Nuclear Hormone Receptor Coregulator GRIP1 Suppresses, whereas SRC1A and p/CIP Coactivate, by Domain-specific Binding of MyoD*

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p160 coregulators were initially identified as nuclear hormone receptor coactivators. In this study, functional data demonstrate that members of the three p160 families can have opposing roles in regulating gene expression by the same transcription factor. Both SRC1A and p/CIP function as coactivators for MyoD-mediated transcription whereas GRIP1 acts negatively as a (co)repressor. SRC1A and p/CIP predominantly interact with distinct sites on the NH2-terminal activation domain of MyoD. GRIP1 binds to both these regions but it alone, and neither SRC1A nor p/CIP, also interacts with specific sites on MyoD that are critical for the binding of the essential MyoD coactivator, p300. This suggests that competition by GRIP1 for SRC1A, p/CIP, and p300 binding sites on a transcription factor may regulate the activity of the factor.

When they were initially discovered, the p160 proteins were thought to act only as coactivators of nuclear hormone receptor-mediated transcription in a ligand-dependent manner (for review, see Refs. 1–5). However, evidence rapidly accumulated to suggest that their functions are not limited to interactions with nuclear receptor-activated transcription but extended to potentiation of other transcriptional activators, e.g. Sp1 (6), NFkB (7–9), serum response factor (SRF) (10), AP1 (11), CREB (12), and CREM (13, 15, 22–24) and the coactivator p300 (14, 15, 18–21), also associate with the coactivator p300. This suggests that competition by GRIP1 for SRC1A, p/CIP, and p300 binding sites on a transcription factor may regulate the activity of the factor.

Experimental Procedures

Plasmids—The cDNAs for GRIP1 and p/CIP generously provided by M. Stallcup (University of Southern California) and M. Rosenfeld (University of California, San Diego, CA) and the cDNA for SRC1A (31) were subcloned into pEG202 (a gift from E. Golemis, Fox Chase Cancer Center). pCMX-LexA, respectively. pCMX-LexA was made by substituting the chloramphenicol acetyltransferase (CAT)1 cassette of HCACAT with a luciferase (LUC) cassette derived from pGL2-Basic (Promega). pCDNA3MyoD has been described previously (26). The cDNAs for GRIP1, SRC1A, and p/CIP were subcloned into pCMX-Gal4N (a gift from R. Evans, Salk Institute) or pCMX-LexA, respectively. pCMX-LexA was made by substituting the Gal4 DNA binding domain of pCMX-Gal4N with the LexA DNA binding domain of pEG202 (a gift from E. Golem, Fox Chase Cancer Center). LgS6Luc was generously provided by S. Holland (Volum Institute). G4TALUC was made by cleaving out a 17-mer-TATA fragment from a 17-TATA poly(A) vector (generous gift of Ming-Ter Tsai, Baylor) and subcloning it into pGL2-Basic. GST-MyoD was originally provided by H. Stallcup (University of Southern California). HCA-LUC was generously provided by S. Hollenberg (Volum Institute). HCACAT was made by substituting the chloramphenicol acetyltransferase (CAT)1 cassette of HCACAT with a luciferase (LUC) cassette derived from pGL2-Basic (Promega). pCDNA3MyoDFlag has been described previously (26). The cDNAs for GRIP1, SRC1A, and p/CIP were subcloned into pCMX-Gal4N (a gift from R. Evans, Salk Institute) or pCMX-LexA, respectively. pCMX-LexA was made by substituting the Gal4 DNA binding domain of pCMX-Gal4N with the LexA DNA binding domain of pEG202 (a gift from E. Golem, Fox Chase Cancer Center). LgS6Luc was generously provided by S. Hollenberg (Volum Institute). G4TALUC was made by cleaving out a 17-mer-TATA fragment from a 17-TATA poly(A) vector (generous gift of Ming-Ter Tsai, Baylor) and subcloning it into pGL2-Basic. GST-MyoD was originally provided by H.

The abbreviations used were: CAT, chloramphenicol acetyltransferase; LUC, luciferase; GST, glutathione S-transferase; BES, 2-hydroxyethylaminomethanesulfonic acid; MEF, mouse embryonic fibroblast; aa, amino acids; HAT, histone acetyltransferase; MCK, muscle creatine kinase; HCA, human cardiac α-actin; GR, glucocorticoid receptor.

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Weintraub (Hutchinson Cancer Institute). pCMXVP16MyoD was made by subcloning PCR amplified MyoD fragment into pCMXVP16 (a gift from R. Evans, Salk Institute). Various deletions as well as site-specific mutants of the MYOD cDNA were created by subcloning different PCR amplified DNA fragments into pGEX2TK. Each set of deletion mutants of GRIP1, SRC1A, PCIP, as well as Gal4DBD fusion constructs were made by subcloning various PCR amplified DNA fragments into either pSG5, pCDNA3, or pCMX-Gal4N expression vectors. The nucleotide sequences of all the mutants have been determined at least for the critical regions subjected to changes. All the expression vectors, either prokaryotic or eukaryotic, were tested for production of a protein of the expected molecular size determined either by Coomassie Blue staining for bacterially produced proteins or in vitro translation for proteins expressed in mammalian cells.

Cell Culture, Transient Transfections, and Luciferase Reporter Assays—Mouse C3H10T1/2 embryoblasts obtained from the American Type Culture Collection were maintained in Dulbecco's modified Eagle's medium supplemented with fetal bovine serum (10%) and β-mercaptoethanol (10−4 M). MEFs were refed with fresh medium without 2-mercaptoethanol when transient transfections were carried out. To measure the transcription activity of MyoD in SRC1A+/−MEFs, 4RELUC, MCKLUC, or HCA-LUC (1 μg each) reporter, plus pCDNA3/Myc expression vectors (amounts as shown in the figures), were cotransfected into the MEFs.

In Vitro Protein-Protein Interaction Assays—In vitro transcription-translation was carried out using 1 μg of various supercoiled DNA plasmids, TNT® coupled transcription/translation system (Promega), and li-18[S]methionine (>1000 Ci/mmol; Amersham Biosciences) according to the manufacturer's protocol. The expression and purification of various glutathione S-transferase (GST) fusion proteins as well as protein-protein interaction assays were performed as described previously (26–28) with slight modifications. The GST and GST fusion proteins were expressed in Escherichia coli (BL21) and purified using glutathione-agarose affinity matrix (Sigma). The molecular weight of each individual purified protein was analyzed and confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

RESULTS

GRIP1 Suppresses MyoD on Muscle-specific Promoters—We first investigated the effects of p160s on MyoD dependent transcription using muscle-specific promoters fused to a reporter gene. 4RELUC contains four tandem E-box motifs and is activated only by MyoD family members but not other myogenic activators. MCKLUC and HCA-LUC carry the muscle specific regulatory enhancers of the muscle creatine kinase gene and the human cardiac α-actin gene, respectively. Mouse CH310T1/2 fibroblasts were transiently transfected with one of the reporters plus an expression vector for MyoD and for a p160 protein (Fig. 1).

As expected, MyoD alone specifically activates all three promoters (Fig. 1, A–C). Importantly, both SRC1A and PCIP were capable of potentiating MyoD transactivation on all three promoters, establishing them as coactivators of MyoD. Very surprisingly, however, GRIP1 not only failed to increase MyoD-mediated induction of reporter activity but repressed it strongly on all three promoters (and nearly completely on the 4RELUC and MCKLUC promoters). Similar effects on MyoD-dependent transcription by p160s also occurs in C2C12 myoblasts and in differentiated C2C12 myotubes. These results strongly imply that SRC1A and p/CIP function in myogenic cells as positive regulators for MyoD, whereas GRIP1 acts negatively in both myogenically undifferentiated and differentiated cells. Furthermore, that these effects are seen using the 4RELUC reporter supports the notion that the effects are mediated through direct interactions with MyoD and not secondarily through effects on other myogenic transcription factors.

The Transactivation Activity of MyoD Is Diminished In SRC1A+/−/− Mouse Embryonic Fibroblasts—We used SRC1A+/−MEFs derived from the SRC1A+/− mice to test whether SRC1A is essential for MyoD transactivation of muscle-specific promoters. We compared the ability of MyoD to transactivate the target reporters in wild type versus SRC1A+/−MEFs. As shown in Fig. 2, the MyoD-dependent reporter activity was many fold less on the 4RELUC reporter (Fig. 2A), the muscle creatine kinase (MCK) promoter (Fig. 2B), and on the human cardiac α-actin (HCA) promoter (Fig. 2C) in the SRC1A+/−MEFs. Indeed, the activation of the muscle creatine kinase promoter mediated by MyoD was completely silenced in the SRC1A+/−MEFs (Fig. 2B). These data demonstrated that the ability of MyoD to transactivate is significantly diminished in the absence of SRC1A and that SRC1A is essential for certain types of muscle specific gene expression.

NH2-Terminus of MyoD Is Targeted by p160 Coregulators In Vitro—We tested the ability of MyoD protein to interact in vitro with all three classes of p160s using GST-affinity binding assays (Fig. 3B). In vitro translated 35S-labeled GRIP1 (lane 1), SRC1A (lane 4), and p/CIP proteins (lane 7) all bind to GST-MyoD significantly when compared with GST alone (lanes 2, 5, and 8). These data establish that the three classes of p160 coregulators can bind directly to MyoD in vitro.

The NH2 terminus of MyoD functions as an activating domain (33), and its bHLH domain is essential for both heterodimerizing with E proteins (34) and DNA binding (35, 36). These domains, or MyoD deleted for these domains, were cloned into GST fusion vectors (Fig. 3A). GST affinity assays reveal that the NH2-terminal domain of MyoD is both required and sufficient to interact fully with any of the three p160 coregulators and with p300 (Fig. 3, C and D), whereas deletion of the MyoD bHLH domain does not significantly effect its ability to bind (Fig. 3E). However, when tested alone (Fig. 3F), the bHLH domain retained minimal ability to interact with p/CIP greater than the binding to GST alone. On the other hand, the COOH terminal of MyoD does not appear to be involved in interactions with p300 or with SRC1A and GRIP1 although p/CIP may require it for full binding activity to some extent (Fig. 3G). Taken together, these data suggest that the NH2 terminus of MyoD is the segment of MyoD that primarily interacts with the p160 coregulators and with p300.

p160 Coregulators Associate with MyoD In Vivo—Mammalian two-hybrid assays were used next to determine whether these in vitro interactions between p160 coregulators and MyoD also can occur in cells. Full-length GRIP1 and p/CIP were fused to the GAL4-DNA binding domain to generate GAL-GRIP1 and GAL-CIP; SRC1A was fused to the LexA-DNA binding domain to generate LexASRC1A. These constructs were individually tested for their ability to effect the transcriptional activity of VP16MyoD. As reported previously (37, 38), GAL-

GRIP1, LexASRC1A, and GALCIP can themselves contribute substantially to reporter activity (column 2 of Fig. 4, A–C). Significantly, all reporter activities were increased in a dose dependent manner when cotransfected with VP16MyoD (columns 3–5 of Fig. 4, A–C). The results of these mammalian two-hybrid experiments establish that all three classes of p160 coregulators can directly associate with MyoD in living cells.

FIG. 1. SRC1A and CIP coactivate, whereas GRIP1 represses, MyoD on muscle specific promoters. A, mouse C3H10T1/2 cells were transiently transfected with 4RELUC reporter (1 µg), expression vectors of MyoD (0.1 µg), and the indicated coregulators GRIP1/SRC1A/CIP (the amounts as shown in the panel). The coregulators were cloned in equivalent vectors. The -fold change of MyoD transactivation (means ± S.E.) was measured by luciferase activity 48 h after transfection relative to the basal reporter gene expression set at a value of 1. B, transient transfection experiments were carried out as shown in A with MCKLUC reporter instead. C, transient transfection experiments were carried out as shown in A with HCALUC reporter instead.

FIG. 2. The transactivation of MyoD is significantly diminished in SRC1A−/− MEFs. A, SRC1A+/+ and SRC1A−/− MEFs were transiently transfected with 4RELUC reporter (1 µg) and various amounts of a MyoD expression vector as indicated in the figure. The transactivation by MyoD was measured by luciferase assay 48 h after transfection. SW3T3 are SRC1A+/+ MEFs (black bars). SM3T3 are SRC1A−/− MEFs (gray bars). B, transient transfection experiments were carried out as shown in A with MCKLUC reporter instead. C, transient transfection experiments were carried out as shown in A with HCALUC reporter instead.

GRIP1, LexASRC1A, and GALCIP can themselves contribute substantially to reporter activity (column 2 of Fig. 4, A–C). Significantly, all reporter activities were increased in a dose dependent manner when cotransfected with VP16MyoD (columns 3–5 of Fig. 4, A–C). The results of these mammalian two-hybrid experiments establish that all three classes of p160 coregulators can directly associate with MyoD in living cells.

SRC1A and CIP Deleted of Analogous Domains Fail to Coactivate MyoD, whereas GRIP1 with the Same Deletion Upregulates MyoD—We recently identified specific segments of
each of the p160s that interact with MyoD both in vitro and in vivo (32). Two separated short domains in the middle of each p160 (designated as MID1 and MID2) as well as the C termini appear to be crucial MyoD interaction domains. We have synthesized in vitro and in vivo the proteins encoded by mutant constructs that lack these elements (designated as MID1/MID2-C). Significantly all these mutant proteins lose the ability to interact with MyoD both in vitro and vivo (32). We have now tested these constructs to determine whether the essential interaction domains also play functional roles in regulating the effects of the p160s on MyoD-mediated transcription. Indeed, the mutant constructs all lose their effects on MyoD-mediated expression (Fig. 5, A–C). SRC1A and p/CIP/MID1/2-C mutants totally lose their ability to augment MyoD-mediated expression on the 4RE, MCK, and HCA reporter constructs (Fig. 5, A and B). Most notably, the GRIP1 mutant loses its suppressive effects and is associated with an intriguing increase in the transcriptional activity of MyoD (Fig. 5C). Thus the functional effects of the p160 coregulators on MyoD mediated transcription, as well as their protein-protein interactions with MyoD,
requires the presence of a combination of the MID1, MID2, and COOH-terminal segments. This is consistent with the idea that these functional effects, whether activating or suppressing gene expression, require direct p160-MyoD physical interaction.

\[ p300 \text{ and the p160 Coregulators Bind to Different Subdomains of the MyoD Amino Terminus—} \]

The NH₂ terminus domain of MyoD is activated by the binding there of p300/CPB (26–28) involving an FYD motif and phenylalanine 42. When these sites are mutated, the NH₂ terminus loses its ability to transactivate or to bind and be coactivated by p300 (26–28). We speculated that the differential effects of the p160s on MyoD activity might be related to their distinctive contacts with the activation domain and with the p300/CPB binding sites.

To begin to test this possibility, we evaluated the GST binding affinity of p300 and of the p160s for three subsegments (and their deletions) of the MyoD NH₂-terminal region designated in Fig. 6A as N1 (aa 1–36), N2 (aa 37–72), and N3 (aa 73–109). We tested as well a MyoD NH₂ terminus bearing a mutation at the critical residue 42. Consistent with previous results (26–28) it appears that both the N3 and N2 regions, including wild type residue 42, are involved in full binding of p300 to the MyoD activation domain (Fig. 6B). As shown in Fig. 6, C–E, the N1 subsegment of MyoD is neither required nor capable of interacting with GRIP1, SRC1A, or p/CIP. The N2 region binds to GRIP1 and SRC1A but not p/CIP. The N3 subsegment appears to interact with both GRIP1 and p/CIP but not SRC1A. Also of importance is the discovery that whereas binding of SRC1A and p/CIP are unaffected by mutation of MyoD at residue 42, both p300 and GRIP1 binding is reduced (lane 7 in Fig. 6, B–E). These results suggest that SRC1A and p/CIP each interact with two different amino acid subsets of the NH₂-terminal activation domain of MyoD. The binding targets of GRIP1 could well overlap those of both SRC1A and p/CIP.

The functional repression of MyoD activated transcription by GRIP1 stands in stark contrast to the coactivation properties of p300, p/CIP, and SRC1A. That GRIP1, the (co)repressor, binds to the activation domain of MyoD at regions that fully overlap those required for binding by the coactivators p300, SRC1A, and p/CIP has not escaped our attention. Whereas the precise mechanisms of these effects remain to be established, it is attractive to speculate that SRC1A and p/CIP can positively effect the simultaneous interactions of the MyoD activation domain with p300 and pCAF. Conversely, the overlap of the GRIP1 binding sites on MyoD with those of p300, SRC1A, and p/CIP suggest that an interference mechanism may explain the repression effects of GRIP1.

The HAT Domain of SRC1A Is Required to Coactivate MyoD—A histone acetyltransferase (HAT) activity has been demonstrated in both SRC1A and ACTR (human homologue of p/CIP) (18, 25). It is also known that the HAT domain of pCAF is required to coactivate MyoD-dependent transcription (27). We tested whether this HAT activity of SRC1A was essential for its coactivation of MyoD-activated transcription. The full HAT activity of SRC1A has been located at its COOH terminus (aa 1107–1441) (25). Thus, SRC1A/H9004C, a mutant devoid of its COOH terminus (aa 1105–1441), was initially employed to test this hypothesis. As shown in Fig. 7A, it appeared that the coactivation ability of the SRC1A mutant devoid of its HAT domain (lanes 6–8) is greatly diminished compared with wild type SRC1A (lanes 3–5). The importance of the HAT domain was further confirmed by testing its effect on MyoD-mediated transcription. We cotransfected a segment of SRC1A (encoding amino acids 1028–1441) carrying both the MID2 region and the HAT COOH-terminal domain. This segment potentiated transcription from the MyoD activated MCK promoter (Fig. 7B, lane 5 and 6). The HAT domain of SRC1A thus appears to be
DISCUSSION

Our examination of the effects of p160 proteins not only demonstrates their functions in modulating MyoD-dependent muscle specific gene expression but reveals their independent and different roles in regulating the same transcriptional activator, MyoD. We found that SRC1A and p/CIP are capable of potentiating MyoD transactivation in C3H10T1/2 cells. We observed increases by SRC1A of up to 9-fold and 4-fold by p/CIP using the natural HCA promoter in pluripotent 10T1/2 cells (Fig. 1C). Thus, we concluded that both SRC1A and p/CIP function as coactivators for MyoD-dependent transcription, activities completely consistent with their known positive effects on other transcription factors.

In marked contrast, GRIP1, which has been extensively characterized as a coactivator of ligand-mediated nuclear hormone receptor-dependent transcription, significantly down-regulates MyoD-reporter activity on both 4RE and MCK promoters. It does this not only in 10T1/2 cells but in differentiating myoblasts as well (32) by at least 80–100% when compared with MyoD tested alone. The extent of such negative regulation is less extreme on the HCA promoter, but it reduces MyoD transactivation by 40–50%. This appears to be the first report in which any p160 protein acts as a repressor of a transcription factor. Our observations are operationally quite different from the one reported instance in which a p160 protein was discovered to repress transcription (39). In that paper it was shown that the glucocorticoid receptor (GR) repressed transcription of the collagenase promoter (col3A) and that GRIP1, binding to the GR, further suppressed transcription. Thus GRIP1, by increasing the already negative activity of GR, was acting to enhance GR activity. In the case of the transcriptional activator MyoD, GRIP1 works to oppose its activity.

Chen et al. (40) have analyzed extensively the effects of GRIP1 on transcription mediated by the other major class of myogenic transactivators, MEF2. When transcription is dependent on the presence of MEF2, GRIP1 strongly enhances transcription from many of the same promoters we have tested (40). MEF2 contributes to E-box-dependent transcription by binding to MyoD and helping tether other coactivators including p300/CBP and, as shown by Chen et al. (40), GRIP1. Indeed,
among the experimental paradigms tested by Chen et al. (40), they concluded that GRIP1 had no effect on transcription from MyoD dependent promoters in the absence of MEF2. However, a review of their data indeed shows that in the absence of MEF2 GRIP1 suppresses transcription mediated by MyoD family members on both the MCK promoter and on the artificial E-box promoter, 4RE. MyoD transactivation is significantly decreased on both 4RE and HCA promoters in SRC1A/H11002/H11002 MEFs, suggesting that SRC1A is required for full MyoD transactivation (Fig. 2, A and C). The transactivation activity of both GALMyoD (full-length of MyoD fused to Gal-DBD, 4-fold decreased) and GALMyoD-N (NH2-terminal activation domain of MyoD fused to Gal-DBD, 2-fold decreased) was also decreased using a G4TATALUC reporter in SRC1A/H11002/H11002 MEFs when compared with wild type MEFs (data not shown). In addition, SRC1A appears to be essential for MyoD transactivation activity on the MCK promoter in SRC1A/H11002/H11002 MEFs (Fig. 2B). The mechanisms by which MyoD acts differently on various muscle specific promoters in SRC1A/H11002/H11002 MEFs still remains to be investigated.

MyoD is active only when it is acetylated (27, 29). The HAT activity of either pCAF (27, 29) or p300 (32) can acetylate MyoD. However, it is the HAT activity of pCAF but not that of p300/CBP that is required to coactivate MyoD-dependent transcription (27). Given that 1) the in vitro interactions between MyoD and pCAF are much weaker than between MyoD and p300 and 2) there is no detectable interaction between pCAF and MyoD in mammalian two-hybrid assays, it seems likely that the role of pCAF in MyoD-activated transcription must be mediated by other associated factors. To date it has been concluded that 1) the in vitro translated p160 product that was used in the binding reaction. C, GST affinity binding assays were carried out as shown in E with GRIP1. D, GST affinity binding assays were carried out as shown in E with SRC1A. E, GST affinity binding assays were carried out as shown in E with CIP.

Fig. 6. Identification of sites on MyoD that interact with p160s. A, schematic representation of GST-MyoD-NH2 terminus and subsegments used in GST-affinity binding experiments. The coordinates (amino acids) of the segments evaluated are indicated in the figure. F42P represents the intact NH2-terminal GST-MyoD-N (aa 1–109) with proline substituted for phenylalanine at amino acid 42. B, in vitro translated 35S-labeled p300 was incubated with various GST-MyoD-NH2-terminal segments immobilized on glutathione-agarose beads. The bound proteins were eluted and resolved by autoradiography after SDS-PAGE. Glutathione-agarose-immobilized GST protein serves as control. 10% Input indicates that the lane was loaded with 10% of the amount in vitro translated p160 product that was used in the binding reaction. C, GST affinity binding assays were carried out as shown in E with GRIP1. D, GST affinity binding assays were carried out as shown in E with SRC1A. E, GST affinity binding assays were carried out as shown in E with CIP.

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regulators and MyoD in vitro (Fig. 3). Importantly, these physical interactions between p160s and MyoD are able to take place independently of both E2A gene products, the heterodimer partners of myogenic bHLH proteins, and MEF2, since they are not necessarily included in the in vitro GST binding reactions.

The activation domains of transcription factors are the binding targets of co-activating adaptor proteins such as p300/CBP. SRC1A and p/CIP coactivators follow this rule, since they bind to the NH2-terminal activation domain of MyoD (Fig. 3, C and D). p300, SRC1A, and p/CIP all coactivate MyoD-dependent transcription through interactions at the same NH2-terminal activation domain of MyoD. This raises the question of whether their binding is coordinated and cooperative or whether their binding activities and docking sites are mutually interchangeable. Our experiments suggest that each has its individual binding positions on the NH2 terminus of MyoD (Fig. 6). Thus, it is likely that these molecules interact and regulate MyoD transactivation in a coordinated manner rather than competing for binding. GRIP1 appears to be an exception since it binds throughout the NH2 terminus (Fig. 6C). This suggests that it could compete for binding by the coactivators thus providing a logical and testable explanation for the (co)repressor activity of GRIP1 on MyoD-mediated transcription.

MyoD protein must be acetylated to function as a transcriptional activator. The HAT domains of both p300 and PCAF are capable of acetylating MyoD. However, only the HAT domain of PCAF, which binds to the MyoD transcription complex most strongly through its affinity for p300, is essential (27, 29). SRC1A and p/CIP contain HAT domains (25) and the HAT activities of these domains might possibly play a positive regulatory role in MyoD activity (Fig. 7). In that regard, GRIP1 has no known HAT activity. Indeed, if GRIP1 binding to the MyoD NH2 terminus displaces p300/CBP and SRC1A and p/CIP, there would then be no known HAT domain bound strongly to MyoD. Accordingly, MyoD would then remain in an unacetylated and, hence, inactive state. Experiments to directly assess these possibilities are in progress.

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Fig. 7. HAT domain of SRC1A is required for MyoD-dependent transcription. A, mouse C3H10T1/2 cells were transiently transfected with MCKLUC reporter (1 μg), expression vectors of MyoD (0.1 μg), and either the wild type SRC1A or mutant SRC1AΔC (aa 1–1104; amounts as indicated in the figure). The coregulators were cloned in equivalent vectors. The -fold change of MyoD transactivation (means ± S.E.) was measured by luciferase activity 48 h after transfection relative to the basal reporter gene expression set at a value of 1. B, transient transfection experiments were carried out as shown in A with the wild type SRC1A and mutant SRC1A-MID2+ C (aa 1028–1441).

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