J. Biochem. **135**, 695–700 (2004)
DOI: 10.1093/jb/mvh084

Human Histone Deacetylase SIRT2 Interacts with the Homeobox Transcription Factor HOXA10

Nancy S. Bae[1,*], Mark J. Swanson[2,†], Alex Vassilev[1] and Bruce H. Howard[1,‡]

[1]Laboratory of Molecular and Growth Regulation, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, 20892, USA; and [2]Laboratory of Eukaryotic Gene Regulation, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, 20892, USA

Received February 10, 2004; accepted April 9, 2004

Histone deacetylases are required for transcriptional repression in eukaryotes. *Saccharomyces cerevisiae* has several histone deacetylases, of which ySir2p is the most conserved throughout evolution. Currently, there is no report on the interacting protein partner of a human Sir2 homolog, SIRT2. Here we show for the first time that SIRT2 interacts with the homeobox transcription factor, HOXA10, which was identified in a two-hybrid screen. Interactions were confirmed by co-immunoprecipitation from *in vitro* translations as well as in human cell-free extracts. Taken together with mouse knockout studies, our results raise the intriguing possibility that SIRT2 plays a role in mammalian development.

Key words: HOXA10, SIRT2.

Chromatin structure can be altered by the covalent, post-translational modification of the amino-terminal tails of the core histone proteins, including acetylation on highly conserved lysine residues in response to appropriate upstream signals (*1*). Such modifications are thought to serve as signals for downstream functions, including recruitment or stabilization of chromatin remodeling complexes (*2*). Histone acetyltransferases modify the positively charged lysine residues of the histones by adding an acetyl group. The subsequent removal of these acetyl groups, or deacetylation, is mediated by histone deacetylases (HDACs), and deacetylation is associated with repression of transcription and recombination (*3*). Long-range repression involving regional inactivation of chromatin is referred to as 'silencing,' since the repressed transcriptional state can be stably inherited by subsequent generations (*4*).

In *Saccharomyces cerevisiae*, silencing occurs at the silent mating loci, telomeres, and the *rDNA* locus. Silencing is mediated by the proteins encoded by the four *S*ilent *I*nformation *R*egulator (*SIR1-4*) genes (*5*), which are brought to the DNA *via* Rap1p, a sequence-specific DNA binding protein (*6*). Of the four yeast Sir proteins, Sir2p (ySir2p) is unique because it is the most evolutionarily conserved and is the only Sir protein that is required at all three different silent loci in yeast (*7*). ySir2p also possesses NAD^+-dependent histone deacetylase activity (*8–10*), which is necessary for its function in silencing.

In human cells, there are seven ySir2p homologs, termed sirtuins (SIRTs) 1 through 7 (*11*, *12*). The gene encoding human SIRT2 produces a transcript of 2.1 kb (*11*). A potential role of SIRT2 in transcriptional regulation was suggested by domain-swapping experiments (*13*), in which a chimera between ySir2p and SIRT2 was shown to function in yeast silencing at a subset of target loci. Recently, SIRT2 was shown to co-localize with microtubules and deacetylate α-tubulin (*14*), suggesting SIRT2 may play a role in cell structural integrity.

Initially identified in *Drosophila*, homeobox (HOX) proteins are evolutionarily conserved transcription factors that are important in developmental regulation through pattern formation by activating and repressing genes required for cell type determination (*15*). The HOX family of proteins contains a common domain called the homeodomain that is responsible for sequence-specific DNA binding (*16*). Differential cofactor binding to HOX proteins can alter the function of the HOX protein from activation to repression (*17*). Human HOXA10, located at the 5′ end of the HOX-A cluster, plays a key role in myeloid and B-lymphoid progenitor cell differentiation (*18*), and the knockout mouse model exhibits defects in reproductive organs (*28*).

To date there is no report of interacting protein partners of SIRT2. The goal of this work was to isolate interacting proteins of SIRT2 that might lead us to understand the biological function(s) of SIRT2. Here, for the first time, HOXA10 was identified as a specific interacting protein of SIRT2. Interactions between SIRT2 and HOXA10 were confirmed by co-immunoprecipitation of *in vitro* transcriptional translation products. Interestingly, subcellular fractionation revealed that SIRT2 and HOXA10 interact in the cytoplasm as well as in the nucleus of human cells. Combined with previous knockout mouse studies (*28*, *29*), these data suggest that SIRT2 may play a role in adult reproductive tissue formation.

*To whom correspondence should be addressed at the present address: Stowers Institute for Medical Research, Kansas City, MO 64110, USA. E-mail: NSB@stowers-institute.org, Fax: +1-816-926-2089
†Present address: School of Biological Sciences, Louisiana Tech University, Ruston, LA 71272, USA.
‡E-mail: howard@helix.nih.gov, Fax: 301-480-9354

MATERIALS AND METHODS

Yeast Two-Hybrid Screening —A human skeletal muscle cDNA library was used for two-hybrid screening (Invitrogen). SIRT2 was fused to pDBLeu at the *Sal*I and *Not*I sites (Invitrogen). SIRT2FL was amplified with 5′TTCTTGTCGACGCACGATGCAGCCTCAAGGGCTTCA3′ and 5′GGCAGGACTAGTTCACTGGGGTTTCTCCTCTCTGT3′. SIRT2(37–244) was amplified with 5′TTCTTGTCGACCGAACGCTGTCGCAGAGTCATCTGT3′ and 5′TTGAACACTAGTAGGGGTGGAGAGGGGTGCCTTGCT3′ (Primer 833). SIRT2(146–244) was amplified with 5′TTATTGTCGACATTGGTGGAGGCGCACGGCACCTTC3′ and Primer 833. The two-hybrid screen was done at the 1× complexity of the library.

In Vitro Transcription / Translation Assays—In both the SIRT2 and SIRT2(210–833) constructs, the gene was cloned as a *Sal*I–*Not*I fragment into the pET-28b plasmid (Novagen). The HOXA10 gene was cloned into pET-41b plasmid (Novagen) as a *Spe*I–*Avr*II fragment *via* PCR amplification. The HOXA10$_{(c\text{-}term)}$ was amplified with primers 5′ACACACACTAGTCCGGAGAAGGATTCCCTGGGCAAT3′ and 5′ACACACCCTAGGTCAGGAAAAATTAAAGTTGGCTGT3′. TnT Quick Coupled Transcription/Translation Systems (Promega) was used. The reactions were incubated with either 5ul of a 50% slurry of Ni-NTA agarose (Qiagen) in binding buffer (100mM KOAc, 10% glycerol, 20 mM Tris pH 7.9, 0.1% NP-40, 5 mM $MgCl_2$, 10 mM imidazole, and Complete protease inhibitor cocktail (Roche)) or 5 μl of a 50% slurry of glutathione-sepharose beads (Amersham) in binding buffer (without imidazole). The incubation was done with rotation at 4°C from 4 h to overnight. The beads were boiled in the presence of SDS to elute the bound fraction. The products were resolved on 10% Bis-Tris NuPage gels using 1× MOPS buffer (Invitrogen). The SIRT1 gene was cloned as a *BamH*I–*Not*I fragment into the pET-28a plasmid (Novagen).

Cell Culture—U937 cells were obtained from Dr. Keiko Ozato (NIH, MD). Cells were maintained in RPMI with 10% heat-inactivated Fetal Calf Serum, 10 mM HEPES buffer solution, 100 μM MEM non-essential amino acids solution, and 1 mM MEM sodium pyruvate solution, in 5% CO_2 at 37°C.

Immunoblotting Analyses—Proteins were separated on 10% Bis-Tris NuPage gels using 1× MOPS buffer (Invitrogen). α-SIRT2 antibody was made against the peptide sequence WKKELEDLVRREH, then affinity purified using the SulfoLink Kit (Pierce). α-HOXA10 antibody was purchased from Covance, and α-HA and α-PDGFR-β antibodies were purchased from Santa Cruz Biotechnology, Inc.

Stable Cell Lines and Retroviral Transduction—SIRT2 constructs were cloned into a bi-cistronic retroviral vector (unpublished) at *Xho*I (5′) and *Not*I (3′) sites in frame with FLAG and HA epitope tags at its 5′ end. A $CaPO_4$ transfection protocol was used to create viral supernatants in the Phoenix-A packaging cell line. The infection protocol is from: http://www.standford.edu/group/nolan/protocols/pro_helper_free.html. Retroviral supernatants were collected at 48 h post-transfection. Cells were transduced by spinoculation (3,000 rpm, 25°C, 1.5 h) in a retroviral supernatant supplemented with 4 μg/ml polybrene.

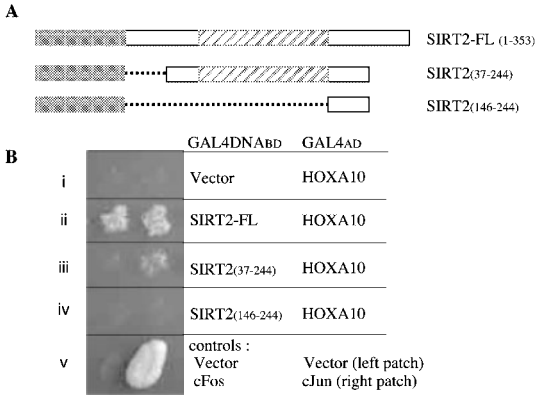

Fig. 1. **HOXA10 interacts with SIRT2.** (A) The GAL4DNA$_{BD}$-SIRT2-FL fusion was used in yeast two-hybrid library screening. The numbers refer to the SIRT2 amino acid residues present in the fusion proteins. The GAL4DNA$_{BD}$-SIRT2$_{(37–244)}$ and GAL4DNA$_{BD}$-SIRT2$_{(146–244)}$ were used to identify the interacting domain with positive library candidates. The grey box denotes the GAL4DNA$_{BD}$. The stippled box indicates the SIRT2 conserved core domain, and the open boxes represent the non-conserved SIRT2 sequences. The dashed lines indicate a fusion where sequences between the two portions are deleted. (B) GAL4$_{AD}$-HOXA10 was coexpressed with GAL4DNA$_{BD}$-SIRT2 fusions and assayed for interaction.

Positively transduced cells were selected using 2 μg/ml puromycin 48 h after spinoculation.

Immunoprecipitation (IP) and Subcellular Fractionation—U937 cells (1×10^8) expressing either FHP-SIRT2 or vector were used for sub-cellular fractionation (*30*). For IP, fractions were incubated with 10 μl of a 50% slurry suspension of α-FLAG M2 affinity gel (Sigma) at 4°C rotating overnight in appropriate buffers. Whole-cell extracts equaling 2×10^5 cells were loaded per lane for the input and flow-through samples, and 1% of the final protein eluted from the beads was loaded as the eluate sample. The bound fraction was eluted from the beads by boiling the beads in sample loading buffer.

RESULTS

The Homeodomain Protein HOXA10 Interacts with SIRT2—A cDNA library from skeletal muscle tissue fused to the GAL4 activation domain (GAL4$_{AD}$) was used in a two-hybrid screen to identify proteins interacting with the SIRT2 (GAL4DNA$_{DB}$-SIRT2-FL, Fig. 1A). The HOXA10 gene (GenBank AF040714.1) was positively identified four times, representing at least two independent clones. Though another protein was identified as a positive interacting protein, only HOXA10 was isolated in full-length in frame with the GAL4$_{AD}$ with variable length non-coding regions. The HOXA10 fusion with the GAL4$_{AD}$ was re-transformed into the host strain along with various GAL4DNA$_{BD}$-SIRT2 constructs to map the interaction domain of SIRT2 (Fig. 1B). The two-hybrid reporter strain was unable to grow on 3-AT when the GAL4$_{AD}$-HOXA10 and GAL4DNA$_{BD}$-vector alone were co-transformed (Fig. 1B, row i). Co-transformation of GAL4$_{AD}$-HOXA10 and GAL4DNA$_{BD}$-SIRT2-FL gave a positive interaction result (Fig. 1B, row ii). In addition, a much weaker growth phenotype was observed between

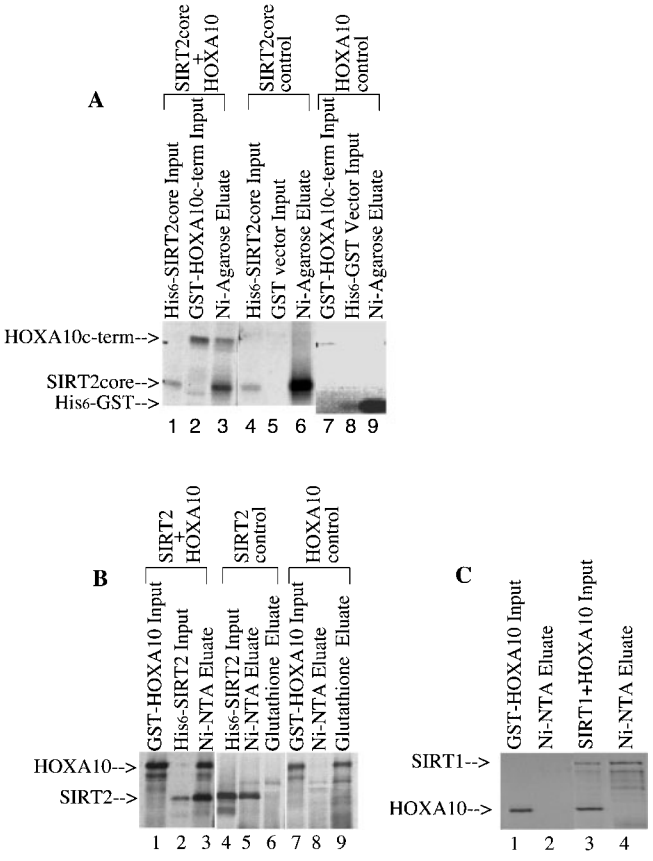

Fig. 2. **SIRT2 and HOXA10 interact *in vitro* transcription/translation reaction.** In all reactions, input lanes represent 5% of the total reaction, and eluate lanes show 20% of the final elution. (A) HIS_6-SIRT2core and GST-HOXA10c-term constructs were used. The products were recovered with Ni-NTA-agarose conjugated resin. Lanes 3, 6, and 9 are the elutions of mixtures of lanes 1 and 2, 4 and 5, and 7 and 8, respectively. Lanes 4 through 9 are the control experiments for the reactions shown in lanes 1 through 3. (B) HIS_6-SIRT2 and GST-HOXA10 full-length constructs were expressed, and the products were recovered with Ni-NTA-agarose resin. Lane 3 is the elution of the mixture of lanes 1 and 2. Lanes 4 though 9 are the control experiments for the reactions shown in lanes 1 through 3. (C) HIS_6-SIRT1 and GST-HOXA10 constructs were expressed. The products were recovered with Ni-NTA-agarose resin. Lanes 2 and 4 are the elutions of lanes 1 and 3.

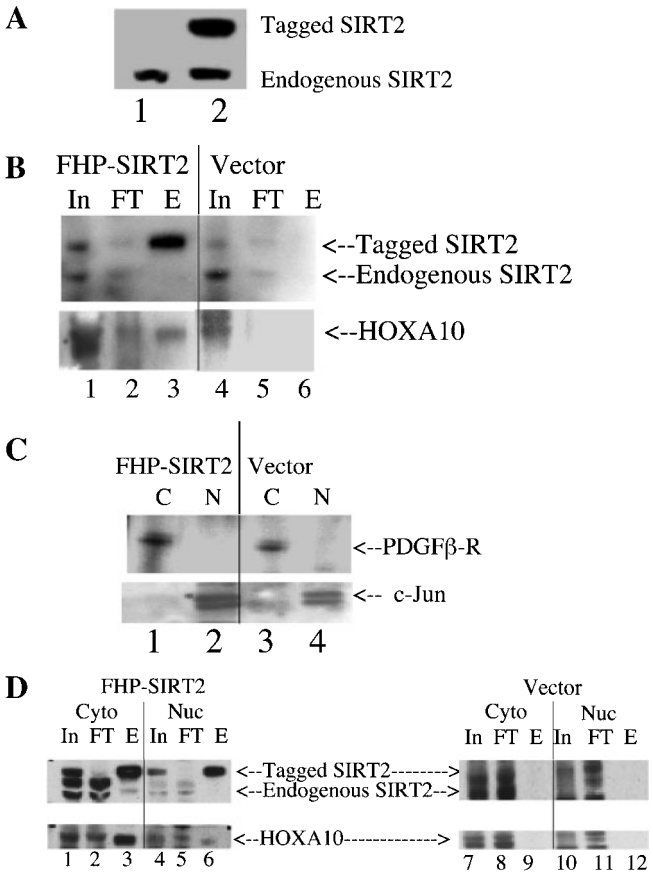

Fig. 3. **Co-immunoprecipitation of SIRT2 and HOXA10 from ectopically expressed U937 cells.** (A) Stable exogenous expression of SIRT2 in U937 cells. Whole cell extracts were isolated from either vector-transduced cells (lane 1) or SIRT2-transduced cells (lane 2). The exogenous expression of the SIRT2 construct was verified by SDS-PAGE and immunoblot analysis. The blot was probed with the α-SIRT2 antibody. (B) Whole cell extracts of FHP-SIRT2 transduced U937 cells were used for immunoprecipitation with α-FLAG M2 agarose. "In" denotes input (lanes 1 and 4), "FT" denotes flow-through (lanes 2 and 5), and "E" denotes eluate from the beads (lanes 3 and 6). Extracts were analyzed by SDS-PAGE and immunoblotting. The blots were probed with either α-SIRT2 (top panel) or α-HOXA10 (bottom panel) antibodies. (C) Transduced U937 cells were fractionated into cytoplasmic and nuclear portions. The fractions were analyzed by SDS-PAGE and immunoblotting. Blots were probed with either α-PDGFβ-R or α-c-Jun. "C" denotes the cytoplasmic fraction (lanes 1 and 3), and "N" denotes the nuclear fraction of the cells (lanes 2 and 4). (D) The same fractions from Panel C were immunoprecipitated using α-FLAG M2 agarose. The samples were analyzed by SDS-PAGE and immunoblotting. "Cyto" denotes the cytoplasmic fraction, and "Nuc" denotes the nuclear fraction of the cells. The blots were probed with α-SIRT2 and α-HOXA10 antibodies. The lane legends are same as in panel (B).

$GAL4_{AD}$-HOXA10 and $GAL4DNA_{BD}$-SIRT2(37–244), comprised primarily of the SIRT2 conserved core domain (Fig. 1B, row iii). When the conserved domain of SIRT2 was deleted, the interaction was abolished (Fig. 1B, row iv). The expression of the fusion proteins was verified by immunoblot analyses (data not shown). These data indicate that, minimally, the conserved core of SIRT2 contributes to the interaction with HOXA10.

HOXA10 and SIRT2 Interact In Vitro—HOXA10 is known to form complexes with other proteins through its heterodimerization domain that contains a highly conserved hexapeptide (*19*). SIRT2 contains a highly conserved domain that possesses deacetylase activity (*11*). Our yeast two-hybrid data revealed an interaction between these two conserved regions. To test the interaction *in vitro*, SIRT2 was cloned into a vector fused to a polyhistidine (HIS_6) tag and HOXA10 was fused to a glutathione-S-transferase (GST) tag. Each construct was translated independently in rabbit reticulocyte lysates and labeled with [^{35}S]-methionine. The resulting proteins were incubated with Ni-NTA-agarose beads or with glutathione-sepharose beads.

The HIS_6-tagged core domain of SIRT2 (Fig. 2A, lane 1) was mixed with the GST-$HOXA10_{(c\text{-terminal})}$ fusion protein (Fig. 2A, lane 2) and incubated with Ni-agarose. $HOXA10_{(c\text{-terminal})}$ co-precipitated with the SIRT2 core domain (Fig. 2A, lane 3). A vector containing both of the HIS_6 and GST tags was used as a control for this set of

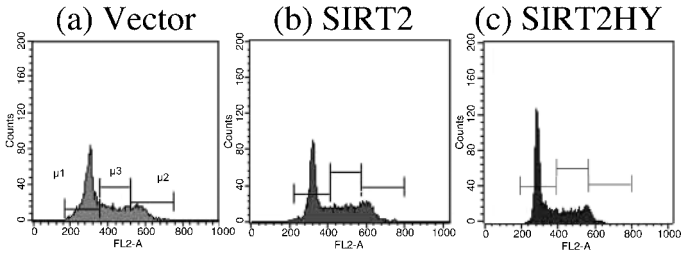

Fig. 4. ***In vivo* effects of SIRT2 over-expressed in U937 cells were examined.** Cell-cycle profiles were examined by staining the cells with propidium iodide followed by flow cytometric analysis. This is a representative profile obtained from the analysis. The percentage of cells in the G_1, S, and G_2 phases of the cell cycle are indicated.

experiments. The GST- $HOXA10_{(c\text{-terminal})}$ fusion protein was not precipitated when incubated with Ni-agarose alone, though the HIS_6-GST control was precipitated (Fig. 2A, lane 9).

The full-length constructs of each protein were also tested for interaction. The results showed that GST-HOXA10 co-precipitates with His_6-SIRT2 (Fig. 2B, lane 3). Appropriate controls showed that this reaction also was specific (Fig. 2B, lanes 4 though 9). Reciprocal co-precipitation with glutathione-beads was performed and gave the same results (data not shown).

To verify the specificity of the interaction, another human homolog of ySir2p, SIRT1, was used as a control. Interactions between SIRT1 and HOXA10 were tested (Fig. 2C). When incubated with Ni-NTA agarose, SIRT1 and HOXA10 did not co-precipitate (Fig. 2C, lane 4). Thus, though both SIRT1 and SIRT2 are homologs of ySir2p, the interaction with HOXA10 was specific for SIRT2.

HOXA10 Co-Immunoprecipitates with SIRT2 In Vivo—An *in vivo* interaction between SIRT2 and HOXA10 was tested using cell lines expressing tagged SIRT2 proteins (FHP-SIRT2). Immunoblotting analysis using α-SIRT2 antibody showed an approximately two-fold increase in the exogenously expressing cell line compared to the vector control (Fig. 3A).

Whole-cell extracts from U937 cells expressing either the FHP-SIRT2 or the vector construct were incubated with α-FLAG M2 agarose beads to precipitate SIRT2. Immunoblotting was done on eluates from these beads using both α-SIRT2 and α-HOXA10 antibodies (Fig. 3B). When extracts from cells expressing the FHP-SIRT2 construct were used, both the tagged SIRT2 and HOXA10 were precipitated (Fig. 3B, lane 3, upper and lower panels, respectively). In this reaction, the endogenous SIRT2 was not precipitated, indicating that SIRT2 does not form homomultimers. In vector-transduced cells, neither HOXA10 nor the endogenous, untagged SIRT2 protein was precipitated (Fig. 3B, lane 6, upper and lower panels, respectively).

Cells were then fractionated into cytoplasmic and nuclear portions, and immunoprecipitations were carried out using α-FLAG M2 agarose beads. To confirm the purity of the fractions, blots were probed with α-PDGFβ-R and α-c-Jun. PDGFβ-R is a cytoplasmic protein and c-Jun, a nuclear protein. Only the cytoplasmic fractions showed positive signals when probed with α-PDGFβ-R (Fig. 3C, upper panel), and only the nuclear fractions showed positive signals with α-c-Jun (Fig. 3C, lower panel).

Once the purity of the fractions was confirmed, these same fractions were incubated with α-FLAG M2 agarose beads. The FHP-SIRT2 expressed in the U937 cells was found in both the cytoplasmic and nuclear fractions (Fig. 3D, lanes 1 and 4, respectively, in the upper panel). Immunoprecipitation reactions with α-FLAG M2 agarose beads showed that HOXA10 co-precipitates with the FHP-SIRT2 protein from both the cytoplasmic and nuclear fractions (Fig. 3D, lanes 3 and 6, respectively in the lower panel). HOXA10 is found predominately in the cytoplasmic fractions and partially in the nuclear fractions. The mobility of the protein varies slightly from sample to sample, presumably due to the different salt concentrations used in the fractionation protocol (Fig. 3D, compare lanes 1, 2 and 3). When vector-transduced U937 sub-cellular fractions were probed with α-SIRT2 and α-HOXA10 antibodies, the endogenous proteins were detected both in the cytoplasmic and nuclear fractions (Fig. 3D, lanes 7 and 10, upper and lower panel). Neither the endogenous SIRT2 nor the HOXA10 protein was precipitated from the control cell fractions on α-FLAG M2 agarose beads (Fig. 3D, lanes 9 and 12, upper and lower panels).

Previously SIRT2 was reported to be a cytoplasmic protein (*20*, *21*), but these experiments could not exclude the possibility of the protein also residing in the nucleus. Recently, North *et al.* (*14*) reported that some portion of SIRT2 is found in the nucleus. In our study, immunoprecipitation following subcellular fractionation further supported the findings of North and colleagues that some portion of SIRT2 is localized to the nucleus. SIRT2 in the nuclear fraction interacts with HOXA10 *in vivo* (Fig. 3D).

In Vivo Effects of the Exogenously Expressed SIRT2-Transduced Cells—Previous studies reported that when HOXA10 was over-expressed in U937 cells, the cells were arrested in the G_1 phase of the cell cycle (*22*). Since HOXA10 was identified as an interacting protein of SIRT2, the cell cycle effects due to the over-expression of SIRT2 were examined. Actively growing U937 cells were permeabilized with trypsin, stained with propidium iodide, and analyzed with a flow cytometer. Figure 4 shows a typical example of cell cycle profiles of transduced cells. Compared to the vector-transduced cells, SIRT2 transduced cells showed almost twice the number of cells in the G_2 phase of the cell cycle (Fig. 4, compare panels (a) panel (b)). This is similar to a recent report by Dryden *et al.* (*23*). Next, the cell cycle effects due to the exogenous expression of an enzymatically inactive form of SIRT2 (SIRT2HY) were examined. Unlike SIRT2, SIRT2HY transduced cells showed no significant changes in their cell cycle profile (Fig. 4, compare panel s (a) and

(c)). Hence, the enzymatic activity of the protein seems to be responsible for the accumulation of cells in G_2 phase of the cell cycle.

DISCUSSION

In the current study, HOXA10 was identified as an interacting partner of SIRT2 from a human skeletal muscle cDNA library. HOXA10 is a sequence-specific DNA-binding transcription factor that has been shown to activate and repress transcription in a context specific manner. The identification of a DNA-binding transcription factor interacting with SIRT2 indicates that SIRT2 may play a role in gene regulation at the level of transcriptional repression by the deacetylation of histones. In *S. cerevisiae*, ySir2p is recruited to target loci primarily by the DNA-binding activator/repressor protein Rap1p indirectly through the Sir3p and 4p proteins (*6*), and the Sir2p-like Hst1p is targeted by the DNA-binding proteins Sum1p and Ntd80p (*24*). Recent work has also shown that the human SIRT1 protein, another homolog of ySir2p, can interact with basic helix-loop-helix transcription factors (*25*).

Human SIRT2 expressed in yeast cells failed to complement a *sir2*Δ mutation (*13*). However, it was shown that when the ySir2p core domain was replaced with the SIRT2 core, this chimeric protein was partially functional in yeast, indicating that the core domains perform similar functions and that the non-conserved sequences may contribute to specific interactions with targeting proteins. In order to characterize better the regions of SIRT2 that interact with HOXA10, domain deletions of SIRT2 were tested in the two-hybrid system. When the non-conserved amino-terminal end of SIRT2 was deleted along with non-conserved carboxy-terminal sequences, the interaction with HOXA10 was partially retained. When a deletion construct that lacked the core domain was tested, there was no detectable interaction with HOXA10. Taken together with the complementation data, our results suggest that determinants in both the conserved core domain and the non-conserved sequences of SIRT2 contribute to the interaction with HOXA10.

Results using an *in vitro* transcription/translation assay supported our results that SIRT2 and HOXA10 directly interact. Furthermore, it was shown that the conserved regions of the Sir2p-like family proteins and the conserved heterodimerization domain of the HOX proteins were sufficient for this interaction. Though both SIRT1 and SIRT2 are homologs of ySir2p and belong to the same HDAC group, the interaction with HOXA10 was specific for SIRT2.

In stable cell lines expressing exogenous SIRT2, subcellular fractionation followed by immunoprecipitation showed an interaction between SIRT2 and HOXA10 in both the nucleus and cytoplasm. HOXA10 is a known nuclear transcription factor, yet it was immunoprecipitated with SIRT2 from the cytoplasmic fraction of the cells as well as from the nuclear fraction. In fact, it is not unusual to find HOX proteins localized in the cytoplasm; a subset of HOXB proteins illustrates such cases (*27*). The HOXA10 protein is phosphorylated (*26*), and such a modification could regulate the localization of HOXA10. On the other hand, SIRT2 may regulate the localization of HOXA10. SIRT2 may be imported into and exported from the nucleus to regulate the function of HOXA10. It is clear that SIRT2 also exists in the nucleus, and an alternative possible role of SIRT2 in the nucleus could be to deacetylate HOXA10 and perhaps other transcription factors, thus regulating genes involved in temporal developmental regulation.

Support for the importance of the *in vivo* interaction of SIRT2 and HOXA10 comes from knockout mouse models. Though normal in appearance, homozygous HoxA10 null mice are sterile; male HoxA10 knockout mice had spermatogenesis defects, and female HoxA10 mutants exhibited decidualization (*28*). Recently, homozygous knockout mice of a SIRTUIN homolog were created (mSIR2α; (*29*)). The mSIR2α knockout mice were viable with minor physical differences from their wild-type littermates; the most notable exception was that they were sterile. Quite similar to male HoxA10 knockout mice, male *sir2*α mutant animals had spermatogenesis defects, and female *sir2*α null animals failed to ovulate. The similarities of the terminal phenotypes of the null mutations of the HoxA10 and mSIR2α mice are in accordance with the interaction described in this work. It seems likely that these two proteins function together to play a role in the development of adult reproductive tissues.

Exogenously expressed SIRT2 caused a two-fold increase in the number of cells in the G_2/M phase of the cell cycle. This finding is in agreement with a report from Dryden *et al.* (*23*), showing that the over-expression of SIRT2 causes cells to exit mitosis more slowly, causing them to accumulate in the G_2 phase. The enzymatically inactive SIRT2HY mutant did not cause the accumulation of cells in the G_2 phase. This suggests that the G_2 accumulation is related to the deacetylase activity of SIRT2, but whether it is due to transcription regulation or tubulin destabilization is unclear.

Deacetylases are required for repression of transcription, so the ability of SIRT2 to repress the induction of HOXA10-dependent transcription would be of interest. Our preliminary transient transfection transcription assay in HeLaS3 cells using GAL4 promoter data shows that when HOXA10 was transfected into cells stably expressing exogenous SIRT2, there was an approximately 50% decrease in the expression level of the reporter gene, compared to controls (data not shown). These data suggest that SIRT2 may act as a repressor of HOXA10 induced transcription.

Here, for the first time, we have described an interaction between the homeobox transcription factor HOXA10 and human histone deacetylase SIRT2 in both the cytoplasmic and nuclear fractions of cells. This interaction was confirmed by co-precipitation from *in vitro* transcription/translation products and immunoprecipitation. This interaction suggests that SIRT2 might influence the regulation of genes involved in general pattern formation during developmental stages. Though the HOXA10 protein can interact with conserved regions of the SIRTUIN family, its interaction was specific with the full-length SIRT2 and not SIRT1. Such specific interactions between members of families of activators and cofactors might shed light onto the regulatory mechanisms of coordinate gene regulation by multi-protein complexes in eukaryotes.

We thank Dr. Robert V. Intine and Dr. Peter Baumann for their critical reading of the manuscript, and Dr. Tomohiko Tamura for technical advice regarding U937 cells. SIRT2 and SIRT1 construct s were gifts from Drs. Jef Boeke and Roy Frye.

REFERENCES

1. Strahl, B.D. and Allis, C.D. (2000) The language of covalent histone modifications. *Nature* **403**, 41–45
2. Narlikar, G.J., Fan, H.Y., and Kingston, R.E. (2002) Cooperation between complexes that regulate chromatin structure and transcription. *Cell* **108**, 475–487
3. Berger, S.L. (2002) Histone modifications in transcriptional regulation. *Curr. Opin. Genet. Dev. Dev.* **12**, 142–148
4. Loo, S. and Rine, J. (1995) Silencing and heritable domains of gene expression. *Annu. Rev. Cell. Dev. Biol.* **11**, 519–548
5. Rine, J. and Herskowitz, I. (1987) Four genes responsible for a position effect on expression from HML and HMR in *Saccharomyces cerevisiae*. *Genetics* **116**, 9–22
6. Moretti, P. and Shore, D. (2001) Multiple interactions in Sir protein recruitment by Rap1p at silencers and telomeres in yeast. *Mol. Cell. Biol.* **21**, 8082–8094
7. Smith, J.S., Brachmann, C.B., Pillus, L., and Boeke, J.D. (1998) Distribution of a limited Sir2 protein pool regulates the strength of yeast rDNA silencing and is modulated by Sir4p. *Genetics* **149**, 1205–1219
8. Imai, S.-I., Armstrong, C.M., Kaebertein, M., and Guarente, L. (2000) Transcriptional silencing and longevity protein Sir2p is an NAD-dependent histone deacetylase. *Nature* **403**, 795–800
9. Landry, J., Sutton, A., Tafrov, S.T., *et al*. (2000) The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. *Proc. Natl. Acad. Sci. USA* **97**, 5807–5811
10. Smith, J.S., Brachmann, C.B., Celic, I., *et al*. (2000) A phylogenetically conserved NAD+-dependent protein deacetylase activity in the Sir2 protein family. *Proc. Natl Acad. Sci. USA* **97**, 6658–6663
11. Frye, R.A. (1999) Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (Sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase acitivity. *Biochem. Biophys. Res. Commun.* **260**, 273–279
12. Frye, R.A. (2000) Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. *Biochem. Biophys. Res. Commun.* **273**, 793–798
13. Sherman, J.M., Stone, E.M., Freeman-Cook, L.L., Brachmann, C.D., Boeke, J.D., and Pillus, L. (1999) The conserved core of a human SIR2 homologue functions in yeast silencing. *Mol. Biol. Cell.* **10**, 3045–3059
14. North, B.J., Marshall, B.L., Borra, M.T, Denu, J.M., and Verdin, E. (2003) The human Sir2 ortholog, SIRT2, is an NAD(+)-dependent tubulin deacetylase. *Mol. Cell* **11**, 437–444
15. Capecchi, M.R. (1997) *Hox Genes and Mammalian Development*, Cold Spring Harbor Symposia on Quantitative Biology. Vol. LXII, pp273–281, Cold Spring Harbor Laboratory Press, NY
16. McGinnis, W. and Krumlauf, R. (1992) Homeobox genes and axial patterning. *Cell* **68**, 283–302
17. Saleh, M., Rambaldi, I., Yang, X.J., and Featherston, M.S. (2000) Cell signaling switches HOX-PBX complexes from repressors to activators of transcription mediated by histone deacetylases and histone acetyltransferases. *Mol. Cell. Biol.* **20**, 8623–8633
18. Thorsteinsdottir, U., Sauvageau, G., and Humphries, R.K. (1997) Hox homeobox genes as regulators of normal and leukemic hematopoiesis. *Hematol. Oncol. Clin. North Am.* **11**, 1221–1237
19. Chang, C.P., Brocchieri, L., Shen, W.F., Largman, C., and Cleary, M.L. (1996) Pbx modulation of Hoxs homeodomain amino-terminal arms establishes different DNA-binding specificities across the Hox locus. *Mol. Cell. Biol.* **4**, 1734–1745
20. Afshar, G. and Murnane, J.P. (1999) Characterization of a human gene with sequence homology to *Saccharomyces cerevisiae* SIR2. *Gene* **234**, 161–198
21. Perrod, S., Cockell, M.M., Laroche, T., *et al*. (2001) A cytosolic NAD-dependent deacetylase, Hst2p, can modulate nucleolar and telomeric silencing in yeast. *EMBO J.* **20**, 197–209
22. Bromleigh, V.C. and Freedman, L.P. (2000) p21 is a transcriptional target of HoxA10 in differentiating myelomonocytic cells. *Gene Dev.* **14**, 2581–2586
23. Dryden, S.C., Nahhas, F.A., Nowak, J.E., Goustin, A., and Tainsky, M.A. (2003) Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle. *Mol. Cell. Biol.* **23**, 3173–3185
24. Xie, J., M. Pierce, V. gailus-Durner, M. Wagner, E. Winter, and A.K. Vershon (1999) Sum1 and Hst1 repress middle sporulation-specific gene expression during mitosis in *Saccharomyces cerevisiae*. *EMBO J.* **18**, 6448–6454
25. Takata, T. and Ishikawa, F. (2003) Human Sir2-related protein SIRT1 associates with the bHLH repressors HES1 and HEY2 and is involved in HES1- and HEY2-mediated transcriptional repression. *Biochem. Biophys. Res. Commun.* **301**, 250–257
26. Eklund, E.A., Jalava, A., and Kakar, R. (2000) Tyrosine phosphorylation of Hoxa10 decreases DNA binding and transcriptional repression during interferon γ-induced differentiation of myeloid leukemia cell lines. *J. Biol. Chem.* **275**, 20117–20126
27. Komuves, L.G., Ma X.K., Stelnicki, E., Rozenfeld, S., Oda, Y., and Largman, C. (2003) HOXB13 homeodomain protein is cytoplasmic throughout fetal skin development. *Dev. Dyn.* **227**, 192–202
28. Satokata, I, Benson, G., and Maas, R. (1995) Sexually dimorphic sterility phenotypes in Hoxa10-deficient mice. *Nature* **374**, 460–463
29. McBurney, M.W., Yang, X., Jardine, K., *et al*. (2003) The mammalian SIR2α protein has a role in embryogenesis and gametogenesis. *Mol. Cell. Biol.* **23**, 38–54
30. Nikolaev, A.Y., Li, M., Puskas, N., Qin, J., and Gu, W. (2003) Parc: A cytoplasmic anchor for p53. *Cell* **112**, 29–40