A Dynamic Role for HDAC7 in MEF2-mediated Muscle Differentiation*

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The overlapping expression profile of MEF2 and the class-II histone deacetylase, HDAC7, led us to investigate the functional interaction and relationship between these regulatory proteins. HDAC7 expression inhibits the activity of MEF2 (-A, -C, and -D), and in contrast MyoD and Myogenin activities are not affected. Glutathione S-transferase pulldown and immunoprecipitation demonstrate that the repression mechanism involves direct interactions between MEF2 proteins and HDAC7 and is associated with the ability of MEF2 to interact with the N-terminal 121 amino acids of HDAC7 that encode repression domain 1. The MADS domain of MEF2 mediates the direct interaction of MEF2 with HDAC7. MEF2 inhibition by HDAC7 is dependent on the N-terminal repression domain and surprisingly does not involve the C-terminal deacetylase domain. HDAC7 interacts with CtBP and other class-I and -II HDACs suggesting that silencing of MEF2 activity involves corepressor recruitment. Furthermore, we show that induction of muscle differentiation by serum withdrawal leads to the translocation of HDAC7 from the nucleus into the cytoplasm. This work demonstrates that HDAC7 regulates the function of MEF2 proteins and suggests that this class-II HDAC regulates this important transcriptional (and pathophysiological) target in heart and muscle tissue. The nucleocytoplasmic trafficking of HDAC7 and other class-II HDACs during myogenesis provides an ideal mechanism for the regulation of HDAC targets during mammalian development and differentiation.

Skeletal muscle has become a model for understanding many fundamental principles of development. Differentiation of precursor cells into skeletal muscle cells involves two events, determination into myoblasts and the formation of postmitotic, multinucleated myotubes with contractile phenotype. These processes are under control of members of the MyoD family of basic-helix-loop-helix (bHLH) transcription factors (MyoD, Myf5, Myogenin, and MRF4). These proteins can inhibit cell proliferation, regulate a cascade of muscle-specific gene expression, auto- and cross-regulate their own and each other’s expression, and induce muscle differentiation in nonmuscle cells (1–3). Myogenic bHLH proteins activate transcription of muscle-specific genes by forming heterodimers with other, ubiquitously expressed bHLH proteins known as E2A proteins (alternatively spliced products of the E2A gene) (4–7). These heterodimers bind to the E box motif (CANNTG), which functions as the cognate binding site in the regulatory regions of most muscle genes (1, 3, 8). MyoD and Myf5 are required for determination of precursor cells into myoblasts (9), whereas Myogenin is specifically required for differentiation (10, 11). Therefore myoD and myf5 are expressed in proliferating myoblasts and are markers for commitment, whereas the expression of myogenin is a marker of terminal-differentiation.

Even though members of the MyoD family are the key regulators of muscle differentiation, the activation of muscle-specific genes is dependent on the association with members of the MEF2 (myocyte enhancer factor 2) (12) family of transcription factors (8). MEF2 proteins cooperatively increase the activity of myogenic bHLH transcription factors (13). In vertebrates the MEF2 family is encoded by four independent genes, mef2a, mef2b, mef2c, and mef2d (13). MEF2 factors belong to the MADS-(MCM1-agamous deficiens-serum response factor) box family and share a highly conserved 86-amino acid-region that encodes the MADS and MEF2 domains, which mediate DNA binding and dimerization, respectively (2). Loss-of-function mutations in the single Drosophila mef2 gene prevent myoblast differentiation (14, 15), and dominant-negative MEF2 mutants inhibit myoblast differentiation in cell culture (16) demonstrating a critical role of MEF2 proteins in terminal muscle differentiation. MEF2-proteins are expressed in a wide range of tissues, whereas MEF2C is restricted mainly to skeletal muscle, brain, and spleen. However, MEF2C DNA-binding activity is highly enriched in muscle and neural tissue indicating a critical role in muscle differentiation.

Positive and negative regulation of eukaryotic transcription has been shown to be mediated in part by two opposing enzymatic activities, histone acetylases and histone deacetylases.

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† The abbreviations used are: bHLH, basic-helix-loop-helix; HDAC, histone deacetylase; DMDM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; HA, hemagglutinin; GST, glutathione S-transferase; MEF2, myocyte enhancer factor 2; MADS, MCM1-agamous deficiens-serum response factor; CtBP, C-terminal binding protein.
HDAC7 Represses MEF2-dependent Transactivation

In this study we report that the recently identified HDAC7 (43), like other class-II HDACs, represses MEF2-dependent transcription via physical interaction with the MADS domain of MEF2. This repression is dependent on the N-terminal repression domain of HDAC7 and functions independently of the C-terminal deacetylase domain. Furthermore we provide evidence that this functions via the interaction with other corepressors, such as CtBP and class-I and -II HDACs. Finally we demonstrate that the hdac7 mRNA is down-regulated but constitutively expressed during differentiation of muscle cells and that the HDAC7 protein shuttles from the nucleus into the cytoplasm during this process thereby preventing the formation of a MEF2/HDAC7-repressive complex.

**Experimental Procedures**

**Cell Culture and Transient Transfections**—Pluripotent C310T1/2 cells were cultured for 24 h in DMEM supplemented with 10% FCS in 6% CO2 before transfection. Cells grown in 12-well plates to 60–70% confluence were transiently transfected using a DOTAP/DOSPER (3:1) liposome (Roche Molecular Biochemicals) mixture in HEBS (42 mM HEPES, 275 mM NaCl, 10 mM KCl, 0.4 mM Na2HPO4, 11 mM Dextrose, pH 7.1), with 2 μg of total DNA. For elimination of Gal-MEF2C, -A, or -D-mediated transactivation 1 μg of G5-E1b-Luc reporter, 0.3 μg of the respective MEF2 cDNA in the pSV40gal expression vector, and 0.6 μg of HDAC4, -5, or -7 in the pSG5 expression vector/well were used. Empty expression vectors served as controls. The same conditions applied for the experiments carried out with Gal-MyoD and Gal-MyoD.

For elimination of MEF2C-mediated transactivation on MEF2-responsive elements, 1 μg of pGL3-MEF2C3-E1b-Luc reporter (or as control, the pGL3-E1b-Luc plasmid without MEF2-binding sites), 0.5 μg of pSG5-MEF2C, and 0.5 μg of pSG5-HDAC4, -5, or -7 were used. Empty SG5 expression plasmid was used as control. Medium was replaced 24 h after transfection, and cells were further grown for 25–48 h before harvesting and assayed for Luciferase activity using the Lucite kit (Packard Instrument Co.) according to the manufacturer’s protocol.

Mouse myogenic C2C12 cells were cultured in growth medium (DMEM supplemented with 20% FCS) in 6% CO2. For differentiation assays, cells were grown confluent, and the media was changed into differentiation medium (DMEM supplemented with 2% horse serum), and cells were harvested at the time points indicated. For visualizing intracellular localization of HDAC7, 5 μg of pCMX-HDAC7-YFP were used to transiently transfect proliferating C2C12 myoblasts held in growth medium or C2C12 cells that have been grown in differentiation medium for 48 h. The appropriate medium was replaced after 24 h and cells were incubated for an additional 24 h. YFP-HDAC7 was visualized using standard fluorescence procedures after fixation in 4% paraformaldehyde in phosphate-buffered solution.

**Immunoprecipitation**—Cos-7 cells were transfected with expression constructs encoding either full-length FLAG-tagged MEF2C and HA-tagged HDAC4, -5, or -7 as indicated using the FuGENE 6 (Roche Molecular Biochemicals) liposome reagent. 48 h post-transfection cells were lysed in phosphate-buffered solution containing 0.5% Triton X-100, 150 mM NaCl, 1 mM EDTA, and protease inhibitors (complete by Roche Molecular Biochemicals) and subjected to brief sonication, and the resultant cellular debris was pelleted by centrifugation. Lysates were then incubated with a mouse monoclonal anti-FLAG M2 antibody (Sigma) for 2 h at 4°C and precipitated by incubation for a further 1 h

**Fig. 1. HDAC4, -5, and -7 down-regulate transactivation mediated by MEF2-proteins but not that of myogenic HLH proteins.**

A, pluripotent C310T1/2 cells were transfected with G5-E1b-Luc as a reporter, pSV40-gal-MEF2C, pSG5-HDAC4, -5, and -7 or the empty vectors. B, C310T1/2 cells were transfected with the same reporter but also pSV40-gal-MEF2A or -D and pSG5-HDAC4, -5, and -7. Fold activation is expressed relative to Luciferase activity obtained after cotransfection of ds G5-E1b-Luc, pSV40-gal0, and empty pSG5 expression vector alone. C, pluripotent C310T1/2 cells were transfected with either GL3-MEF2C3-E1b-Luc or the GL3-E1b-Luc plasmid with no MEF2 binding sites as reporter, pSG5-MEF2C, pSG5-HDAC4, -5, and -7 or the empty pSG5 expression vector. D, pluripotent C310T1/2 cells were transfected with G5-E1b-Luc as a reporter, pSV40-gal-Myogenin or -MyoD, pSG5-HDAC4, -5, and -7 or the empty vectors. Fold activation is expressed relative to Luciferase activity obtained after cotransfection of G5-E1b-Luc, pSV40-gal0, and empty pSG5 expression vector alone.

(HDACs) (17, 18). Whereas histone acetylases are associated with transcriptional activation, deacetylation of histone leads to a compact chromatin structure to which the accessibility of transcriptional activators is impaired, and thereby transcription is repressed.

Recently, we and others have shown, that the transcriptional activity of MEF2C is modulated by cofactor recruitment. MEF2C recruits chromatin remodeling factors such as the histone acetylases p300/CBP and P/CAF (19–22) to activate gene expression. This recruitment is probably mediated by the coactivator GRIP1, which has been shown to directly interact with MEF2C and to be necessary for MEF2C-dependent gene expression and skeletal muscle differentiation (23). Former studies revealed that GRIP1-mediated activation is due to interaction with p300/CBP and P/CAF (24, 25). Interestingly MEF2 proteins also recruit HDACs, which leads to a repression of
with protein A/G affinity resin (Pierce). Precipitated proteins were resolved by SDS-polyacrylamide gel electrophoresis and subsequently immunoblotted with a rabbit polyclonal anti-HA antibody (Bab. Co.).

RNA Extraction, Northern Hybridization, and Probe Preparation—Total RNA was extracted by guanidinium thiocyanate-phenol-chloroform method. Northern blots, random priming, and hybridization were performed as described previously (44, 45). The 1.8-kilobase pair HindIII fragment of pCMX-m HDAC7-HA (43) was used as a probe for HDAC7. As a probe for HDAC5 the KpnI/SmaI 1.7-kilobase pair fragment of pCMX-mHDAC5-HA was used. Other cDNA probes have been described previously (23).

cDNA probes were radioactively labeled by random priming. DNA fragments (200 ng) were boiled with 20 ng of random hexamers (pdN6; Amersham Pharmacia Biotech) and then incubated overnight with EcoPol buffer (New England Biolabs), 200 μM dGTP/dTTP, 10 μM [γ-32P]dATP/[γ-32P]dCTP (Bresatec), and 5–10 units of Klenow polymerase (New England Biolabs). Probes were purified using NICK columns (Amersham Pharmacia Biotech) according to the manufacturer’s protocol.

GST Pulldowns—GST and GST fusion proteins were expressed in Escherichia coli (BL21) and purified using glutathione-agarose affinity chromatography as described previously (45, 46). Amounts and integrity of GST fusion proteins were checked by SDS-polyacrylamide gel electrophoresis and Coomassie staining. The TnT-coupled transcription/translation kit (Promega) was used to produce [35S]methionine-labeled proteins. Pulldowns were carried out as described previously (23).

RESULTS AND DISCUSSION

HDAC7 Represses MEF2-mediated Transactivation: MyoD and Myogenin Activity Is Refractory to Class-II HDAC-mediated Inhibition—The class-II HDAC4 and -5 interact with MEF2 and suppress its ability to activate transcription and cell specific differentiation (26, 27, 29, 31). Therefore we tested if the recently discovered HDAC7 (43), which is expressed in a cell-specific manner and colocalizes with other class-I and -II HDACs and corepressors in distinct nuclear compartments, regulates MEF2-mediated transcription.

MEF2C is highly expressed in developing skeletal, cardiac, and smooth muscle cells. Weak expression is detected in endothelial cells and surrounding mesenchyme during embryo development (47–49). Because HDAC7 and MEF2C are expressed in a similar cell-specific manner, we initially tested the effect of HDAC7 expression on MEF2C activity.

C3H10T1/2 pluripotent cells were cotransfected with GAL-MEF2C and the G5E1b-LUC reporter in the presence and absence of the class-II HDAC4, -5, and -7. HDAC7 interacts with MEF2C in vivo and in vitro. A, COS-7 cells were transfected with HA-tagged HDAC4, -5, and -7 and FLAG-tagged MEF2C (amino acids 1–117) or the empty expression vectors as control. Immunoprecipitation with a FLAG antibody followed by Western blot analysis with a HA antibody show that class-II HDACs interact with MEF2C in vivo. B, glutathione-agarose-immobilized, bacterial expressed GST and GST-MEF2C proteins were incubated with either [35S]radiolaabeled full-length HDAC7 or fragments of HDAC7 encompassing the indicated amino acid positions. The input lanes represent ~20% of total radiolabeled HDAC7 protein. C, schematic representation showing the HDAC7 regulatory domains and the deletion fragments used. R1, R2, repression domains 1 and 2.
the addition of HDAC4 and -7, whereas no significant effect is seen on the basal activity of the GAL4-expression plasmid. Surprisingly, HDAC5 poorly repressed MEF2C activity at the amounts transfected in this assay.

The MEF2 family is composed of a group of proteins encoded by four vertebrate genes mef2a, mef2b, mef2c and mef2d (50). All of these factors bind DNA, recruit cofactors, and trans-activate gene expression in a similar manner. Hence we analyzed the effect of HDAC4, -5, and -7 expression on the transcriptional activation mediated by MEF2A and MEF2D (Fig. 1B). Similarly to the results obtained with MEF2C, we observed that HDAC4 and -7 dramatically reduced the activity of MEF2A and MEF2D. Again, HDAC5 weakly repressed MEF2A and MEF2D (Fig. 1B). Thus class-II HDACs, including HDAC7, repress MEF2-dependent gene expression.

The cell culture experiments presented above suggest that HDAC7 inhibits MEF2 activity. Therefore, we examined whether HDAC7 repressed MEF2C-dependent activation of a luciferase reporter with 3 tandem copies of the MEF2 cognate binding sites upstream of a basal E1b promoter (Fig. 1C). These experiments clearly demonstrate again that HDAC7 represses MEF2C-mediated transactivation of a MEF2-dependent reporter. The specificity of this repression was supported by the inability of class-II HDACs alone to repress the MEF2C-dependent reporter (MEF2[1-121]-E1b-LUC) and the failure of MEF2C and class-II HDACs to regulate the expression of the vehicle, i.e. the basal E1b promoter linked to luciferase (Fig. 1C).

MEF2 proteins belong to the MADS box family of transcription factors that cooperate with the myogenic bHLH proteins, MyoD and Myogenin, in the activation of the contractile protein gene expression and function within a regulatory network that establishes the differentiated phenotype. Therefore, we investigated whether HDAC7 regulates the transactivation of gene expression mediated by myogenic bHLH proteins. C310T1/2 cells were transfected with Myogenin or MyoD fused to the DNA-binding domain of GAL4 and examined for the effect of class-II HDAC expression on the activity of these transcriptional activators. We observed that neither HDAC4, -5, or -7 had any significant effect on the activity of these bHLH factors (Fig. 1D).

This data suggest that HDAC7 specifically represses the activity of the MADS box proteins, MEF2A, -C, and -D, but not that of the bHLH factors, MyoD and Myogenin. HDAC7 functions as a negative regulator of myogenesis by inhibiting MEF2-dependent gene expression and transactivation dependent on the cooperative function between MEF2 and the MyoD family of basic helix-loop-helix transcription factors. This correlates with the observations that have been made with HDAC4 and -5 (26, 27, 29, 31).

**HDAC7 Interacts with MEF2C:** the N-terminal 121 Amino Acids of HDAC7 Mediate the Interaction—

The class-II HDACs have been reported to directly interact with the N terminus of MEF2 in vitro and in vivo (26, 27, 29, 31). Therefore we tested if HDAC7 interacts with MEF2C in vivo. COS-7 cells were transfected with FLAG-tagged MEF2C (amino acids 1–117), HA-tagged HDAC7, and the other class-II HDACs (HDAC4 and -5). Coimmunoprecipitation with the FLAG-antibody demonstrates that ectopic MEF2C interacts with HA-HDAC7, as well as with HDAC4 and -5 in vivo (Fig. 2A).

The regulation of MEF2-dependent transcription by the class-II HDACs and the demonstration of interaction between
these factors in the immunoprecipitation assay strongly suggests that these proteins interact by a direct mechanism. However, it does not completely eliminate the possibility of an indirect mechanism in which additional factor(s) mediate the interaction, although we postulated that HDAC7 represses MEF2C activity/function by direct interaction. We tested this hypothesis using a biochemical approach, the in vitro GST pulldown assay to confirm the direct interaction between MEF2C and HDAC7.

Glutathione-agarose-immobilized, bacterial expressed GST and GST-HDAC7 proteins were incubated with either 35S-radiolabeled CtBP or HDAC7 encompassing the indicated amino acid positions. The input lanes represent ~10% of total radiolabeled protein.

Fig. 5. HDAC7 interacts with other corepressors. Glutathione-agarose-immobilized, bacterial expressed GST and GST-HDAC7 proteins were incubated with either 35S-radiolabeled CtBP or HDAC7 encompassing the indicated amino acid positions. The input lanes represent ~10% of total radiolabeled protein.

Fig. 6. HDAC7 is constitutively expressed and shuttles into the cytoplasm during myoblast differentiation. A, total RNA was isolated from proliferating C2C12 myoblasts (PMB), confluent myoblasts (CMB) cultured in growth medium (DMEM containing 20% FCS), and developing myotubes 4, 8, 24, 72, and 120 h after serum exchange into differentiation medium (DMEM containing 2% adult horse serum). After blotting, RNA was probed with 32P-radiolabeled cDNAs encoding glyceraldehyde-3-phosphate dehydrogenase, HDAC5 and -7, Myogenin, MyoD, p21, and cyclinD1. Induction of Myogenin and p21 as well as down-regulation of cyclinD1 confirm that these cells have undergone terminal differentiation into myotubes. B, C2C12 myoblasts were transfected with HDAC7 fused to the yellow fluorescent protein. Cells kept in growth medium show an exclusive nuclear staining of HDAC7-YFP. Cells 72 h after serum withdrawal into differentiation medium show a cytoplasmatic localization of HDAC7-YFP.

To delimit the minimal region of HDAC7 required to mediate the interaction with MEF2C we incubated a number of 35S-labeled C-terminal unidirectional deletions of HDAC7 with GST-MEF2C. We observed that the N-terminal fragment of HDAC7 encompassing amino acids 1–121 is sufficient to interact with MEF2C (Fig. 2B). This fragment encodes repression domain 1 of HDAC7 and contains a highly conserved motif of 17 amino acids (Fig. 2C), which has been shown to be necessary for the interaction of HDAC4 and -5 with the MEF2 proteins (29).

The MADS Box Domain of MEF2C Mediates the Physical Association with HDAC7—We used the in vitro GST pulldown assay to confirm the direct interaction between MEF2C and GST-HDAC7 and to identify the domain in MEF2C that mediates the interaction with this class-II HDAC.

Glutathione-agarose-immobilized GST-MEF2C was tested for direct interaction with in vitro 35S-radiolabeled native HDAC7 (Fig. 2B). Native HDAC7 showed a very strong direct interaction with GST-MEF2C relative to GST alone, suggesting a direct protein/protein interaction between these two transcription factors as it has been shown for HDAC4 and -5 (29, 31).

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Repression of MEF2C-mediated Transcription by HDAC7 Is Mediated by the N-terminal Repression Domain: the Deacetylase Domain Is Not Required—HDACs have been shown to repress transcription at least partially via deacetylation of histones but also to harbor other repression domains (43).
Therefore we were interested to examine whether the downregulation of MEF2-mediated transactivation via HDAC7 requires the enzymatic deacetylation activity. Two deletion mutants of HDAC7 that still interact with MEF2C but lack the HDAC domain (amino acids 1–372 and 1–121; Fig. 2) and a point mutation in the HDAC domain that interferes with the ability to deacetylate histones (H657A, Ref. 51) were tested for their influence on MEF2C-mediated transactivation. Interestingly, these mutants were also able to inhibit MEF2C-mediated transactivation and did not interfere with the full-length HDAC7 (Fig. 4A). No significant effect of HDAC7 mutants or the full-length construct was observed on the basal activity when no MEF2C was transfected (Fig. 4B).

Because HDAC7 mutants and deletions lacking deacetylase function are able to repress MEF2C-mediated transactivation we wanted to gain an insight in how RD1 and RD2 of HDAC7 mediate repression. We therefore tested if HDAC7 interacts with other class-I and -II HDACs and corepressors as it has been shown for HDAC -4 and -5. GST pulldown experiments using bacterial expressed GST-HDAC7 and in vitro-translated 35S-labeled class-I and -II HDACs or the corepressor CtBP demonstrate that HDAC7 interacts with all tested HDACs (-1, -2, -4, -5, and -7) as well as with the corepressor CtBP (Fig. 5). This observation provides evidence that HDAC7-mediated repression of MEF2 activity requires the recruitment of corepressors and other class-I and -II HDACs.

This is consistent with the identification of a distinct matrix-associated nuclear structure that contains corepressors (SMRT, members of the Sin 3 and NuRD complexes), class-I and -II HDACs (43, 51). Furthermore the interaction of CtBP with HDAC7 correlates with several observations that demonstrate that HDAC4, MTR, and Cabin-1 constitute a family of calcium-sensitive transcriptional repressors of MEF2 and recruit CtBP via the PXDLR motif conserved in the N-terminal region of HDAC4, -5, and -7 (32–34, 52).

**HDAC7 Is Expressed during Skeletal Muscle Differentiation and Is Regulated via Nucleo-Cytoplasmic Trafficking.**—To gain insight into how class-II HDACs regulate MEF2C-dependent gene activation during muscle differentiation, we examined the expression pattern of the mRNAs encoding HDAC5 and -7 during the conversion of mouse myoblast C2C12 cells into terminal differentiated myotubes. Proliferating C2C12 myoblasts were induced to biochemically and morphologically differentiate into postmitotic, multinucleated myotubes by serum withdrawal in culture over a period of 4–120 h. Total RNA was isolated from proliferating myoblasts, confluent myoblasts, and postmitotic myotubes after 4, 8, 24, 48, 72, and 120 h of serum withdrawal and examined by Northern blot analysis. The mRNAs for HDAC7 and -5 are expressed in proliferating myoblasts and suppressed as the cells exit the cell cycle and fuse into terminal differentiated myotubes that have acquired a muscle-specific phenotype (Fig. 6A). The repression of the cyclinD1 mRNA and the induction of the mRNAs encoding myogenin and p21 relative to GAPDH confirms that these cells were exiting from the cell cycle and activating the differentiation program. However, expression of the class-II HDACs is still observed in postmitotic cells.

If the HDAC7 protein acts as a transcriptional repressor of MEF2-mediated gene activation it should be found in the nucleus when MEF2 differentiation-dependant genes are inactive. During myogenesis, when these genes are activated, HDAC7 should be unable to suppress the function of MEF2 in the context of HDAC7 expression in postmitotic differentiated cells. The HDAC7 function as a repressor of MEF2 activity must be overcome by another mechanism such as cellular trafficking, localization, and/or proteolytic degradation. Observations reported by others have shown that other class-II HDACs dissociate from MEF2 proteins and shuttle into the cytoplasm in differentiation involving calcium/calmodulin and 14–3–3-dependant phosphorylation (27, 29, 32, 35, 42, 52). To address this question we transfected C2C12 myoblasts with a construct encoding a fusion protein of HDAC7 and the yellow fluorescent protein (pCMX-HDAC7-YFP) and examined its sub-cellular localization in C2C12 cells cultured in growth medium (DMEM supplemented with 20% FCS) or differentiation medium (DMEM supplemented with 2% horse serum), which leads to withdrawal from the cell cycle and the induction of the differentiation program. As seen in Fig. 6B, HDAC7-YFP is predominantly localized in the nucleus when cells were held in growth medium. After 48 h of serum withdrawal (i.e. culturing in 2% horse serum), the HDAC7 protein is localized within the cytoplasm, where it cannot form an inhibitory complex with MEF2 factors. This observation provides a plausible mechanism for the regulation of MEF2 function during muscle differentiation: serum withdrawal leads to nucleo-cytoplasmic trafficking of HDAC7 and therefore allows the differentiation process to proceed.

The class-II HDACs are very homologous to each other and show many similar features in the context of transcriptional regulation; however, they are different proteins with (i) unique amino acid sequences and (ii) spatio-temporal-specific expression patterns. Currently, we still remains unclear what their specific roles in vivo are and whether functional redundancy occurs in the class-II HDACs.

**REFERENCES**
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