Many aspects of neurogenesis and neuronal differentiation are controlled by basic helix-loop-helix (bHLH) proteins. One such factor is SHARP-1, initially identified on the basis of its sequence similarity to hairy. Unlike hairy, and atypically for bHLHs, SHARP-1 is expressed late in development, suggestive of a role in terminal aspects of differentiation. Nevertheless, the role of SHARP-1 and the identity of its target genes remain unknown. During the course of a one-hybrid screen for transcription factors that bind to regulatory domains of the M subfamily of acetylcholine receptor gene, we isolated the bHLH transcription factor SHARP-1. In this study, we investigated the functional role of SHARP-1 in regulating transcription. Fusion proteins of SHARP-1 tethered to the gal4 DNA binding domain repress both basal and activated transcription when recruited to either a TATA-containing or a TATAless promoter. Furthermore, we identified two independent repression domains that operate via distinct mechanisms. Repression by a domain in the C terminus is sensitive to the histone deacetylase inhibitor trichostatin A, whereas repression by the bHLH domain is insensitive to TSA. Furthermore, overexpression of SHARP-1 represses transcription from the M1 promoter. This study represents the first report to assign a function to, and to identify a target gene for, the bHLH transcription factor SHARP-1.

Transcription factors of the basic helix-loop-helix (bHLH) family play an important role in neuronal determination and early differentiation in all phyla that have been examined. Numerous vertebrate bHLHs (for reviews see Refs. 1–3) have been identified on the basis of homology to their Drosophila counterparts (reviewed in Refs. 4–6). Some, such as Mash-1 (mouse achaete scute homologue), are transcriptional activators and act as positive regulators of neurogenesis, whereas others, such as HES-1 (homologue of hairy and Enhancer of Split), are transcriptional repressors and act as negative regulators of neurogenesis.

Structurally, bHLH proteins share a number of common features. The HLH domain mediates homomeric or heteromeric dimerization (7), and the adjacent basic region mediates DNA binding. Three groups of bHLH proteins can be recognized, according to the target binding site they recognize (8–10). Class A and Class C bHLHs function as transcriptional activators and repressors, respectively, whereas class B bHLH proteins can be either activators or repressors.

Recently, the cDNA for SHARP-1, a novel bHLH protein, was isolated on the basis of its homology to Hairy and Enhancer of Split (11). However, sequence alignment showed that SHARP-1 is only distantly related to these proteins, exhibiting 37–42% sequence identity within the bHLH domain. Unlike most bHLH proteins, SHARP-1 is not expressed in neuronal progenitor cells or early differentiating neurons but is restricted to a subset of neurons of the postnatal central nervous system (11), suggestive of a role in terminal neuronal differentiation rather than in neural determination. Unlike all other Hairy/E(spl)/HES proteins, SHARP-1 lacks the hallmark WRPW domain, which binds the co-repressor GROUCHO (or GROUCHO-like proteins) and is required for both transcriptional repression and suppression of neurogenesis. Absence of the WRPW motif suggests that SHARP-1 functions by recruiting transcriptional machinery other than GROUCHO. All class B bHLH proteins contain an arginine at position 13 in the basic region, essential for these proteins to bind to class B sites. The presence of an arginine at this position in SHARP-1 suggests that it belongs in this group, but because this group contains both activators and repressors, SHARP-1 function cannot be predicted on the basis of protein sequence.

At present, almost nothing is known about the role of bHLH proteins in differentiated neurons, and in common with many other bHLH proteins, no target genes of SHARP-1 are known. In the present study, we ascribe a transcriptional function to SHARP-1 and identify the M1 muscarinic acetylcholine receptor gene as a target gene. We show that SHARP-1 is able to repress transcription of both TATA-containing and TATAless promoters when recruited via a Gal4 DNA binding domain (DBD). Repression occurs when SHARP-1 is bound either proximally or more distally to the promoter. Furthermore, we show that repression by SHARP-1 is bimodal. One mode of repression requires the bHLH domain and is insensitive to the histone deacetylase inhibitor, TSA, whereas the other is mediated via the C-terminal domain and represses transcription through a TSA-sensitive mechanism. We also show that overexpression of SHARP-1 represses transcription of a reporter construct containing the M1 promoter. These results show that within the Hairy-related/HES family, SHARP-1 is unique in its com-
bination of presumed biological function and transcriptional mechanism.

**EXPERIMENTAL PROCEDURES**

**Reporter Plasmid Construction**—The plasmid pBM2389 +417/+166 M1 was generated as follows. A PCR product generated by using as template pGL3 +166/+603 M1 and the primers M1 235 (12) and RV3 (Promega) was cloned into pGem-T easy (Promega). The fragment was excised with EcoRI and cloned into pBM2389 (15). The SHARP-1 coding region was generated by PCR with the primers SHARP-1.11s and SHARP-1.762a (numbers are relative to the translation start site of SHARP-1) containing NcoI linkers and cloned in frame into the NcoI site of pCS2+ MT (14) to give pMT SHARP-1. To generate pMT G4 SHARP-1, a PCR product obtained using the primers SHARP-1.4s and SHARP-1.763a, consisting of the SHARP-1 coding region flanked by EcoRI linkers, was cloned into the EcoRI site of pMT G4 (15). PCR products with EcoRI linkers were generated using the sense primer SHARP-1.4s, and the antisense primers SHARP-1.519a, SHARP-1.306a, or SHARP-1.147a (numbers are relative to the translation start site of SHARP-1) were similarly used to generate pMT G4 NhHO-SHARP-1 (residues 1–173), pMT G4 NhSHARP-1 (residues 1–102), and pMT G4 NH1-SHARP-1 (residues 1–49), respectively. PCR products containing SHARP-1 fragments between positions 520 and 702, 307 and 519, and 127 and 306 with EcoRI linkers were cloned into pMT G4 to generate pMT G4 C-SHARP-1 (residues 174–253), pMT G4 O-SHARP-1 (residues 103–173), and pMT G4 BH-SHARP-1 (residues 43–102), respectively. To clone pSHARP-1 myc, a PCR fragment containing the open reading frame of SHARP-1 was generated using a sense primer containing a BamHI linker and an antisense primer containing an EcoRI linker and cloned into pCS2+ MT (14). The reporter plasmid pGL3–372/+602 M1 has been reported previously (12). The reporter plasmids pTRE UAS TATA, pGL3 UAS TRE TATA, and pGL3 UAS TRE Inr have been described previously (15).

**Yeast One-hybrid Screening**—pBM2389 +417/+166 M1 was transformed into the yeast strain SFY526 (16). This yeast strain was then transformed with DNA from an adult rat brain cDNA yeast expression library (Clontech), using the protocol of Schiestl and Gietz (17), and transformants were grown on complete supplement mixture (Wako Chemical), cells were treated with the indicated concentrations of TSA for 24 h prior to transfection and fed with media containing TSA for 24 h.

**Chemical**), cells were treated with the indicated concentrations of TSA for 24 h prior to transfection and fed with media containing TSA for 24 h. For transfections in the presence of TSA (Wako Chemical), cells were treated with the indicated concentrations of TSA for 24 h prior to transfection and fed with media containing TSA for 24 h. For transfections in the presence of TSA (Wako Chemical), cells were treated with the indicated concentrations of TSA for 24 h prior to transfection and fed with media containing TSA for 24 h.

**Immunoprecipitation Assay**—Neuro 2a cells were plated onto 10-cm plates to a density of 50%. Cells were incubated for 3–4 h with 10 μg of DNA and 22.5 μl of Tkt® 50 in a final volume of 4.8 ml. Cells were harvested after 2 days into 1 ml of 1 x phosphate-buffered saline containing 0.5% Nonidet P-40 and protease inhibitors Block (Roche Molecular Biochemicals), sonicated for 90 s, and centrifuged at maximum speed for 10 min at 4 °C. The supernatant was preclarified for 2 h with 80 μl of protein G-Sepharose. For each immunoprecipitation, half of the total sample was incubated with 3 μl of Gal4 DBD antiserum (Santa Cruz Biotechnology) overnight. Beads were added, and samples were incubated for an additional 2 h. Samples were washed four times with 20 mL Tris, pH 8.0, 1 mM EDTA, 100 mM NaCl, 0.5 mM Nonidet P-40, 1% glycerol, and 0.1% SDS. Proteins were eluted with 15 μl of loading dye. Samples were run on a 10% SDS-polyacrylamide gel electrophoresis gel and blotted onto a Hybond C + nylon membrane (Amersham Pharmacia Biotech). The membrane was subjected to Western blot analysis using a 1/1000 dilution of c-myc antiserum (Santa Cruz Biotechnology).

**RESULTS**

**SHARP-1 Binds to the M1 promoter**—In our previous studies, we have shown that transcription of the M1 muscarinic acetylcholine receptor gene is regulated by several domains within the first exon (12, 18). In particular, the region between +166 and +412 (relative to the transcriptional start site) appears to contain both enhancer and repressor elements. To identify transcription factors that bind to this region of the M1 gene we used the yeast one-hybrid approach (19, 20). Using the +166/+417 domain as bait to screen an adult rat brain cDNA library, we isolated two independent positive clones (Fig. 1a,
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A data base search for proteins with mammalian bHLH homologies identified SHARP-2, Stra13, and DEC1 (Fig. 2). SHARP-2 is a bHLH protein isolated in the same screen as that used to identify SHARP-1 (11), and its function is also unknown. Stra13 was isolated as a retinoic acid-inducible gene in mouse P19 embryonic carcinoma cells and has been shown to be able to repress the thymidine kinase promoter when fused to Gal4 DBD (21). Finally, DEC1 is a protein that was cloned by subtractive hybridization to identify mRNAs expressed in cAMP-differentiated human embryonic chondrocytes (22). Again, no function for DEC1 has been reported. Inspection of amino acid sequences shows that SHARP-2, Stra13, and DEC1 contain 411 or 412 amino acids, of which 366 are conserved, showing a sequence identity between them of 89%, suggesting that they are, in fact, rat, mouse, and human homologues. SHARP-1 is more divergent and contains only 253 amino acids. The highest sequence identity is seen in the bHLH domain and in helices 3 and 4 (also called Orange domain), whereas within the C-terminal domain only two stretches of 8 and 11 amino acids are conserved.

SHARP-1 Homodimerizes—All bHLH proteins dimerize to bind DNA (25). Because SHARP-1 was identified in the present study using a yeast one-hybrid screen, it seemed likely that SHARP-1 could either homodimerize or heterodimerize with a yeast partner. To distinguish between these possibilities, we carried out an immunoprecipitation assay using differentially tagged recombinant SHARP-1. Neuro 2a cells were transfected with either myc-tagged Gal4DBD (pMT G4) and myc-tagged SHARP-1 (pMT SHARP-1) (lane 1) or myc-tagged Gal4 SHARP-1 (pMT G4 SHARP-1) and myc-tagged SHARP-1 (pMT G4 SHARP-1) (lane 2). Cells were harvested after 2 days, and cell extracts were immunoprecipitated with Gal4 DBD antibody and analyzed by Western blotting with c-myc antibody (Ab). SHARP-1 was not immunoprecipitated in the presence of the Gal4 DBD (lane 3), but G4-SHARP-1 was immunoprecipitated (lane 4), showing that SHARP-1 is able to homodimerize. IP, immunoprecipitation.

Expression of SHARP-1 in Different Cell Lines—It has been shown previously that expression of SHARP-1 is largely restricted to differentiated neurons in the postnatal central nervous system, predominantly in the cerebellum and hippocampus, although it is also detectable at a reduced level in heart, muscle, and lung (11). We examined expression of SHARP-1 in different cell lines and cerebellum using reverse transcription-PCR. As seen in Fig. 4a, SHARP-1 is highly expressed in IMR32 cells, a human neuroblastoma cell line that also expresses M1, Neuro 2a cells, and NB4 1A3, two mouse M1 neurons (11). Although SHARP-1 was isolated by homology to hairy and Enhancer of Split, sequence alignment with these proteins shows that they are quite distantly related, sharing only 37–42% homology within the bHLH domain (11). As a first step toward identification of a function for SHARP-1, we carried out a data base search for proteins with homology to SHARP-1 and identified three proteins: SHARP-2, Stra13, and DEC1 (Fig. 2). SHARP-2 is a bHLH protein isolated in the same screen as that used to identify SHARP-1 (11), and its function is also unknown. Stra13 was isolated as a retinoic acid-inducible gene in mouse P19 embryonic carcinoma cells and has been shown to be able to repress the thymidine kinase promoter when fused to Gal4 DBD (21). Finally, DEC1 is a protein that was cloned by subtractive hybridization to identify mRNAs expressed in cAMP-differentiated human embryonic chondrocytes (22). Again, no function for DEC1 has been reported. Inspection of amino acid sequences shows that SHARP-2, Stra13, and DEC1 contain 411 or 412 amino acids, of which 366 are conserved, showing a sequence identity between them of 89%, suggesting that they are, in fact, rat, mouse, and human homologues. SHARP-1 is more divergent and contains only 253 amino acids. The highest sequence identity is seen in the bHLH domain and in helices 3 and 4 (also called Orange domain), whereas within the C-terminal domain only two stretches of 8 and 11 amino acids are conserved.
non-expressing neuroblastoma cell lines. Low levels of expression were detected in the 3T3 fibroblast cell line. PCR was carried out using hprt primers as a cDNA loading control (Fig. 4b).

**SHARP-1 Acts as Transcriptional Repressor**—bHLH proteins can act as transcriptional activators or repressors (reviewed in Ref. 1). Because the transcriptional function of SHARP-1 is unknown, we assessed its ability to (a) regulate transcription from both TATA-containing and TATAless promoters, (b) regulate transcription when bound either proximally or distally, and (c) regulate basal and activated transcription. IMR32, 3T3, and Neuro 2a cells were transfected with plasmids expressing SHARP-1 fused to Gal4 DBD with each of the reporter genes showed in Fig. 5. pTRE UAS TATA contains seven TRE and five Gal4 binding sites 21 base pairs upstream of the E1b TATA box. pGL3 UAS TRE TATA contains five Gal4 binding sites (placed 350 base pairs from the TATA box) and seven TRE upstream of the TATA box. In pGL3 TRE UAS Inr, the TATA box from pGL3 TRE UAS TATA, was replaced by the adenovirus major late promoter initiator. Expression values of all reporter constructs were normalized to expression in the presence of Gal4 DBD alone. SHARP-1 was able to repress transcription of a TATA-containing promoter when bound proximally or to the transcription start site in all cell lines (Fig. 5a, left). SHARP-1 was also able to repress activated transcription by Tet-VP16 (activation domain of the herpes simplex virus transcriptional activator VP-16 fused to the binding domain of the tetracycline-responsive factor) in all cell lines (Fig. 5a, right). In addition, SHARP-1 was able to repress both basal and activated transcription from a TATA-containing promoter when bound distally to the transcription start site (Fig. 5b). We also tested the ability of SHARP-1 to regulate transcription from a TATAless promoter. As can be seen in Fig. 5c, SHARP-1 can repress basal and activated transcription from a TATAless promoter. We therefore conclude that SHARP-1 acts as a repressor of both basal and activated transcription of both TATA-containing and TATAless promoters. In the case of the TATA-containing promoter, repression is evident when SHARP-1 is bound either proximally or distally to the promoter, although the degree of repression is more marked when SHARP-1 is tethered proximally.

**SHARP-1 Represses Transcription through Two Independent Domains**—To map the domain(s) responsible for the repression function of SHARP-1, we generated deletion mutants of the Gal4-SHARP-1 fusion protein. The ability of these fusion proteins to repress transcription was analyzed using the reporter gene driven by a TATA-containing promoter with five Gal4 binding sites proximal to the transcription start site (pTRE UAS TATA) in Neuro 2a cells (Fig. 6). Results were normalized to expression of the reporter gene in the presence of Gal4 DBD alone. Western blot analysis showed that all constructs were expressed at similar levels (data not shown). Deletion of the C-terminal domain of SHARP-1 (to give pMT G4 NbHO-SHARP-1) slightly relieved repression by SHARP-1, but the C-terminal domain (residues 174–253) of SHARP-1 fused to Gal4 DBD (pMT G4 C-SHARP-1) was able to repress transcription as robustly as full-length SHARP-1. Therefore, it would appear that SHARP-1 must contain at least two independent repression domains, one in the C-terminal domain and another in the remaining fragment. To map the second repression domain of SHARP-1, more deletion mutants were examined. Deletion of the Orange domain and C-terminal domain to give pMT G4 NbH-SHARP-1 still gave robust repression, but further deletion of the bHLH domain to leave only the N-terminal domain (pMT G4 N-SHARP-1) led to relief of most of the repression activity, suggesting that the bHLH domain mediates repression. This was confirmed by analysis of two further constructs. Fusion of the Orange domain and flanking sequence (residues 103–173) and the Gal4 DBD (pMT G4 O-SHARP-1) showed some degree of repression, but fusion of the bHLH domain (residues 43–102) and Gal4 DBD (pMT G4 bH-SHARP-1) indicated that the bulk of repression in this second region was mediated by the bHLH domain. In summary, we identified two independent repression domains in SHARP-1, one in the bHLH domain and the other in the C terminus.

**SHARP-1 Represses Transcription through Two Different Mechanisms**—Recent studies have shown that many transcriptional repressors exert their action through recruitment of histone deacetylase activity (see Ref. 26 for review). To test whether SHARP-1 represses transcription through such a mechanism, we treated Neuro 2a cells with the histone deacetylase inhibitor TSA (27) and examined the effect on SHARP-1-mediated repression (Fig. 7). For each concentration of TSA used, expression values of the reporter gene were normalized to those obtained in the presence of Gal4 DBD alone, and results were expressed as fold over untreated cells. Repression by full-length SHARP-1 is partially relieved by TSA, because expression of the reporter gene was derepressed 4-fold in the presence of 100 nM TSA. Deletion of the C-terminal domain of SHARP-1 (to give pMT G4 NbHLLHO-SHARP-1) showed that repression mediated by the bHLH domains was much less sensitive to TSA, resulting in a 1.6-fold derepression by 100 nM TSA. However, repression by the C-terminal domain alone was relieved by 6.5-fold with 100 nM TSA, and 2-fold derepression could be seen in the presence of 10 nM TSA. These results show that the C-terminal domain of SHARP-1 represses transcription via a mechanism that is likely to involve histone deacetylase activity but that the bHLH domain represses transcription in a histone deacetylase-independent manner.

**SHARP-1 Controls the Levels of M1 Expression**—To test the
functional effect of SHARP-1 on M1 expression, IMR32, 3T3, and Neuro 2a cells were transfected with a reporter vector containing the region of the M1 gene between 2372 and 1602 (relative to the transcription start site). This construct has been shown before to be capable of driving expression in IMR32 cells, a neuronal cell line that expresses the M1 gene (18). The same construct does not drive expression in the non-M1-expressing neuronal cell line Neuro 2a and drives only low levels of expression in 3T3 cells (18). Overexpression of SHARP-1 (pSHARP-1myc) had no effect on expression of the promoterless reporter vector pGL3 basic but reduced expression driven by the M1 promoter by 55% in IMR32 cells (Fig. 8a). A similar effect was seen in 3T3 cells (Fig. 8b). No effect was seen in Neuro 2a, the neuronal cell line that does not express M1 (data not shown). These results show that SHARP-1 is able to repress expression of the M1 gene in an M1-expressing cell line.

**DISCUSSION**

bHLH proteins are key players that regulate many aspects of development and differentiation in all tissues and phyla. To date, no target genes or function of SHARP-1 has been identified. SHARP-1 is unusual in two respects. First, SHARP-1 is related to, but distinct from, HAIRY/E(spl)/HES bHLH proteins. Second, expression of SHARP-1 appears to be restricted to postnatal neurons of the central nervous system, rather than neural progenitors, implying its role in late neuronal differentiation rather than neurogenesis. These features suggested that SHARP-1 may affect transcriptional regulation and target promoters distinct from those used by other members of the HAIRY/E(spl)/HES bHLH family.

SHARP-1 contains an Arg in position 13 of the basic region present in all class B bHLH proteins. This residue is essential for class B proteins to bind the consensus sequence CA(C/T)GTG (9). Sequence analysis of the region between 1166 and 1417 of the M1 gene does not indicate the presence of any known recognition consensus motif (class A, B, or C) for bHLH (8–10), suggesting that SHARP-1 may recognize a novel binding site. Gel electrophoresis mobility shift assays have failed to demonstrate an ability of Stra13 to bind to either an E-box or N-box (21). Because SHARP-1 and SHARP-2/Stra13/DEC1 are all highly conserved within their basic regions or presumptive DNA binding domain, it is possible that both SHARP-1 and SHARP-2 recognize a common binding site, distinct from class A, B, or C sites.

By recruiting SHARP-1 to heterologous promoter constructs through the Gal4 DBD, we have shown that SHARP-1 acts as a transcriptional repressor and furthermore that SHARP-1 is able to repress both basal and activated transcription (Fig. 5). It is well documented that arrangement of basal promoter elements can profoundly influence the response of a promoter to different factors. The evolutionarily conserved Kruppel-associated box present in the N-terminal regions of most Kruppel-class zinc finger proteins specifically silences the activity of promoters whose initiation is dependent on the presence of a TATA box (28), whereas initiator-containing promoters are relatively unaffected. Similarly, Oct2 isoforms repress transcription only from TATA-containing promoters (29). Here, we show that SHARP-1 is more promiscuous and can repress transcription driven from both TATA-containing and TATA-less promoters (Fig. 5). In addition to promoter type, the relative position of its DNA binding site to the promoter can also influence repressor action. This can be seen in the case of the neuron-restrictive silencer factor (20, 30), which represses transcriptional derepression.

![SHARP-1 schematic](image-url)

**FIG. 5.** SHARP-1 acts as transcriptional repressor. IMR32, 3T3, and Neuro 2a cells were transfected with vectors expressing Gal4 DBD alone or fused to SHARP-1 together with or without a vector expressing Tet-VP16 and the reporter plasmid shown in each figure. Equal amounts of Gal4 DBD fusion, Tet-VP16/stuffer, and reporter plasmids were used. Values are the averages of three independent transfections, each performed in triplicate, normalized to cotransfected CMV-Renilla, and expressed as a percentage of the values obtained with Gal4 alone for each cell line in the absence or presence of Tet-VP16 (open bars). a shows repression by proximal recruitment of SHARP-1 to a TATA-containing promoter, whereas b shows the effect when distally recruited. c shows repression of a TATA-less promoter. In each case the left hand panel shows repression of basal promoter activity, whereas the right panel shows repression of VP16-activated transcription.
tion when recruited distally but can activate transcription when recruited proximally (31). Here again, SHARP-1 appears to act as a more global repressor and is capable of repressing transcription from a TATA-containing promoter when recruited either proximally or distally. Nevertheless, repression is more marked when SHARP-1 is tethered proximal to the TATA box (Fig. 5).

Transcriptional repression can take many forms (32). Several repressors function by deacetylating the N-terminal tails of histones that are thought to render chromatin inaccessible to the transcriptional machinery. Such is the case of the Mad family of bHLH-ZIP proteins, whose members bind to mSIN3A, an adapter molecule that links histone deacetylases to DNA-bound transcription factors (33, 34). Other repressors appear to interact directly with the transcriptional machinery itself, for example Kruppel, which at high concentrations homodimerizes and becomes a potent repressor by interacting directly with TFIIE (35, 36). Others such as retinoblastoma can interact both with general transcription factors or with histone deacetylase (37–40). Repression by SHARP-1 appears to be bimodal. One mode of repression is mediated via the bHLH domain and is TSA-insensitive, whereas the other mode is mediated by the C-terminal domain and is TSA-sensitive (probably due to recruitment of HDAC). Interestingly, Stra13 has been shown to repress expression of the c-myc gene through an HDAC-independent pathway and to negatively autoregulate its own promoter through an HDAC-dependent mechanism (41). The region responsible for the HDAC-independent repression of Stra13 has not been mapped, but glutathione S-transferase pull-down studies showed that residues 111–343 are required for interaction with HDAC-1, Sin3, and NcoR (41), whereas functional analysis of mutated Stra13 demonstrated that residues 147–354 were required for Stra13 repression of VP16-activated transcription. As shown in Fig. 2, this region contains the C-terminal domain and part of the Orange domain of SHARP-2/Stra13/DEC1. We have mapped the domain responsible for the TSA-sensitive component of SHARP-1 repression to the C-terminal domain (residues 174–253). Within this region, SHARP-1 and Stra13 display pockets of homology, principally within two stretches of 8 (residues 205–211) and 15 amino acids (residues 221–236), suggesting that these may mediate the interaction with HDAC. The HDAC-independent repression domain of SHARP-2/Stra13 has not been reported, but the GROUCHO-independent mode of repression by HAIRY/E(spl) requires the bHLH and Orange domains (23).

Although SHARP-1 and SHARP-2 are almost identical in their bHLH domains (95% over 59 residues), they diverge more markedly from HAIRY (37% over 59 residues). It remains to be seen whether the same domains or mechanisms are involved in mediating HDAC-independent repression by SHARP-1, SHARP-2/Stra13/DEC1, and HAIRY/E(spl) proteins.

Bimodal repression is not uncommon. Repression by the neuron-restrictive silencer factor (20, 30) is mediated by an N-terminal domain that recruits Sin3/HDAC-1 (15, 42–44) and

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2 A. Roopra and N. J. Buckley, unpublished observations.
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Fig. 8. SHARP-1 represses \( M_3 \) expression. IMR32 cells (a) and 3T3 cells (b) were cotransfected with a reporter construct containing the region of the \( M_3 \) gene between -372 and +602 and either pMT (open bars) or a vector expressing SHARP-1 (shaded bars). Reporter and expression vectors were in a ratio of 1:5. Values are the averages of three independent transfections, each performed in triplicate, normalized to cotransfected CMV-RENilla, and expressed as fold over luciferase activity obtained with the empty reporter vector, pGL3 basic cotransfected with empty expression vector, pMT.

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The Basic Helix-Loop-Helix Protein, SHARP-1, Represses Transcription by a Histone Deacetylase-dependent and Histone Deacetylase-independent Mechanism

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