Mirk/dyrk1B Decreases the Nuclear Accumulation of Class II Histone Deacetylases during Skeletal Muscle Differentiation*

Xiaobing Deng, Daina Z. Ewton, Stephen E. Mercer, and Eileen Friedman‡

From the Department of Pathology, Upstate Medical University, State University of New York, Syracuse, New York 13210

Mirk/dyrk1B is a member of the dyrk/minibrain family of serine/threonine kinases that mediate the transition from growth to differentiation in lower eukaryotes and mammals. Depletion of endogenous Mirk from C2C12 myoblasts by RNA interference blocks skeletal muscle differentiation (Deng, X., Ewton, D., Pawlikowski, B., Maimone, M., and Friedman, E. (2003) J. Biol. Chem. 278, 41347–41354). We now demonstrate that knockdown of Mirk blocks transcription of the muscle regulatory factor myogenin. Co-expression of Mirk with MEF2C, but not MyoD or Myf5, enhanced activation of the myogenin promoter in a Mirk kinase-dependent manner. Mirk activated MEF2 not through direct phosphorylation of MEF2 but by phosphorylation of its inhibitors, the class II histone deacetylases (HDACs). MEF2 is sequestered by class II HDACs such as HDAC5 and MEF2-interacting transcriptional repressor (MITR). Mirk antagonized the inhibition of MEF2C by MITR, whereas kinase-inactive Mirk was ineffective. Mirk phosphorylates class II HDACs at a conserved site within the nuclear localization region, reducing their nuclear accumulation in a dose-dependent and kinase-dependent manner. Moreover, less mutant MITR phosphomimetic at the Mirk phosphorylation site localized in the nucleus than wild-type MITR. Regulation of class II HDACs occurs by multiple mechanisms. Others have shown that calcium signaling leads to phosphorylation of HDACs at 14-3-3-binding sites, blocking their association with MEF2 within the nucleus. Mirk provides another level of regulation. Mirk is induced within the initial 24 h of myogenic differentiation and enables MEF2 to transcribe the myogenin gene by decreasing the nuclear accumulation of class II HDACs.

The availability of in vitro culture systems that recapitulate the major features of muscle cell differentiation has allowed much progress toward the elucidation of the molecular basis for muscle maturation and regeneration. An improved understanding of these processes might have significant clinical ramifications, such as the development of strategies to ameliorate muscle function as a transcriptional activator of MEF2C, a known mediator of myogenin expression, by decreasing the nuclear concentration of class II HDACs that function as MEF2C inhibitors. Mirk phosphorylates these HDACs at a conserved serine within their nuclear localization signal.

EXPERIMENTAL PROCEDURES

Small Interfering RNA (RNAi) to Mirk—The RNAi sequence to Mirk, GACCTACAAGCACATCATT, was inserted into the pSilencer plasmid

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† To whom correspondence should be addressed: Upstate Medical University, Pathology Dept., 2303 Weiskotten Hall, 750 East Adams St., Syracuse, NY 13210. Tel.: 315-464-7138; Fax: 315-464-8419; E-mail: friedmae@upstate.edu.

‡ To whom correspondence should be addressed: Upstate Medical University, Pathology Dept., 2303 Weiskotten Hall, 750 East Adams St., Syracuse, NY 13210. Tel.: 315-464-7138; Fax: 315-464-8419; E-mail: friedmae@upstate.edu.

The abbreviations used are: CDK, cyclin-dependent kinase; HDACs, histone deacetylases; CaMK, calcium/calmodulin-dependent protein kinase; HNF1α, hepatocyte nuclear factor α; RNAi, RNA interference; MITR, MEF2-interacting transcriptional repressor; NLS, nuclear localization sequence; EGFP, enhanced green fluorescent protein; PBS, phosphate-buffered saline; GM, growth medium.
Affymetrix Gene Chips—The MOE430A chip (mouse expression) was utilized. The chips contain 22,690 unique probe identifier oligonucleotide sequence sets. Data were analyzed by using the Microarray Suite 4.0 and, for a more stringent program, the Robust Multi-Choice Analysis program (Gene Traffic, Iobion) to identify genes exhibiting at least a 2-fold reduction, comparing the RNA populations treated with RNAi to Mirk to those treated with the vector control. 12,435 probe sets were identified in the RNAi population and 12,718 in the vector control. 193 transcripts were decreased, and 86 transcripts were increased.

Materials—Antibodies to myogenin, MEF2C, MyoD, and β-tubulin were from Santa Cruz Biotechnology. Antibody to the N terminus of HDAC9 from ABGENT was used to detect MTR. Rabbit polyclonal antibody to a unique sequence at the C terminus of Mirk was as described (2). Polyvinylidene difluoride transfer paper Immobilon-P was purchased from Millipore. PLUS reagent and Lipofectamine were from Invitrogen; all radioactive materials were purchased from PerkinElmer Life Sciences; ECL reagents were from Amersham Biosciences, and tissue culture reagents were from Mediatech (Fishier). Alexa Fluor 594 (highly cross-adsorbed) secondary antibody conjugates were from Santa Cruz Biotechnology. Antibody to the N terminus of HDAC-9 was purchased from Millipore. PLUS reagent and Lipofectamine were from Invitrogen; all radioactive materials were purchased from PerkinElmer Life Sciences; ECL reagents were from Amersham Biosciences, and tissue culture reagents were from Mediatech (Fishier). Alexa Fluor 594 (highly cross-adsorbed) secondary antibody conjugates were from Santa Cruz Biotechnology. Antibody to the N terminus of HDAC-9 was purchased from Millipore. PLUS reagent and Lipofectamine were from Invitrogen; all radioactive materials were purchased from PerkinElmer Life Sciences; ECL reagents were from Amersham Biosciences, and tissue culture reagents were from Mediatech (Fishier).

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(2) C2C12 cells were transfected with a 10 to 1 ratio (μg/μg) of RNAi plasmid to EGFP selection plasmid in serum-free medium and then isolated by fluorescence-activated cell sorting for EGFP by using a BD Biosciences FACSVantage SE cell sorter.

Northern Analysis for Myogenin mRNA—Total RNA was prepared by the RNeasy protocol (Qiagen) and analyzed for mRNA composition by microarray analysis. 4 μg of the remaining total RNA from each cell lysate was electrophoresed in a 1.1% agarose-formaldehyde gel, transferred to nylon membranes by downward capillary transfer, and cross-linked by baking in an oven. The membranes were hybridized to a 32P-labeled 1-kb EcoRI fragment of the myogenin cDNA construct. The probe was labeled with random primers and was hybridized overnight at 68 °C with at least 10⁷ cpm of the labeled probe, washed twice at room temperature for 15 min with 1× SSC, 0.1% SDS, and then washed for 20 min at 65 °C in 0.2× SSC, 0.1% SDS and autoradiographed.

In Vitro Kinase Assay—GST-Mirk or GST-YF-Mirk preparations were incubated for 5 min at 30 °C with 20 μl of kinase buffer (50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 0.5 mM dithiothreitol), containing 50 μM cold ATP plus 5 μCi of [γ³²P]ATP and 1 μg of purified recombinant GST-HDAC5 protein as substrate, and then analyzed by PAGE and autoradiography.

Glutathione S-Transferase Fusion Proteins—Mirk and YF-Mirk were subcloned into the pGEX-6P1 vector (Amersham Biosciences), and the HDAC5 constructs were subcloned into the pGEX 4T1 vector (Amersham Biosciences), and both were expressed and purified as described.²

Metabolic Labeling—C2C12 cells were incubated for 6 h in serum-free low phosphate Dulbecco’s modified Eagle’s medium (9:1 ratio phosphate-free medium/normal medium) with 150 μCi of [³²P]orthophosphate/2 ml of medium.

Immunodetection—Following treatment as indicated and washing twice with PBS, cells were lysed in hot SDS-PAGE sample buffer (50 mM Tris, pH 6.8, 2% SDS, 10% glycerol), boiled for 5 min, and vortexed vigorously. Protein determinations were made using Coomasie protein assay reagent from Pierce. Depending upon the experiment, 30–50 μg of cell lysate were blotted onto polyvinylidene difluoride membranes after separation on SDS-PAGE. The blots were blocked in 5% milk in TBST (0.1% Triton in PBS) for 1 h at room temperature and incubated for 1–2 h at room temperature with primary antibody in TBST buffer, 3% milk, and proteins were subsequently detected by enhanced chemiluminescence.

Band Analysis—Immunoblots were scanned using a Lacie Silver scanner, and densitometry was performed using the IP Lab Gel program (Scanalytics).

RESULTS

Use of Small Interfering RNA to Mirk Substantially Decreased the Levels of Myogenin mRNA and Protein in C2C12 Myoblasts—In our recent study (3), we utilized RNAi to demonstrate that Mirk helps to mediate skeletal muscle cell differentiation. Western blotting analysis showed that knockdown of endogenous Mirk led to depletion of other proteins that mediate the myotube phenotype. In the current study we used microarray analysis to identify the genes down-regulated when Mirk levels were reduced by RNA interference. Two RNA populations were compared by microarray analysis as follows: RNA from differentiating C2C12 myoblasts in which Mirk was induced as a natural result of differentiation, and RNA from myoblasts cultured under the same conditions but with endogenous Mirk depleted by RNAi. Cells expressing RNAi were

² S. Mercer, D. Ewton, S. Lim, T. Mazur, and E. Friedman, submitted for publication.
in Tables I and II were examined by Northern blotting for myogenin to Mirk.

plasmid for Mirk RNAi and EGFP, or vector DNA and EGFP, selected by cell sorting for EGFP, placed in GM for 1 day, and then switched to differentiation medium for 1 day. Vc, vector; si, small interfering RNA to Mirk. A, cell lysates examined by Western blotting for Mirk and myogenin. NS, nonspecific cross-reacting protein to demonstrate equal loading and transfer. B, RNA preparations used for microarray analysis in Tables I and II were examined by Northern blotting for myogenin mRNA. 18 S and 28 S rRNA bands are indicated.

selected by cell sorting for the co-transfected EGFP marker gene, with cells transfected with vector DNA serving as the control. The sorted C2C12 cells were then plated for 1 day and then switched to differentiation medium for 1 day to induce Mirk (vector control, Vc cells) or to allow the RNAi to deplete endogenous Mirk (si cells). Western blotting confirmed that endogenous Mirk levels were decreased 60-fold by RNA interference (Fig. 1A).

The MOE430A Affymetrix chip (mouse expression) was utilized for microarray analysis of the RNA populations from Mirk-depleted and control cells. The chips contain 22,690 unique probe identifier oligonucleotide sequence sets. Data were analyzed using the Microarray Suite 4.0 and, a more stringent program, the Robust Multi-Chip Analysis program (Gene Traffic, Iobion) to identify genes exhibiting at least a 2-fold reduction when the mRNA population from the RNAi-treated cells was compared with the mRNA population from vector-control-treated cells. 12,433 probe sets were identified in the RNAi population and 12,718 in the vector control. 193 transcripts were decreased, and 86 transcripts were increased. The genes whose expression was most strongly reduced when Mirk was depleted by RNAi are shown in Table I.

Most of the genes reduced by knockdown of endogenous Mirk by RNA interference were skeletal muscle differentiation genes. None of the Id family of differentiation inhibitor genes was increased when Mirk was depleted (Table II). One of the genes reduced most in expression was myogenin, whose expression was reduced 7.5-fold. Northern blotting confirmed the microarray analysis. Myogenin mRNA was undetectable by Northern blotting, although similar levels of 18 S and 28 S rRNAs were found in the RNA preparations (Fig. 1B). As an additional control, cyclin D1 mRNA levels were measured in the Northern blots and were identical in control and RNA interference samples (data not shown). Therefore, Mirk controls the transcription of myogenin. Our prior study supports the hypothesis that Mirk mediates expression of myogenin. Myogenin and Mirk are induced at the protein level with similar kinetics during the initial differentiation of primary cultured murine hindlimb muscle satellite cells, C2C12 cells, and L6 rat myoblasts placed in differentiation medium (3). Furthermore, depletion of Mirk by RNAi led to a 13-fold reduction in myogenin protein levels (Fig. 1A), and overexpression of Mirk induced a more rapid appearance of myogenin (3). Thus myogenin down-regulation following loss of Mirk is mediated at the transcriptional level.

RNAi to Mirk Blocks Activation of the Myogenin Promoter—Myogenesis is regulated by the following four transcription factors called muscle regulatory factors: Myf5, MyoD, myogenin, and MRF-4, which function in a sequential program, and a second group of transcription factors termed myocyte enhancer-2 (MEF2A through 2D). Myf5 and MyoD are essential for establishing the myogenic lineage, before Mirk is induced through RhoA action at the onset of differentiation (3). Myogenin is up-regulated later during the process of terminal differentiation. Both an E box (CANNTG) and an MEF2-binding site within the myogenin promoter are essential for myogenin transcription. The transcription factors MyoD, MEF-2, Myf5, and the ubiquitous E box proteins have been implicated in myogenin regulation (13–15) and could serve as potential Mirk substrates. The muscle regulatory factor Myf-5 is co-expressed with MyoD only in dividing myoblasts but is lost within 12 h of the start of differentiation and is not seen in cells expressing myogenin, indicating that MyoD, not Myf-5, mediates myogenin transcription (16).

We first determined whether endogenous Mirk could up-regulate myogenin expression by transfecting a myogenin promoter reporter construct into growing C2C12 myoblasts. The cells were then switched to differentiation medium for 24 h to induce the up-regulation of Mirk (3). The cells were not sorted in this experiment, and the transfection efficiency was at best 30%, so some background was detected due to the residual endogenous Mirk activity. However, a clear difference was observed. The myogenin promoter reporter construct was activated 4-fold in differentiating C2C12 cells that had up-regulated endogenous Mirk expression as part of their differentiation program (Fig. 2). However, when the myogenin reporter construct was co-transfected with an expression plasmid for RNAi to Mirk to knockdown endogenous Mirk levels and cells were then cultured in differentiation medium, little increase in myogenin reporter activity was observed. Therefore, reduction of endogenous Mirk levels blocked the activation of the myogenin promoter.

Mirk Activates a Myogenin Reporter Construct in a Kinase-dependent Manner in Concert with MEF2C but Not MyoD or Myf5—Mirk activates the transcription factor HNF1α and phosphorylates HNF1α at a residue within its CBP-binding domain (6). We postulated that Mirk would also phosphorylate, and thus activate, one of the transcription factors that mediate transcription of the myogenin gene. We co-transfected a myogenin promoter reporter construct together with expression plasmids for either Mirk, kinase-inactive YF-Mirk, or the vector, and we then performed parallel experiments with the Mirk constructs in the presence of expression constructs for MEF2C, MEF2D, MyoD, Myf5, or combinations. Mirk activated the myogenin promoter reporter construct about 2-fold, whereas YF-Mirk had no effect (Fig. 3A, vector lanes). When either MEF2C or MEF2D were co-expressed with wild-type Mirk, there was a further 2-fold increase in reporter activity. MEF2D, and to a lesser extent MEF2C, increased myogenin reporter expression when co-expressed with kinase-inactive YF-Mirk, probably because each factor complexed with endogenous transcription factors. However, MEF2C clearly activated the myogenin reporter in a Mirk kinase-dependent manner. In contrast, MyoD increased the activity of the myogenin reporter construct about 10-fold, but expression of Mirk did not alter this activation (Fig. 3B). When MyoD and MEF2C were co-expressed, the stimulatory effect of Mirk was seen again (Fig. 3C). As in the case with MyoD, Myf5 increased the activity of the myogenin reporter in a Mirk-independent manner (Fig. 3D). However, when MEF2C was co-expressed with Myf5, the large activation of the myogenin reporter that was seen was further enhanced by expression of wild-type Mirk but not ki-
nase-inactive Mirk. These data, taken together, demonstrated that Mirk functioned by activating MEF2C.

Mirk Does Not Directly Activate MEF2C by Phosphorylation—In an earlier study, we had found that Mirk activated transcription mediated by HNF1α/H9251 in a kinase-dependent manner and that Mirk phosphorylated HNF1α at Ser-247 (6). We scanned MEF2C for residues conserved between human and mouse that were similar to the amino acids around the site phosphorylated by Mirk in HNF1α and other substrates (4, 5).2 No consensus sequences were detected in MEF2C, suggesting that Mirk activated MEF2C in an indirect manner. We tested this hypothesis by expressing MEF2C in C2C12 cells with either wild-type Mirk or kinase-inactive YF-Mirk for 24 h, then culturing cells in medium containing [32P]orthophosphate for 6 h, and immunoprecipitating the labeled MEF2C (Fig. 3E).

Western blotting after autoradiography demonstrated that equal amounts of MEF2C were immunoprecipitated, but no difference in 32P labeling of the protein was seen between cells expressing ectopic wild-type Mirk and those expressing kinase-inactive YF-Mirk. Endogenous kinases phosphorylated MEF2C, but the abundant ectopic Mirk did not. In contrast, wild-type Mirk strongly phosphorylated co-expressed HNF1α, H9251, and other substrates (4, 5).

| Genes down-regulated by RNAi to Mirk                          | UniGene name                                               |
|---------------------------------------------------------------|-------------------------------------------------------------|
| -11.53                                                        | Collagenous repeat-containing sequence, skeletal development protein |
| -9.99                                                         | H19 fetal liver mRNA, oct-binding maintenance sequence for unmethylated DNA |
| -8.63                                                         | Myosin light chain, phosphorylatable, fast skeletal muscle |
| -7.57                                                         | Myogenin                                                   |
| -7.40                                                         | Myosin light chain, alcal, fast skeletal muscle             |
| -6.57                                                         | Troponin C, fast skeletal                                   |
| -6.24                                                         | Actin, α1, skeletal muscle                                   |
| -5.59                                                         | Suppressor of cytokine signaling 2                          |
| -5.49                                                         | Actin, α, cardiac                                           |
| -4.87                                                         | Osteglycin                                                  |
| -4.58                                                         | Placenta-specific 8                                         |
| -4.43                                                         | Lymphocyte antigen α6 complex, locus A                      |
| -4.32                                                         | Troponin C, cardiac/slow skeletal                            |
| -4.17                                                         | Actinin α3                                                  |
| -4.04                                                         | Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1a |
| -4.02                                                         | CD60 antigen                                                |
| -4.00                                                         | Inhibitor of DNA binding 3                                  |
| -3.96                                                         | Calcium channel, voltage-dependent, γ subunit 1             |
| -3.78                                                         | Strathmin-like 2                                             |
| -3.68                                                         | Titin                                                       |
| -3.67                                                         | Myosin, light polypeptide 9, regulatory                      |
| -3.62                                                         | Biglycan                                                    |
| -3.47                                                         | IGFBP7                                                      |
| -3.43                                                         | Early growth response 1 (EGR1)                              |
| -3.38                                                         | Enolase 3, β muscle                                          |
| -3.36                                                         | Dynein, cytoplasmic, intermediate chain I                    |
| -3.35                                                         | SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin d |
| -3.31                                                         | Granzyme E                                                  |
| -3.29                                                         | Cadherin 15                                                  |
| -3.26                                                         | Myomesin 2                                                  |
| -3.23                                                         | Troponin T1, skeletal slow                                   |
| -3.28                                                         | Thioredoxin-interacting protein                              |
| -3.21                                                         | IGFBP5                                                      |
| -3.16                                                         | Sarcoglycan, β (43-kDa dystrophin-associated glycoprotein)  |

| Genes up-regulated by RNAi to Mirk                            | UniGene name                                               |
|---------------------------------------------------------------|-------------------------------------------------------------|
| 7.29                                                          | Cubilin (intrinsic factor-cobalamin receptor)               |
| 6.14                                                          | Niban protein                                               |
| 5.93                                                          | Serine (or cysteine) proteinase inhibitor, clade B, member 2 |
| 5.66                                                          | Pyruvate dehydrogenase kinase, isoenzyme 4                  |
| 4.95                                                          | CD53 antigen                                                |
| 4.85                                                          | RIKEN cDNA 1190002H23 gene                                   |
| 3.77                                                          | Serine protease inhibitor 13                                 |
| 3.55                                                          | Calcitonin-related polypeptide, β                            |
| 3.40                                                          | Neurofilament, light polypeptide                             |
| 3.29                                                          | Activated leukocyte cell adhesion molecule                   |
| 3.27                                                          | ESTs                                                        |
| 3.11                                                          | Histamine N-methyltransferase                               |
| 2.89                                                          | Mannosidase 1, α                                            |
| 2.89                                                          | Mus musculus, similar to protein phosphatase 1, regulatory subunit 10, clone IMAGE:5012018, mRNA |
| 2.83                                                          | src-like adaptor                                            |
| 2.77                                                          | Serum/glucocorticoid-regulated kinase                       |
| 2.76                                                          | Hedgehog-interacting protein                                |
| 2.75                                                          | Occludin                                                    |
| 2.73                                                          | Methyltetrahydrofolate dehydrogenase (NAD+-dependent), M ethnyltetrahydrofolate cyclohydrolase |
| 2.67                                                          | RIKEN cDNA 130001921 gene                                    |
| 2.66                                                          | Asparagine synthetase                                        |
| 2.63                                                          | RAR-related orphan receptor β                               |
| 2.60                                                          | Branched chain aminotransferase 1, cytosolic                |
Depletion of Endogenous Mirk by RNA Interference Blocks Activation of the Myogenin Promoter

![Graph showing the effect of Mirk depletion on myogenin promoter activation.](image)

**Fig. 2. Depletion of endogenous Mirk by RNA interference blocks activation of the myogenin promoter.** C2C12 cells were transfected with the pSilencer expression plasmid encoding RNAi to Mirk or vector control, cultured in growth medium for 1 day, and then switched to differentiation medium for 1 and 2 days. Myogenin reporter-luciferase activity was assayed. Mean of three values ± S.D. is shown if S.D. is >5%. One of duplicate experiments is shown.

whereas this phosphorylation was greatly diminished when kinase-inactive YF Mirk was co-expressed with HNF1α (data not shown). Because Mirk activates the transcriptional activity of MEF2C in a kinase-dependent manner (Fig. 3A), Mirk must phosphorylate one of the many factors that associate with MEF2C, either to release its inhibition, such as class II histone deacetylases (HDACs), or to potentiate its activation, such as nuclear histone acetyltransferases.

**Mirk Opposes Transcriptional Inhibition Caused by HDACs**—In myoblasts, HDACs associate with muscle regulatory factors such as myogenin and MyoD, whereas in differentiating myotubes HDACs have lost association with these regulatory factors and either bind preferentially to the retinoblastoma protein or are excluded from the nucleus (for a review see Ref. 17). Deacetylase inhibitors such as sodium butyrate are commonly used to enhance differentiation. However, if added to myoblasts just as they are signaled to differentiate by a switch from growth medium to differentiation medium, deacetylase inhibitors have been observed to do the opposite, to block myogenesis and to block the induction of myogenin (18, 19). It has been proposed that the release of HDACs from association with muscle regulatory factors at the start of differentiation can be blocked by the addition of a deacetylase inhibitor (20). We tested whether elevated levels of Mirk in stable C2C12 transfectants (3) would prevent a deacetylase inhibitor from blocking the release of HDACs from MEF2C in transcriptional complexes at the myogenin promoter and thus enhance the transcription of myogenin. C2C12 cells that had been stably transfected with Mirk and control transfectants were transferred to differentiation medium in the presence of an increasing concentration of the histone deacetylase inhibitor sodium butyrate, and the induction of myogenin was measured by Western blotting. Higher levels of myogenin protein were observed in the presence of overexpressed Mirk (Fig. 4A). Thus Mirk diminished the effect of the deacetylase inhibitor and restored some myogenin transcription, with the greatest effect seen at the lower levels of sodium butyrate. The hypothesis that Mirk antagonized the function of endogenous histone deacetylases was next tested directly.

**Mirk Opposes the Transcriptional Repression of the Myogenin Promoter by MTR**—Class II histone deacetylases (HDAC-4, -5, -7, and -9) have been shown to repress the transcriptional activity of the MEF2 transcription factors through an MEF2 binding domain (20). We postulated that Mirk might activate MEF2C by opposing the effect of class II HDACs. The natural C-terminal deleted form of HDAC9, MITR, is known to antagonize MEF2 activation of the 3xMEF2-luciferase reporter construct that contains three binding sites for MEF2 (21). By increasing the amount of MITR that was expressed led to a 3-fold decrease in the activity of co-expressed MEF2 (Fig. 4, B and C, first 4 bars) whether the cells were cultured in growth medium (Fig. 4B) or in serum-free medium (Fig. 4C). However, co-expression of increasing concentrations of wild-type Mirk with the highest concentration of MITR added (0.1 μg) partially reversed this inhibition under both culture conditions (Fig. 4, B and C, 4th to 8th bars). In contrast, co-expression of increasing concentrations of kinase-inactive YF-Mirk did not reverse the inhibition of MEF2 by MITR. Therefore, Mirk activated co-expressed MEF2 by counteracting the inhibition caused by MITR.

We next tested whether MITR would antagonize myogenin reporter activation by Mirk. Increasing concentrations of MITR inhibited the activation of the myogenin reporter (Fig. 4D), as has been reported by many investigators. The highest concentration of MITR expression plasmid tested inhibited the activity of the myogenin reporter 3-fold (Fig. 4D). Wild-type Mirk partially blocked the transcriptional inhibition caused by MITR, whereas no such effect was seen when kinase-inactive Mirk was co-expressed (Fig. 4D). Thus Mirk activated the myogenin promoter reporter by blocking, in a kinase-dependent manner, the transcriptional repression caused by the class II HDAC MITR.

**Mirk Phosphorylates HDAC5 at a Site Conserved in Class II HDACs—Activated CaMK regulates MEF2-HDAC interactions by phosphorylating two conserved HDAC sites flanking a nuclear localization sequence near their N termini in response to calcium signaling (22, 23). These sites serve as binding sites for 14-3-3 proteins when phosphorylated by CaMK (23). In HDAC5, one CaMK site is Ser-259. We found a consensus region for Mirk phosphorylation 20 amino acids from the CaMK site at serine 279, which is conserved within all class II HDACs (Fig. 5A). To test whether Mirk phosphorylated this site, we mutated this site in HDAC5 to S279A both in the presence of the S259A mutation or with this site unmutated. Neither HDAC5-S259S/S279A nor HDAC5-S259A/S279A was a substrate for Mirk. However, Mirk strongly phosphorylated both wild-type HDAC5 and HDAC5/S259A/S279S (Fig. 5B), demonstrating that Mirk phosphorylates HDAC5 at Ser-279 but not at the CaMK site of Ser-259. Mutation of serine 279 in HDAC5 to the nonphosphorylatable alanine did not prevent nuclear export in the presence of activated CaMK (22) in transient transfection experiments in COS cells. However, serine 279 is within the nuclear localization region, so we next tested whether phosphorylation of a class II HDAC by Mirk would block its import into the nucleus.

**Phosphorylation of MITR by Mirk Decreases the Nuclear Abundance of MITR**—Phosphorylation of a protein within its nuclear localization site often causes retention of the protein within the cytoplasm. For example, phosphorylation of the CDK inhibitor p21 by activated Akt increases the amount of p21 in the cytoplasm in breast cancers (24, 25). We tested the hypothesis that co-expression of MITR with Mirk would reduce the amount of MITR that reached the nucleus. MITR was transiently expressed in C2C12 myoblasts in growth medium for 24 h, either in the presence of wild-type Mirk, kinase-inactive Mirk, or no exogenous Mirk. Endogenous MITR is expressed at low levels in undifferentiated C2C12 cells (26) and was not detected by Western blotting (Fig. 6A, last 2 lanes).
Cells were separated into nuclear and cytoplasmic fractions, which were then analyzed by Western blotting for exogenous MITR. The fractionation was effective as only the nuclear fractions contained MyoD, whereas the cytoplasmic fractions were highly enriched in β-tubulin (Fig. 6A). There is very little endogenous Mirk in growing myoblasts, and when MITR was expressed in the absence of exogenous Mirk, about half of the MITR was found in the nucleus (Fig. 6A, 1st 2 lanes, also note

### Fig. 3. Mirk activation of the myogenin promoter is increased by co-expressed MEF2C, but Mirk does not phosphorylate MEF2C in vivo. A–D, C2C12 cells in growth medium were co-transfected with the myogenin promoter reporter construct together with an expression plasmid for Mirk, kinase-inactive YF-Mirk, or the pCDNA vector, together with either the pEMSV vector or an expression plasmid for MEF2C (A), an expression plasmid for MyoD (B), expression plasmids for MyoD and MEF2C (C), and expression plasmids for Myf5 and MEF2C (D), as noted. After 24 h, cells were transferred to differentiation medium for an additional 24 h before assay. Myogenin reporter-luciferase activity was assayed. Mean of three values ± S.D. is shown if S.D. is > 5%. One of duplicate experiments shown.

E, C2C12 cells were co-transfected with expression plasmids for MEF2C and either wild-type or kinase-inactive (YF) Mirk, allowed to express, and then labeled in [32P]orthophosphate-containing medium. MEF2C was immunoprecipitated (IP); the level of phosphorylation in MEF2C was determined by autoradiography, and the relative abundance of the immunoprecipitated MEF2C was determined by Western blotting (WB). One of duplicate experiments is shown.
Mirk opposes the action of histone deacetylases by increasing myogenin expression. A, inhibition of induction of myogenin by the class II histone deacetylase inhibitor sodium butyrate is limited by increased expression of Mirk. Mirk stable transfectants of C2C12 cells and control transfectants were treated with 0.5 to 5 mM sodium butyrate for 24 and 48 h, and the abundance of myogenin protein at each time point was determined by Western blotting. The mean of four experiments, two at each time point, is shown ± S.D., if S.D. > 5%. B, Mirk opposes the
the lack of endogenous Mirk). However, when wild-type MITR was co-expressed with Mirk, there was a dramatic 6-fold decrease in the amount of MITR in the nuclear fraction but no increase in the amount of cytoplasmic MITR. In sharp contrast, when MITR was co-expressed with kinase-inactive Mirk, the amount of MITR in the nucleus doubled. These data suggest the following model: Mirk phosphorylates the class II HDAC MITR within its nuclear localization domain thus decreasing the rate of its translocation into the nucleus. Exogenous kinase-dead YF-Mirk did not block MITR from entering the nucleus. In fact, both MITR and kinase-inactive YF-Mirk were enriched in the nucleus. This unusual pattern was seen in duplicate experiments and may suggest some continued association of YF-Mirk and MITR.

We extended this study by next investigating whether the amount of Mirk that reached the nucleus was dependent on the ratio between Mirk and MITR. In Fig. 6A, equal amounts of expression plasmids for Mirk and for MITR were transfected. The ratio of MITR to Mirk was now varied from 1:1 to 2:1 to 4:1 (Fig. 6B). In the absence of ectopic Mirk, the ratio of cytoplasmic to nuclear MITR was 0.5. When the Mirk/MITR ratio was 4:1 (0.5 μg of Mirk/2 μg of MITR), the ratio of MITR in the cytoplasm to the nucleus increased 8-fold. When the Mirk level was increased further (1 μg of Mirk/2 μg of MITR), the ratio of MITR in the cytoplasm to the nucleus increased 20-fold. There was no further increase in the amount of Mirk synthesized even when the input of Mirk was increased to 2 μg (see Fig. 6B, total lysates). However, when Mirk was co-expressed, the MITR in the nucleus exhibited a decreased electrophoretic mobility (Fig. 6B, arrows at right), which was observed in duplicate experiments. Therefore, increasing the ratio of Mirk to MITR caused a dose-dependent decrease in the amount of MITR that reached the nucleus, and this MITR exhibited decreased mobility consistent with post-translational modification such as phosphorylation.

When MITR was expressed in COS cells, C2 myoblasts, and

The inhibition of MEF2 by MITR in a kinase-dependent manner in NIH3T3 cells cultured in growth medium. NIH3T3 cells in 12-well plates were co-transfected using Lipofectamine Plus in serum-free medium with the 3x-MEF2-luciferase reporter plasmid (0.1 μg), an expression plasmid (0.05 μg) for MEF2C (pcDNA3-MEF2C), an expression plasmid for β-galactosidase (0.1 μg), and expression plasmids encoding either MITR (H) alone (0–0.1 μg as noted, 1st 4 bars) or 0.1 μg of MITR and increasing concentrations of wild-type Mirk (M) or kinase-inactive YF-Mirk (YF, 0.01–0.05 μg as noted). The amount of DNA transfected was kept constant by the addition of vector DNA as needed. After 5 h in serum-free medium, serum was added to 7%, and the cells were incubated overnight before assay. Data shown is the mean ± S.D. of triplicate measurements. The asterisks mark the values statistically different (t test) between the Mirk plus MITR transfectants versus the 0.01 μg of MITR-treated cells. C, Mirk opposes the inhibition of MEF2 by MITR in a kinase-dependent manner in NIH3T3 cells cultured in serum-free medium. Transient transfections were performed in parallel to the experiment in B, but the transfected NIH3T3 cells were maintained in medium containing 0.1% fetal calf serum for 24 h before assay. Data shown are the mean ± S.D. of triplicate measurements. The asterisks mark the values statistically different (t test) between the Mirk plus MITR transfectants versus the 0.01 μg of MITR-treated cells. D, C2C12 myoblasts were transfected with the myogenin promoter reporter construct, increasing concentrations of the expression plasmid for MITR, with either wild-type Mirk, kinase-inactive YF-Mirk, or an equal amount of vector DNA. After 24 h, cells were transferred to differentiation medium for an additional 24 h before assay. Myogenin reporter-luciferase activity was assayed. Mean of three values ± S.D. is shown if S.D. is >5%. One of triplicate experiments is shown.
10T1/2 fibroblasts, MITR localized to discrete nuclear bodies, but in response to CaMK signaling, MITR remained in the nucleus but was evenly distributed, as determined by immunofluorescence studies (26, 27). Ectopic MITR was seen in the cytoplasm of C2 skeletal myotubes after 9 days in differentiation medium (26). In contrast to these experiments, MITR was readily detected biochemically in the cytoplasmic fractions of C2C12 skeletal myoblasts (Fig. 6, A and B). To resolve these apparent differences, we examined the location of ectopic MITR in C2C12 myoblasts by immunofluorescence analysis using the same antibody, anti-HDAC9, which readily detected MITR in the cytoplasm of fractionated cells in multiple experiments. In each case, MITR was only detected in the nucleus by immunofluorescence (Fig. 6C). We repeated this experiment using FLAG epitope-tagged MITR, and we detected the FLAG epitope in immunofluorescence experiments. Again, the majority of FLAG epitope-tagged MITR was found localized in the nucleus. MITR occasionally was detected throughout the entire cell (Fig. 6C), as expected from the fractionation experiments. This cytoplasmic and nuclear localization was observed in 7% of the cells (74 of 1053 cells examined). This low frequency prevented analysis of the effect of Mirk on MITR nuclear localization by immunofluorescence, parallel to the studies shown in Fig. 6, A and B. In our fractionation scheme a known transcription factor, MyoD, was localized only in the nuclei, and a known cytoskeletal protein, β-tubulin, was found almost exclusively in the cytoplasmic fractions in multiple experiments (Fig. 6, A and B). We postulate that cytoplasmic MITR is complexed with other cellular proteins in vivo which block the epitopes recognized by the anti-HDAC9 antibody and, to a lesser extent, the FLAG epitope. In contrast cytoplasmic MITR was readily detected when samples were boiled in SDS-PAGE loading buffer before Western blotting. We have demonstrated previously that Mirk, which dimerizes to a 140-kDa form in vivo (28), is localized in higher molecular weight complexes in vivo, up to at least 660 kDa by analysis by fast protein liquid chromatography (7). MKK3, the activator kinase for Mirk, can readily be detected biochemically in the cytoplasmic fractions of such large complexes, preventing their detection by immunofluorescence techniques.

**Decreased Abundance of Phosphomimetic MITR in the Nucleus**—The results of the fractionation experiments (Fig. 6) implied that a wild-type MITR construct would accumulate in the nucleus to a greater extent than a MITR construct that was mutated to the phosphomimetic aspartic acid residue at the Mirk phosphorylation site. We tested this model by mutating MITR at Ser-243, the Mirk phosphorylation site. We tested this model by mutating MITR at Ser-243, the Mirk phosphorylation site homologous to the HDAC5 site of Ser-279 (Fig. 5A). Wild-type MITR, MITR mutated at the Mirk site to the nonphosphorylatable residue alanine (S243A), or a MITR phosphomimetic site mutant (S243D) was transiently transfected into C2C12 cells and maintained for 24 h in growth medium, and then one set of cells was switched to differentiation medium for 24 h. Each set of cells was then separated into nuclear and cytoplasmic fractions that were analyzed by Western blotting. The fractionation was effective as the nuclear fractions contained MyoD, whereas the cytoplasmic fractions were highly enriched in β-tubulin, and the amounts of either MyoD or tubulin were similar in cells expressing each construct (Fig. 7A).

In myoblasts in GM, similar amounts of wild-type MITR and both mutant MITR constructs were seen in the cytoplasmic fractions. However, there was less of the phosphomimetic MITR-S243D construct in the nucleus (Fig. 7A, last lane) even though there was no decrease in the amount of MyoD (compare MyoD levels in nuclei from cells in GM). After myoblasts were switched to differentiation medium, MITR was lost from the cytoplasm both in the wild-type and mutant constructs. The level of nuclear MITR was lowest in the phosphomimetic construct, although the levels of MyoD were similar in each transfectant. These data supported the hypothesis that phosphoryl-
Less Phosphomimetic MTR-S243D Is Found in the Nucleus

B Similar Amounts of MITR Mutants Made

C Mutation of MITR at the Mirk Phosphorylation Site Does Not Alter the Inhibition of MEF2 by MITR

FIG. 7. Phosphomimetic MTR is lost from the nucleus. A, C2C12 myoblasts were transiently transfected with expression plasmids (2 μg of DNA each) for either wild-type MITR, MITR mutated to alanine at the Mirk phosphorylation site (S243A), or MITR phosphomimetic at the Mirk phosphorylation site (S243D) and were allowed to express for 24 h in growth medium. The cells were either immediately partitioned into nuclear and cytoplasmic fractions or transfected into differentiation medium (DM) for 24 h before fractionation. Both sets were then analyzed for the abundance of MITR by Western blotting. Fractionation was monitored by Western blotting for tubulin as a cytoplasmic (Cyto) marker and MyoD as a nuclear (N) marker. One of duplicate experiments is shown. B, parallel cultures were prepared at the same time as those transfected above, allowed to express for 24 h in growth medium (GM), and then analyzed by Western blotting for MITR without cell fractionation. C, MITR mutated at the Mirk phosphorylation site to alanine has the same inhibitory action as wild-type or phosphomimetic MITR. NIH3T3 cells were transiently co-transfected with the 3x-MEF2-luciferase reporter plasmid, expression plasmids for either wild-type MITR, MITR mutated to alanine at the Mirk phosphorylation site (S243A), or MITR phosphomimetic at the Mirk phosphorylation site (S243D), an expression plasmid for β-galactosidase, and an expression plasmid for β-galactosidase. Therefore, phosphorylation of MITR by Mirk inhibited the nuclear transport of MITR, therefore limiting the amount of MITR that could accumulate in the nucleus.

We had considered the possibility that the phosphomimetic construct might be expressed at a lower level than the wild-type construct. Parallel cultures were transfected with each construct, maintained in growth medium for 24 h, and harvested at the same time as the GM experiment shown in Fig. 7A. However, these cells were not fractionated before lysis. Western blotting demonstrated that the three constructs were expressed at similar levels, and there was no decrease in expression of MITR-S243D, which could explain the decrease in its nuclear abundance in fractionated cells (Fig. 7B). Therefore, studies using the mutant MITR constructs gave results generally similar to the co-expression studies. When MITR was phosphorylated by Mirk, its transport to the nucleus was inhibited so less MITR accumulated in the nucleus, and thus less was available to inhibit MEF2C.

Two hypotheses were considered to account for the lack of accumulation of MITR within the cytoplasm when Mirk was overexpressed (Fig. 6A). In the first hypothesis, Mirk only blocked MITR transport into the nucleus and had no effect on the proteolysis of MITR within the cytoplasm. Alternatively, the phosphorylation of MITR by Mirk could also trigger the rapid turnover of MITR within the cytoplasm. We have shown previously that Mirk can mark proteins for proteolysis. Phosphorylation by Mirk shortens the half-life of cyclin D1 in myoblasts. Mirk phosphorylates cyclin D1 at Thr-288 when cyclin D1 is bound to GSK3β (5). GSK3β itself is well known to destabilize cyclin D1 by phosphorylation at the adjacent site of Thr-286 (29).

MITR that did not reach the nucleus was rapidly turned over within the cytoplasm when cells began to differentiate (Fig. 7A). However, the hypothesis that phosphorylation of MITR at Ser-243 by Mirk would directly trigger the rapid turnover of MITR within the cytoplasm was not supported. There was no increase in the amount of MITR-S243A in the cytoplasm in either growing or differentiating myoblasts, compared with wild-type MITR. Therefore, phosphorylation by Mirk at this site was not essential for proteolysis of MITR.

Mutation of the Mirk Phosphorylation Site in MITR Does Not Alter the Capacity of MITR to Function as a Transcriptional Inhibitor—Three MITR constructs were compared for their ability to inhibit the activity of an MEF2-dependent luciferase reporter (3xMEF2-luc). MEF2C activates this reporter though the class II HDAC, HDAC4, blocks its activity in a dose-dependent manner (21). NIH3T3 cells were transiently co-transfected with the 3xMEF2-luc reporter plasmid together with an expression plasmid for MEF2C and expression plasmids for either wild-type MITR, MITR mutated at the Mirk phosphorylation site to the nonphosphorylated form (S243A), or MITR phosphomimetic at the Mirk phosphorylation site (S243D), or MITR mutated at the Mirk phosphorylation site to the phosphomimetic form (S243D), or MITR phosphomimetic at the Mirk phosphorylation site (S243D), or MITR mutated at the Mirk phosphorylation site to the nonphosphorylatable form (S243A). All three constructs inhibited MEF2C to the same extent, about 6-fold (Fig. 7C). However, these cells were not fractionated before lysis. Therefore, phosphorylation of MITR by Mirk did not alter the capacity of MITR to inhibit MEF2C, but instead modulated the amount of MITR which reached the nucleus.

DISCUSSION

Transcriptional activation can occur when histone acetyltransferases acetylate the core histones of nucleosomes, resulting in chromatin relaxation. Conversely, deacetylation of histones by HDACs results in chromatin condensation and transcriptional repression. Histone deacetylases block transcription as part of transcriptional co-repressor complexes. Class II HDACs are structurally related (HDAC4, -5, -7, and
-9 and MITR, the natural C-terminal deleted form of HDAC9) and are restricted in distribution, with the highest expression found in muscle, heart, and brain (reviewed in Ref. 17). Class II HDACs bind to members of the MEF2 family of MADS box transcription factors and repress their activity. HDAC4 is either cytoplasmic or pancellular in proliferating C2.7 myoblasts, whereas HDAC5, HDAC7, and MITR are largely nuclear (30–32). During myogenesis, class II HDACs dissociate from MEF2, relieving its repression. HDAC4 remains distributed through the myotube, whereas HDAC5 and HDAC7 exit the nucleus (22, 30, 32). MITR is initially found in punctate "speckles" in the nucleus, but early in myogenesis transitions to a diffuse nuclear localization, then some MITR exits the nucleus when myotubes are formed (27, 26, 33). CaMK signaling mimics the effect of myogenic signals on HDAC dissociation from MEF2 (22). Dissociation of class II HDACs such as HDAC5 and MITR from MEF2 depends on phosphorylation of two conserved serines (22). Phosphorylation of these residues creates binding sites for the 14-3-3 chaperone protein (31, 34).

The abundance of nuclear HDACs is regulated by nuclear localization and nuclear export signals as well as by phosphorylation of 14-3-3-binding sites. Nuclear/cytoplasmic shuttling of class II HDACs depends on a C-terminal nuclear export sequence and a nuclear localization sequence (NLS) in the N terminus (22). Microinjected HDAC4-GFP was previously (35) to be localized to the cytoplasm of undifferentiated C2C12 myoblasts, consistent with our observation that some of the ectopic MITR molecules are found in the cytoplasm (Fig. 6, A and B). Microinjection of full-length HDAC4 resulted in cytoplasmic localization in 30–40% of IMR90-EIA fibroblasts, whereas expression of the C-terminal fragment, which lacks the NLS as well as the MEF2 binding region, resulted in cytoplasmic localization in 90% of the cells (12). Formation of an MEF2-HDAC4 complex efficiently targets this complex to the nucleus, whereas deletion of the MEF2 NLS results in cytoplasmic retention of this complex (30).

In the current study we have found that the kinase Mirk/dyrk1B is essential for the transcription of myogenin and that Mirk induces myogenin transcription through indirect activation of the MEF2 transcription factor. Mirk relieves the inhibition of MEF2 by the class II HDAC Mirk in a kinase-dependent manner by phosphorylating the class II HDACs on a conserved serine within the highly conserved nuclear localization sequence. Separation of C2C12 cells into nuclear and cytoplasmic fractions and Western blotting analysis demonstrated that the nuclear entry of the HDAC MIRK was inhibited when MIRK was co-expressed with Mirk. The higher the ratio of Mirk to MITR expressed, the more MITR was constrained to the cytoplasm. In contrast, when kinase-inactive Mirk was co-expressed, MITR was able to concentrate in the nucleus. Cell fractionation studies with mutant MITR constructs confirmed the interpretation that phosphorylation by Mirk decreased the rate of accumulation of MITR in the nucleus. A phosphomimetic construct of MITR mutated at the Mirk phosphorylation site exhibited less accumulation in the nucleus than wild-type MITR. When C2C12 cells were induced to differentiate, MITR was solely detected in the nucleus, but more wild-type MITR was found there than the phosphomimetic construct. Mirk phosphorylated class II HDACs at a conserved serine within the nuclear localization domain and inhibited accumulation of MITR in the nucleus, presumably by blocking a step in nuclear import. Another example of a serum/threonine kinase that blocks nuclear import is Akt/protein kinase B. Akt is well known for its ability to block the nuclear import of the CDK inhibitor p21 Cip1 by phosphorylating p21 at threonine 145 within its nuclear localization domain in Her-2/neu-transformed NIH3T3 cells (24).

The ability of Mirk to activate myogenin transcription complements the various other functions of Mirk during muscle differentiation. Terminal myoblast differentiation occurs when myoblasts cease cycling and become arrested in G0/G1. Myoblast growth arrest is mediated by increased expression of the CDK inhibitor p27 Kip1 and by decreased expression of the G1 cyclin, cyclin D1. However, cell cycle regulation occurs at both the transcriptional level and the post-translational level in myoblasts. Functions at the post-translational level to maintain p27 protein levels while reducing the abundance of cyclin D1. Mirk phosphorylates p27 at Ser-10, thus stabilizing p27 and blocking its nuclear export and degradation (4). At the same time, Mirk acts together with GSK3β to induce cyclin D1 degradation by phosphorylating cyclin D1 at Thr-288, whereas GSK3β phosphorylates the adjacent site of Thr-286 (5). Supporting the results of these phosphorylation studies was the observation that depletion of endogenous Mirk by RNAi in differentiating myoblasts increased cyclin D1 protein and decreased p27 protein without modulating the mRNA levels of either molecule (4, 5). The current study demonstrates the multifunctional role of Mirk in myoblast differentiation. In addition to the previously established role for Mirk in facilitating cell cycle arrest, Mirk is now shown to facilitate progression of the subsequent differentiation program through transcriptional activation of the muscle regulatory factor myogenin.

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REFERENCES

1. Lee, K.-M., and Friedman, E. (1998) Proc. Am. Assoc. Cancer Res. 39, 273
2. Lee, K., Deng, X., and Friedman, E. (2000) Cancer Res. 60, 3631–3637
3. Deng, X., Ewton, D., Pawlikowski, B., Mainonne, M., and Friedman, E. (2003) J. Biol. Chem. 278, 41347–41354
4. Deng, X., Mercer, S., Shah, S., Ewton, D., and Friedman, E. (2004) J. Biol. Chem. 279, 23488–23504
5. Zou, Y., Ewton, D., Deng, M., Mercer, S., and Friedman, E. (2004) J. Biol. Chem. 279, 27790–27798
6. Lim, S., Jin, K., and Friedman, E. (2002) J. Biol. Chem. 277, 25040–25046
7. Lim, S., Zou, Y., and Friedman, E. (2002) J. Biol. Chem. 277, 49438–49445
8. Kestrup, H., Becker, W., Heukelbach, J., Wilmes, A., Schurman, A., Huppertz, C., Kainulainen, H., and Joost, H.-G. (1996) J. Biol. Chem. 271, 3488–3495
9. Becker, W., Weber, Y., Wetzol, K., Ehrhardt, K., Toedler, F., and Joost, H.-G. (1996) J. Biol. Chem. 271, 25883–25890
10. Himmel, S., Tege, W., Frank, R., Leder, S., Joost, H., and Becker, W. (2000) J. Biol. Chem. 275, 24341–24346
11. Leder, S., Weber, X., Alafajq, J., Estivill, X., Joost, H.-G., and Becker, W. (1999) Biochem. Biophys. Res. Commun. 254, 474–479
12. Paroni, G., Mizuori, M., Henderson, C., Del Sal, G., Schneider, C., and Brancolini, C. (2004) Mol. Cell. Biol. 15, 2804–2818
13. Edmondson, D., Cheng, T., Cserjesi, P., Chakraborthy, T., and Olson, E. (1992) Mol. Cell. Biol. 12, 3665–3677
14. Malik, S., Huang, C., and Schmidt, J. (1995) Eur. J. Biochem. 250, 88–96
15. Lim, B., Mitchell, P., Regley, K., and Pavlath, G. (2003) Differentiation 71, 217–227
16. Kitzmann, M., Carnacc, G., Vandroome, M., Primig, M., Lamb, N., and Fernandez, A. (1998) J. Cell Biol. 142, 1447–1459
17. McKinsey, T., Zhang, C., and Olson, E. (2003) Curr. Opin. Cell. Biol. 14, 763–772
18. Johnston, L., Tascott, S., and Eisen, H. (1992) Mol. Cell. Biol. 12, 5133–5139
19. Izzi, S., Cosso, G., Nervi, C., Sartorelli, V., and Puri, P. L. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 7757–7762
20. McKinsey, T., Zhang, C., and Olson, E. (2001) Curr. Opin. Genet. Dev. 11, 397–404
21. Chan, J. K. L., Sun, L., Yang, X.-J., Zhan, G., and Wu, Z. (2003) J. Biol. Chem. 278, 23515–23521
22. McKinsey, T., Zhang, C., Lu, J.-R., and Olson, E. (2000) Nature 408, 106–111
23. Zhang, C. L., McKinsey, T., Chang, S., Antos, C., Hill, J., and Olson, E. (2000) Cell 101, 479–488
24. Zhou, B., Liao, Y., Xia, W., Lee, M.-H., and Hung, M.-C. (2001) Nat. Cell Biol. 3, 245–252
25. Xia, W., Chen, J.-S., Zhou, X., Sun, P.-R., Lee, D.-F., Liao, Y., Zhou, B.-P., and Hung, M.-C. (2004) Clin. Cancer Res. 10, 3815–3824
26. Zhang, C. L., McKinsey, T. A., and Olson, E. N. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 7354–7359
27. McKinsey, T., Zhang, C. L., and Olson, E. (2001) Mol. Cell. Biol. 21, 6312–6321
28. Zou, Y., Lim, S., Lee, K., Deng, X., and Friedman, E. (2003) J. Biol. Chem. 278, 49573–49581
29. Diehl, J., Zindy, F., and Sherr, C. (1997) Genes Dev. 11, 957–972
30. Borghi, S., Molinari, S., Razzini, G., Parise, F., Battini, R., and Ferrari, S. (2001) J. Cell. Sci. 114, 4477–4483
31. McKinsey, T. A., Zhang, C. L., and Olson, E. N. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 14400–14405
32. Dressel, U., Bailey, P. J., Wang, S.-C. M., Downes, M., Evans, R. M., and Muscat, G. E. O. (2001) J. Biol. Chem. 276, 17007–17013
33. Zhang, C. L., McKinsey, T. A., Lu, J.-R., and Olson, E. N. (2001) J. Biol. Chem. 276, 35–39
34. Wang, A. H., Kruhlak, M. J., Wu, J., Bertos, N. R., Vezmar, M., Posner, B. I., Bazett-Jones, D. P., and Yang, X.-J. (2000) Mol. Cell. Biol. 20, 6904–6912
35. Miska, E. A., Langley, E., Wolf, D., Karlsson, C., Pines, J., and Kouzarides, T. (2001) Nucleic Acids Res. 29, 3439–3447
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Xiaobing Deng, Daina Z. Ewton, Stephen E. Mercer and Eileen Friedman

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