125]. Because obesity promotes macrophage infiltration in white adipose tissues and liver, inflammation and subsequent insulin resistance, the negative effect of HDAC4 on NF-κB suggests for a protective role against obesity [125]. In agreement, HDAC4 variants have been associated with both body mass index and waist circumference [125] and its expression is downregulated in the fat from obese subjects [126].

The involvement of the HDACs-SIKs axis in the control of energy supply and metabolism seems to be conserved during evolution. In *Drosophila* HDAC4 nuclear accumulation in the fat body cells is supervised by the LKB1-SIK3 signaling, under the supervision of different dietary conditions. SIK3 controls lipid metabolism by limiting HDAC4-mediated FOXO activation and *ATGL*/*Bmm* transcription (adipose triglyceride lipase). In the control of lipid metabolism HDAC4 behaves as a lipolytic factor [127].

SIKs can monitor class IIa HDACs activities also independently from metabolism. In muscle cells, SIK1 phosphorylates class IIa HDACs thus promoting their export into the cytoplasm [119,128]. In this context, SIK1 could integrate cAMP signaling with the myogenic program [129]. In *C. elegans* the expression of chemoreceptors gene in certain chemosensory neurons is under the regulation of a SIK member (KIN-29), which controls the localization of the class IIa HDAC counterpart (HDA-4). This control releases the repressive influence of HDA-4 on MEF2-target genes. In the circuit participate also the phosphatase Calcineurin, which instead promotes HDA-4 nuclear accumulation and chemoreceptors repression [130].

Finally, in a detailed study performed in HEK293 cells, Walkinshaw *et al.* evaluated 13 kinases of the LKB1 family (MARK1, MARK2, MARK3/C-TAK1, NUAK1/ARK5, NUAK2/SNARK, SNRK, NIM1) for the regulation of class IIa HDACs subcellular localization [94]. They demonstrated that only the ectopic expression of SIK2 or SIK3 but not of SIK1 causes a dramatic cytoplasmic relocalization of HDAC5 and HDAC9 and, at a lesser extent, of HDAC4 and HDAC7. For the cytoplasmic relocalization of class IIa HDACs the catalytic activity of SIK2 is required, while the catalytic activity of SIK3 is dispensable. Moreover, while SIK2 promotes the nuclear export through the phosphorylation of 14-3-3 consensus sites, SIK3 is effective also on the Ser/Ala mutants in the 14-3-3 binding sites of HDAC4. This result proves that the SIK3-mediated export is both kinase activity and classical 14-3-3 binding sites independent [94]. Finally, while SIK2 causes the derepression of MEF2 and stimulates myogenesis in C2C12 cells, SIK3 is incompetent toward MEF2 activation [96]. Whether these differences reflect cell lineage specific features or others conditions is currently unknown.

The large number of studies on the LKB1-SIKs-class IIa HDACs axis underlines the extreme flexibility of the class IIa HDACs, and in particular of the nuclear/cytoplasmic regulation. By a simple operation (nuclear exit) it is possible to reset the transcriptional landscape of cells by both inducing MEF2-target genes and repressing FOXO-target genes.

Phosphatases & nuclear import

More than 15 years ago it was demonstrated that Calyculin A, an inhibitor of the phosphatases PP1 and PP2A, promotes the nuclear export of HDAC4 and reduces its interaction with importin-α [82]. Several years later the contribution of the PP2A complex was proved. PP2A is able to bind the amino-terminal portion of class IIa HDACs, in correspondence of the NLS1 and to dephosphorylate these regulators [84,88,89].

In HDAC4 two residues have been proposed as targets of PP2A: serine 246 and 298. Serine 298 is also regulated by GSK3β and constitutes a signal for polyubiquitylation and degradation [75]. The PP2A-mediated dephosphorylation may therefore also protect HDAC4 from the UPS-mediated nuclear degradation.

The PTH-related peptide suppresses MEF2 and RUNX2 transcriptional activities and chondrocyte hypertrophy, via PP2A, which dephosphorylates HDAC4 at serine 246 [89]. PP2A is sufficient to trigger the import of HDAC4 and inhibition of PP2A, in U2OS cells, causes the cytoplasmic relocalization of the deacetylase [89].

Ataxia telangiectasia is a complex syndrome characterized by neurodegeneration and epigenetic reprogramming and caused by mutations of the ATM kinase [131]. Since ATM phosphorylates and inhibits PP2A, ataxia telangiectasia is characterized by HDAC4 nuclear accumulation in neurons. Here, HDAC4 represses MEF2 and CREB thus inducing heterochromatinization and neurodegeneration [132].

The myosin phosphatase complex, consisting of PP1β and MYPT1, is necessary to repress *NUR77*. This complex is able to dephosphorylate HDAC7, thus stimulating its nuclear relocalization [94,133]. In smooth muscles, the myosin phosphatase dephosphorylates the myosin light chain, thus inducing muscle relaxation and it is inactivated following the phosphorylation of MYPT1 on threonine 696 [134]. Kinases regulated by the GTPase RhoA are able to phosphorylate and inhibit myosin phosphatase on this residue [134] and they are also responsible for the nuclear export of HDAC5 [134]. It is therefore possible that RhoA activation could neutralize MYPT1, thus

resulting in the hyperphosphorylation and export of HDAC5 [134].

PP1α is another phosphatase capable of mediating HDAC4 nuclear import. Its action can overcome the antagonist activating effect of calcineurin on MEF2 [135].

The regulation of class IIa HDACs phosphorylation is a complex issue, not limited to 14-3-3 binding sites but involving additional residues. In the case of HDAC5, at least 17 phosphorylation sites have been characterized, 13 of which do not encompass the consensus for 14-3-3 proteins [136]. In particular, the phosphorylation of serine 279 is essential to induce the nuclear import of the protein. This residue is conserved among all class IIa HDACs, with the exception of HDAC7 [136]. Curiously, this residue was previously characterized as an Mirk/DirkB target, but in this case its phosphorylation causes the nuclear export of class IIa HDACs [137]. Additional kinases responsible for HDAC5 Ser279 phosphorylation have been identified in PKA and CDK5 [138,139]. PKA retains HDAC5 in the nucleus by interfering with 14-3-3 binding, thus causing suppression of MEF2-dependant cardiac fetal gene expression and cardiomyocyte hypertrophy [138]. On the opposite, CDK5 promotes HDAC5 nuclear export in neurons. In this context, cocaine administration activates PP2A, which dephosphorylates Ser279 and determines the nuclear import of HDAC5 [139]. In the nucleus HDAC5 can repress genes regulated by cocaine [139].

The opposite influence on HDAC5 subcellular localization by the phosphate group on serine 279 is puzzling. Additional substrates of these kinases could explain the paradox. It is evident that further studies are necessary to clarify this point.

14-3-3 independent regulation of subcellular localization

There are some indications about a 14-3-3-independent control of class IIa nuclear/cytoplasmic shuttling. An example concerns the import regulated by TRX1 (thioredoxin 1) in cardiomyocytes [140]. TRX1 is able to recruit HDAC4 in the nucleus and to inhibit the activity of some pro-hypertrophic factors such as MEF2 and NFAT [140]. This adjustment of HDAC4 'shuttling' is tissue-specific and mediated through the control of the redox state of two cysteines [140].

Cardiac hypertrophy is characterized by an increase in intracellular reactive oxygen species (ROS) [90]. Under oxidizing conditions, a disulfide bridge between the cysteines 667 and 669 is formed [140]. Cysteine 667 lies in the binding site for the 'structural' zinc ion [141,142]. In a reducing environment, cysteines 667 and 669 and the zinc ion bound to its binding site leads to a protein folding that brings the structural zinc-binding domain in contact with the NES. In this manner the CRM1 binding site is masked and the nuclear export is blocked. In the presence of oxidants, cysteines 667 and 669 are oxidized, the zinc is no longer coordinated, the NES is exposed to CRM1 and the protein is exported into the cytoplasm [140]. TRX1 is able to attenuate cardiac hypertrophy in part by restoring the cytoplasmic reducing environment. Furthermore, after the binding to TBP-2, TRX1 reduces DnaJB5 (Hsp40), which in this state can interact with HDAC4. Next the complex TRX1-TBP-DnaJB5 reduces the disulfide bridge 667–669 of HDAC4. This intervention is sufficient for determining the nuclear accumulation of HDAC4, in spite of its phosphorylation status [140,143]. Similarly to HDAC4, also HDAC5 has been recently described as a redox-sensor in adult heart [144].

As discussed above, the AMPc through PKA owns different options to influence class IIa HDACs subcellular localization. An additional opportunity consists in the PKA-promoted (clearly indirect) dephosphorylation of serines 265/266 in HDAC4, independently from the above-described action on SIK2. These residues are conserved in HDAC5 (278/279) and in HDAC9 (242/243) and lie within the NLS [94]. Their dephosphorylation favors class IIa nuclear accumulation and the subsequent repression of MEF2D and myogenesis [138]. However, since PKA phosphorylates also MEF2D [145], it is difficult to discriminate between the repressive activity of PKA due to the direct phosphorylation of MEF2s or the nuclear import of a class IIa HDACs.

During the cell cycle, HDAC4, HDAC5 and HDAC9, but not HDAC7 can be phosphorylated by Aurora B kinase, respectively on Ser265, Ser278 and Ser242. These phosphorylations allow the relocalization of the deacetylases at the mitotic midzone during late anaphase, and in the midbody during cytokinesis [146]. This phosphorylation-dependent relocalization abolishes the interaction with the NCoR complex, thus limiting part of class IIa deacetylase activity [146].

Overall, the regulation of class IIa HDACs functions during the cell cycle is largely unexplored, although it could provide important hints on the relationships between this gene family and the control of proliferation.

Conclusion

In this review we have discussed about the multiple options that cells have evolved to control class IIa HDACs. Although the phosphorylation-dependent control of nuclear cytoplasmic shuttling takes a chief

role, additional opportunities are emerging such as the control of transcription and translation. In summary, cells dispose of different operating layers to influence class IIa HDACs activities. This complexity reflects the astonishing number of biological processes under the supervision of this gene family. It seems that each control is sculptured under the needs of specific conditions.

Future perspective

Although studies on class IIa HDACs are intense, there are several important questions, which still need to be answered, for example, cytoplasmic versus nuclear functions, redundancy, compensatory regulative circuits, transcriptional repression and activation, histones versus transcription factors modifications and many others issues. Investigation on class IIa HDACs will certainly proceed in the next future and additional pieces to this intricate puzzle will be step by step added.

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Executive summary

- Class IIa histone deacetylases (HDACs) are exposed to multilayered levels of controls, reflecting the multiple biological responses under their supervision.

Transcriptional control

- Few data are available about the transcriptional machinery responsible for class IIa HDACs expression.
- Interrogation of ENCODE database indicates that several transcription factors (TFs) including oncogenes and cell cycle regulators bind the proximal promoter of class IIa HDACs.
- HDAC4 and HDAC7 proximal promoters are exposed to intense TFs and epigenetic modifiers binding.

Translational control

- Different microRNAs can influence class IIa HDACs translation.
- In some cases, class IIa HDACs are part of feedback circuits controlling the transcription of their own regulative microRNA.
- Further studies are necessary to clarify the effective contribution of class IIa HDACs to the biological role of the specific microRNAs.

Proteolytic control

- Class IIa HDACs levels can be regulated by the ubiquitin-proteasome system and further studies are necessary to define the E3 ligases involved.
- Specific proteolytic cleavages can also influence class IIa HDACs activities in a member specific fashion.
- Although the involvement of caspases during apoptosis is well known, additional proteases operating in different circumstances may exist.

Phosphorylation, 14-3-3 binding & subcellular localization

- The most common and widespread mechanism to influence class IIa HDACs activities.
- Several environmental conditions and signaling pathways use this post-translational modification for influencing class IIa HDACs.
- A fast response that allows an immediate and reversible adaptation of the cells to the new environmental conditions.

14-3-3 independent regulation of subcellular localization

- Less characterized, nevertheless experimental evidences indicate that 14-3-3 independent control of class IIa HDACs does exist.
- Up to now the best characterized mechanism involves the redox control of a pair of cysteine residues lied in the deacetylase domain of HDAC4.

Future perspective

- Future studies on class IIa HDACs will certainly provide additional key information about this important gene family. The mysterious change in the catalytic site occurred with the evolution of vertebrates is a challenging and fascinating question. Undoubtedly, the plethora of biological responses supervised by class IIa HDACs guarantees that, thanks to these studies, new tools and opportunities for the treatment of different human diseases will be established.

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