Murine Sall1 Represses Transcription by Recruiting a Histone Deacetylase Complex*

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The multi-zinc finger proteins of the Sal family regulate organogenesis. Genetic evidence from Drosophila has shown that spalt (sal) can alter gene expression in a cell autonomous fashion, but Sal proteins have never been directly analyzed for their ability to activate or repress transcription. In this report, we show that a member of the Sal family, mouse Sall1, is a potent transcriptional repressor. When fused to a heterologous DNA-binding domain, Sall1 represses transcription of a luciferase reporter by over 100-fold. Expression of the N terminus alone is sufficient for dose-responsive repression that, as shown by deletion analysis, requires the extreme N-terminal amino acids of the protein. The N terminus of Sall1 can repress at both short and long range relative to the promoter, and treatment with the histone deacetylase (HDAC) inhibitor, trichostatin A, alleviates repression by 3-fold. The same regions of the protein that are required for repression specifically interact with components of chromatin remodeling complexes, HDAC1, HDAC2, RbAp46/48, MTA-1, and MTA-2. Finally, we demonstrate that Sall1 is localized to discrete nuclear foci and this localization depends on the N-terminal repression domain. Together, these results suggest that the N terminus of mouse Sall1 can recruit HDAC complexes to mediate transcriptional repression.

The transcriptional regulators of the spalt (sal) gene family play an important role in regulating developmental processes of many organisms. The most well studied sal family members, Drosophila spalt-major (salm) and spalt-related (salr), specify cell fate decisions of chordontonal precursors in the peripheral nervous system (1), regulate tracheal development (2), control terminal differentiation of photoreceptors (3), and determine proper placement of wing veins (4). Mutations in the human sal ortholog, SALL1, cause an autosomal dominant disorder, Townes-Brocks Syndrome, and result in ear, limb, anal, renal, and heart anomalies (5). Targeted deletion of mouse Sall1 results in severe renal dysplasia or complete agenesis, indicating that Sall1 has an essential role in kidney development (6).

Sall1 proteins are postulated to be transcriptional regulators because their sequence contains multiple DNA-binding zinc finger motifs. Mouse Sall1 has 10 zinc fingers (Fig. 1A). Nine are the C2H2-type (shaded gray) and are arranged as doublets with a third finger associated with the second pair. The second finger of each pair contains the conserved sequence F(T/S)TKGNLK that has been termed the SAL-box (7). This sequence is located in the predicted α-helical region of the zinc finger that specifies the nucleotide consensus binding sequence and is thought to bind A/T-rich sequences (8, 9). However, the native DNA-binding site and direct target genes regulated by Sal proteins have not yet been determined. The most N-terminal zinc finger is of the C2HC-type (solid gray) and may mediate protein-protein interactions rather than DNA binding (reviewed in Ref. 10).

It has been suggested that Sal proteins may act as transcriptional repressors in a variety of developmental contexts in Drosophila. sal inhibits expression of tracheless (trh), ventral veinless (vul), and knirps (kni) in the developing trachea (2), rhodopsin 1 (rh1) in differentiating photoreceptors (3), and iriquis (iro) and kni during development of the longitudinal veins (11). These genetic experiments also indicate that sal-mediated repression is cell autonomous, raising the possibility that Sal may directly inhibit expression of these downstream targets in Drosophila. Despite this considerable genetic evidence supporting a role for sal family members in the regulation of gene expression, there is currently no evidence that Sal proteins can directly mediate transcriptional repression.

Here we test whether mouse Sall1 is capable of directly affecting transcriptional activity and provide the first direct evidence that Sal proteins may act as transcriptional repressors. When linked to a heterologous DNA-binding domain, the Sall1 protein is capable of repressing transcription over 100-fold. We localize repressor activity within the N terminus of the protein and suggest that histone modification by HDACs is involved in repression by Sall1. The dependence of Sall1-mediated repression on histone deacetylation is further confirmed by demonstrating a physical association between HDAC complex components and the N-terminal Sall1 repression domain. In addition, the ability of Sall1 to repress transcription correlates with localization of Sall1 in focal nuclear subdomains that may be involved in recruiting Sall1 to specific HDAC complexes within the nucleus.

EXPERIMENTAL PROCEDURES

cDNA Isolation—Mouse Sall1 cDNA was isolated by screening a mouse 129/SvJ kidney cDNA library with a Sall1 EST clone (GenBank™ accession number AA117993) by Incyte Genomics. Sequencing of the largest cDNA clone (5.4 kb) showed that this clone encoded all but the initial ATG and 386 bases of the coding region of the mouse ortholog of human SALL1. The remaining 5′-sequence was obtained by performing 5′-rapid amplification of cDNA ends on E17 RNA from mouse kidney using the 5′3′-rapid amplification of cDNA ends kit (Roche Molecular Biochemicals) according to the manufacturer’s specifications. The full cDNA clone was reconstructed by PCR amplification using Sall1-specific primers designed from the subsequently published mouse

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1 The abbreviations used are: HDAC, histone deacetylase; TSA, trichostatin A; trh, tracheal-less; NHL, nuclear foci; GAL4DB, GAL4 DNA-binding domain; GST, glutathione S-transferase; AdMLP, adenovirus major late promoter.
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Sall1 sequence (Ref. 12, GenBankTM accession number A2721914) and an internal KpnI site.

Cell Culture and Transfection—COS-1 cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, penicillin, and streptomycin. Cells were plated in 6-well plates at a density of 3 × 10⁶ per well and transfected using FuGENE (Roche Molecular Biochemicals) according to the manufacturer's directions.

Plasmids—The GAL4DB expression plasmid, BGX-I, and the eukaryotic GST fusion plasmid, pEBG, were graciously provided by R. Maas (Brigham and Women's Hospital) and S. Stefani (McGill University) and used to construct the GAL4DB-Sall1 and GST-Sall1 fusions by PCR using restriction fragment subcloning. The following Sall1 constructs were confirmed by sequencing and detection of the expressed fusion proteins by anti-GAL4DB or anti-GST Western blotting: full-length (amino acids 1–1322), N-terminal (2–435), C-terminal (1104–1322), Zn-finger (436–1103), and the N-terminal deletion constructs encoding amino acids 77–435, 135–435, 205–435, 265–435, and 399–435. The luciferase reporters were generous gifts from J. Milbrandt (SV40Luc, pGVSV40Luc, pGS–450hp-SV40Luc, pG5–2900hp-SV40Luc, and pGSADMLPlae, Washington University), R. Davis (pG551bLuc, University of Massachusetts), and D. Moore (GAL4tkLuc, Baylor). MTA1-T7 and MTA2-FLAG (PCIN4) were provided by R. Kumar (M.D. Anderson Cancer Center) and W. Gu (Columbia University), respectively. The Sall1-FLAG constructs were made using a p300 DNA-binding domain plasmid obtained from J. Merchant (University of Michigan) by removing p300 and replacing it with the full-length Sall1 sequence. The FLAG-tagged N-terminal deletion constructs Sall1-77 (1322), Sall1-130 (1322), Sall1-204 (1322) were generated by excising a 5′-fragment of Sall1-FLAG with HindIII (5′ end of cDNA) and NheI (nucleotide 913) and replacing it with a HindIII-NheI cut PCR fragment. These constructs encode a full-length protein beginning with nucleotide 77, nucleotide 130, or nucleotide 204 and are FLAG-tagged at their C termini.

Reporter Assays—Cells were transiently transfected with 1 µg of GAL4DB fusion plasmid, 2 µg of luciferase reporter plasmid, and 0.05 µg of cytomegalovirus-β-galactosidase control plasmid per well. pcdNA3 (Invitrogen) was used to normalize the DNA content of the transfection. When appropriate, cells were treated with 200 ng trichostatin (TSA, Calbiochem) or an equivalent volume of Me2SO vehicle for 24 h prior to harvesting for reporter assays. After 48 h, the transfected cells were washed with phosphate-buffered saline, lysed with reporter assay lysis buffer (Promega), and spun at 10,000 × g for 10 min, GST-Sall1 fusion proteins for 10 min, GST-Sall1 fusion protein complexes were isolated by precipitation of 50 µg of total protein (or 200 µg for the full-length GST fusion) with glutathione-Sepharose beads (Amersham Bioscience) for 2 h at 4 °C. The beads were washed three times with 10 volumes of lysis buffer and eluted by boiling in Laemmli sample buffer, GST-Sall1 fusions and Sall1-interacting proteins were separated by SDS-polyacrylamide gel electrophoresis on 7.5% gels, transferred to nitrocellulose, blocked in 3% nonfat dry milk, and incubated with appropriate antibodies. Antibody reactivity was detected using horseradish peroxidase-labeled secondary antibodies (horseradish peroxidase anti-mouse (KPL) and horseradish peroxidase anti-rabbit (Sigma)) and ECL detection reagent (Amersham Pharmacia Biotech). Immunofluorescence—Cells were transfected on glass coverslips and allowed to express for 48–72 h. The coverslips were fixed in 3% paraformaldehyde, washed in phosphate-buffered saline containing 50 mM glycine, permeabilized in cold methanol, and blocked in 10% normal goat serum (Sigma). Primary antibodies were diluted in phosphate-buffered saline containing 1% bovine serum albumin (anti-FLAG 1:200, anti-GAL4DB 1:50, and anti-Sall1 1:500) and detected using fluorescein isothiocyanate anti-mouse (ICN) secondary antibodies. All coverslips were incubated with 100 ng/ml Hoechst dye to detect cell nuclei and mounted with Miowiol 488 (Polysciences, Inc.). Images were visualized with a fluorescence (Nikon Eclipse E800) or confocal (MRC1024 Bio-Rad) microscope. Greater than 200 cells from at least 5 independent transfections were scored for each pattern of nuclear staining (punctate, reticular, diffuse, see text).

Antibodies—Polyclonal antibodies were raised against an N-terminal peptide of mouse Sall1, REEGITKEKGPSSRPTKS (Strategic Biosolutions). Anti-GAL4DB (RK5C1), anti-GST (B14), anti-HDAC2 (H-54), and anti-Sin3A (AK-11) (Santa Cruz); anti-FLAG (M2) and anti-HDAC1 (Sigma); anti-T7 (Novagen); and anti-RhAp46/48 (15G12) (Genetex) were all obtained from commercial sources.

RESULTS

Mouse Sall1 Represses Transcription—The ability of mouse Sall1 to affect transcription was analyzed by fusing the GAL4 DNA-binding domain (GAL4DB) to mouse Sall1. As depicted in Fig. 1A, the full-length protein (1–1322), a N-terminal construct containing the C-HC zinc finger (2–435), a C-terminal region containing one double zinc finger pair (1104–1322), and a zinc finger domain containing seven C-HC zinc fingers (436–1103) were expressed as GAL4DB fusion proteins and transfected with a GAL4-responsive luciferase reporter. When assayed for its ability to activate or repress a GAL4-responsive reporter, full-length mouse Sall1 was a very strong transcriptional repressor (>100-fold). This repression activity appears to be largely mediated by the N-terminal domain because it alone is capable of potent repression. The C-terminal domain does not have the ability to repress or activate transcription and the zinc finger domain containing the remaining seven C-HC zinc fingers represses transcription to a lesser extent. DNA binding is required for Sall1 repression because expression of full-length Sall1 that lacks the GAL4 DNA-binding domain does not repress transcription (data not shown).

Repression by Sall1 N Terminus Is Dose Responsive—Because the N terminus alone was shown to be a particularly powerful repressor, it was analyzed in more detail to determine whether transcriptional repression was dependent on Sall1 dosage. Increasing amounts of the GAL4DB-Sall1-N fusion construct were transfected into cells to test for their ability to repress luciferase expression. Increasing doses of Sall1-N repressed luciferase activity in a dose-responsive manner (Fig. 1B). At the lowest amount of Sall1-N transfected (10 ng), the luciferase activity was identical to the activity produced by GAL4DB alone while at the highest amount of Sall1-N transfected (1 µg), the luciferase activity measured was less than 3% of the GAL4DB control. The Sall1 N terminus was capable of repressing transcription from two additional GAL4-responsive reporters containing the adenovirus E1b promoter or the thymidine kinase promoter and in two different cell lines (data not shown).

The Extreme N Terminal Mediates Repression—To localize the repression domain within the N terminus, a series of Sall1-N deletion constructs were coexpressed with the GAL4-responsive 540-luciferase reporter. As depicted in Fig. 2, these deletion constructs sequentially removed the C-HC zinc finger and regions rich in threonine, glutamine, and serine. Interestingly, removal of the extreme N-terminal 76 amino acids including the C-HC zinc finger, Sall1-N (77–435), significantly reduced the ability to repress transcription from over 100-fold to only 10-fold. A further deletion of the first 129 amino acids, Sall1-N (130–435), removed all of the repression activity of the Sall1 N terminus and none of the smaller constructs regained this ability to repress transcription. Our assay was able to detect both transcriptional activation and repression because the GAL4 transactivation domain consistently increased luciferase activity more than 10-fold (data not shown). Yet, despite the resemblance between the glutamine-
rich region of Sall1 and the activator domain of the transcription factor Sp1 (7), none of the Sall1 N-terminal constructs unmasked a region capable of activating transcription.

Repression at a Distance—To determine whether repression was dependent on the distance of the binding sites from the promoter region, Sall1-N was targeted to GAL4-binding sites at different distances from the SV40 promoter. The SV40 reporter used in the previous experiments contained five GAL4-binding

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**FIG. 1. Transcriptional repression by Sall1.** A, the mouse Sall1 protein contains one C2HC zinc finger (solid gray) and nine C2H2 zinc fingers (shaded gray) arranged in pairs. The full-length protein, the N terminus, the C terminus, and a zinc finger region of Sall1 were expressed as GAL4DB fusion proteins along with a reporter in which luciferase gene transcription is under the control of five GAL4DB-binding sites and the SV40 promoter (pG5SV40Luc). Fold repression was calculated by dividing the normalized luciferase activity of cells expressing GAL4DB alone by the activity of the Sall1 fusion protein. Values are plotted as the mean ± S.D. of triplicate transfections from two independent experiments and demonstrate that the full-length and N terminus of Sall1 exhibit potent repression activity.

B, increasing doses of GAL4DB-Sall1-N (10, 100, 250, 500, and 1000 ng) or GAL4DB alone was expressed with the luciferase reporter and cytomegalovirus-β-galactosidase as in A. Normalized luciferase measurements were divided by the activity for GAL4DB alone and multiplied by 100 to calculate percent luciferase activity in arbitrary units. Percent luciferase activity is plotted to highlight the dose response of repression of Sall1-N compared with the control, GAL4DB. Values are plotted as the mean ± S.D. of triplicate transfections from three independent experiments.

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**FIG. 2. Repression is mediated by the N-terminal amino acids of Sall1.** The Sall1 N terminus contains a C2HC zinc finger (gray oval), a threonine-rich domain (Thr), a glutamine-rich domain (Gln), and a serine-rich domain (Ser). The N terminus (amino acids 2–435) and constructs sequentially deleting these domains or their intervening sequences, N-(77–435), N-(130–435), N-(204–435), N-(266–435), N-(399–435), were expressed as GAL4DB fusions, tested for their ability to repress luciferase transcription, and plotted as fold repression as described in the legend to Fig. 1A.
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Repression Is Dependent on Histone Deacetylase Activity—Repression of transcription by many proteins close to and at a distance from the transcription factors (reviewed in Ref. 18). Chromatin remodeling enzymes, such as Ikaros (16) and nuclear hormone receptors (reviewed in Ref. 17), and thereby mediate transcriptional repression. Two HDAC enzymes, HDAC1 and HDAC2, and two associated proteins, RbAp46 and RbAp48, are core components common to both the NuRD and Sin3 repression complexes. NuRD and Sin3 differ by the inclusion of NuRD-specific (MTA1, MTA2, Mi2, and MBd3) and Sin3-specific (Sin3A, SAP18, and SAP30) factors to alter chromatin structure and repress transcription via distinct DNA-binding transcription factors (reviewed in Ref. 18).

To test for a physical interaction between Sall1 and HDAC complexes, the same regions of Sall1 that were expressed as GAL4DB fusion proteins were subcloned into a GST fusion expression vector, pEBG, and overexpressed in COS-1 cells. GST-Sall1 fusion proteins were isolated on glutathione-Sepharose beads and analyzed by Western blotting. All GST-Sall1 proteins were expressed at similar levels except for the largest fusion, full-length Sall1, which was at least 4-fold less well expressed (data not shown) and required 4-fold more of the cell lysate to be used in the precipitation (see “Experimental Procedures”).

When the GST-Sall1 precipitates were analyzed for the ability to directly or indirectly bind HDAC repression complexes, the same regions of Sall1 that mediated strong repression, the full-length protein (1350), interacted with repression components (Fig. 4A). Endogenous HDAC1, HDAC2, and RbAp46/48, core components of all HDAC repression complexes, bound to N-terminal and full-length GST fusion proteins, but not to the C-terminal or zinc finger proteins or to GST alone.

To discern whether Sall1 interacted with the Sin3 or NuRD repression complex, we next tested whether these GST-Sall1 proteins could bind to Sin3A or to the NuRD-specific components, MTA1 and MTA2. MTA1-T7 and MTA2-FLAG were coexpressed with GST-Sall1 fusions and, like HDAC1/2 and RbAp46/48, were able to bind full-length and N-terminal Sall1 fusion proteins, but not the C-terminal or zinc finger regions. Interestingly, another NuRD complex component, Mi2, was not detectable in the GST-Sall1 precipitates (data not shown), suggesting that not all NuRD components were present in the active Sall1 repression complex. However, since Sin3A does not bind any GST-Sall1 fusion proteins, the active Sall1 repressor complex likely resembles NuRD and not Sin3.

The correlation between strong transcriptional repression and NuRD complex interaction was evident for the N-terminal deletions of Sall1 (Fig. 4B). When the N terminus and the three longest deletion constructs (77–435, 130–435, and 204–435)
were expressed as GST fusion proteins in COS-1 cells and purified on glutathione-Sepharose, only Sall1-N was able to interact with HDAC1, HDAC2, RbAp46/48, MTA1, and MTA2, but not Sin3A. A, whole cell extracts prepared from cells transfected with GST alone or GST fused to the N terminus, C terminus, zinc finger, or full-length Sall1 proteins were incubated with glutathione-Sepharose, washed, and eluted in sample buffer. These eluates and one-tenth (5 μg) of the GST expressing input lysate were analyzed for the presence of endogenous HDAC complex components by Western blotting using anti-HDAC1, anti-HDAC2, anti-RbAp46/48, and anti-Sin3A antibodies. MTA1-T7 and MTA2-FLAG were co-expressed with the GST constructs prior to cell lysis and detected by anti-T7 or anti-FLAG antibodies. B, glutathione-Sepharose precipitates from N-terminal GST fusion proteins Sall1-N, the deletion constructs N-(77–435), N-(130–435), N-(204–435), and one-tenth (5 μg) of the Sall1-N-expressing lysate were analyzed for HDAC complex components as in A.

Sall1 Is Localized to Nuclear Foci through Its N Terminus—Because transcriptional regulators have been shown to localize in discrete subdomains within the nucleus (19), we next examined the cellular localization of the Sall1 protein. Immunofluorescence of cells overexpressing a full-length FLAG-tagged Sall1 protein demonstrated that Sall1 is found in the nucleus in discrete punctate foci (Fig. 5A, top panels). This pattern was evident in ~70% of transfected cells using a monoclonal antibody that recognized the C-terminal FLAG epitope and the anti-Sall1 polyclonal antisera directed against an N-terminal peptide (data not shown) indicating that this punctate localization was easily detectable by multiple reagents.

Similar punctate nuclear foci were also detected in cells expressing GAL4DB-Sall1-N (Fig. 5A, bottom panel) demonstrating that the N-terminal domain of Sall1 was sufficient for localization to these discrete nuclear subdomains. Notably, the presence of the canonical nuclear localization sequence in GAL4DB was also required because GST-Sall1-N was not localized to the nucleus (data not shown). Thus, while the N-terminal domain of Sall1 does not appear to contain a functional nuclear localization signal, it does contain a domain that targets Sall1 to discrete nuclear foci once Sall1 has entered the nucleus.

Sall1 Localization Correlates with Repression—Since Sall1-N was an active repressor, bound to HDAC complexes, and was sufficient to localize Sall1 to discrete nuclear foci, we next sought to determine whether the presence of Sall1 in nuclear foci correlated with its ability to repress. In contrast to the punctate staining observed for most cells expressing Sall1-N, N-terminal deletions that significantly reduced or abolished repression by Sall1 altered the subnuclear localization. Three different patterns of Sall1 nuclear staining were observed and are represented in Fig. 5B: punctate (top panels), reticular (middle panels), and diffuse (bottom panels). Deletion of the first 76 amino acids from the N terminus resulted in a 10-fold reduction in repression (Fig. 2) and reduced the number of cells exhibiting punctate nuclear staining (16%) compared with Sall1-N (66%, top left panel). The remainder of the Sall1-N-(77–435) expressing cells exhibited a reticular (32%, middle left panel) or diffuse (52%) nuclear staining pattern and were clearly distinct from the discrete punctate pattern. A larger N-terminal deletion that removed 129 amino acids and completely abrogated repression (Fig. 2) exhibited a predominantly diffuse nuclear pattern of staining (83%, bottom left panel). Thus, deletion of a region of the N-terminal of Sall1 that is required for transcriptional repression alters the nuclear localization of Sall1 from a predominantly punctate nuclear distribution to a diffuse or reticular pattern of nuclear staining.

To confirm these results in the context of the intact Sall1 protein and its native nuclear localization sequence, the same deletion constructs that were expressed as N-terminal Sall1 GAL4DB and GST fusion proteins for the repression and interaction studies were made as FLAG-tagged proteins containing the zinc finger and C-terminal domains. Similar to the results observed with the GAL4DB N-terminal fusion proteins, Sall1-(77–1322) exhibited a punctate pattern of nuclear staining in only 18% of cells compared with 67% for full-length Sall1 (Fig.
The remainder of the Sall1-(77–1322) expressing cells exhibited either diffuse nuclear staining (56%) or a reticular pattern of nuclear staining (26%, middle right panel). Sall1-(130–1322), the deletion analogous to the N-terminal construct that completely abrogated Sall1-mediated repression (Fig. 2), exhibited diffuse nuclear staining in the majority of expressing cells (79%, bottom right panel). Sall1-N-(204–435), a larger N-terminal deletion construct that also does not repress transcription (Fig. 2) displayed a predominantly diffuse nuclear staining pattern that was indistinguishable from Sall1-(130–1322) (data not shown). Together, these results strongly suggest that localization to discrete, punctate nuclear foci is necessary for full transcriptional repression by Sall1.

**DISCUSSION**

In this report we provide the first direct evidence that the multi-zinc finger protein mouse Sall1 is a potent repressor of transcription. This conclusion is consistent with the genetic evidence in *Drosophila* that *sal* inhibits expression of multiple genes in a cell autonomous manner (2, 3, 11). The demonstration that Sall1 is a strong transcriptional repressor does not exclude the possibility that it could also function as a transcriptional activator in some contexts. In patterning *Drosophila* wing veins, the two *sal* family members, *salm* and *salr*, repress genes when they are highly expressed, but also can activate gene transcription at lower expression levels (11). This study examined the ability of Sall1 to control transcription in an overexpression system and thereby highlighted a role for Sall1 in transcriptional repression. However, to date our analysis of Sall1 *in vitro* has not uncovered a domain that activates transcription.

The Sall1 N terminus alone is capable of strong transcriptional repression. This region contains a C 2HC zinc finger and domains rich in threonine, glutamine, and serine. The C 2HC zinc finger region, and not the threonine, glutamine, or serine-rich domains, is necessary for potent repression because truncation of the extreme N terminus reduces repression activity by 10-fold. The glutamine-rich domain has been postulated to mediate transcriptional activation (7), but this domain did not contribute to activation or repression in the experiments presented here. Within the region required for repression, all vertebrate Sal proteins contain a homologous C 2HC zinc finger and a 20-amino acid region at their extreme N termini. Further studies are underway to test whether these regions are sufficient to repress transcription or whether additional regions cooperate to achieve full transcriptional repression by the N terminus of mouse Sall1.

The *Drosophila* Sal family members do not encode a C 2HC zinc finger. S. Kiefer and M. Rauchman, unpublished observations.
zinc finger domain and do not share any apparent homology with vertebrate Sal proteins at their N termini. However, although not investigated in detail in this report, our data also demonstrate that Sall1 contains another repression domain in a region of the protein that includes the DNA-binding C2H2 zinc fingers and this domain appears to repress independently of HDAC (Figs. 1A and 4A). Since the C2H2 zinc fingers are highly conserved between fly and vertebrate Sal, one possibility is that Drosophila Sal mediates repression via this region of the protein. The N-terminal repression domain identified in this study may have been recruited later in evolution and may serve a unique function in vertebrate development. The presence of multiple independent repression domains in transcription factors, in some cases overlapping with the DNA-binding domain, has been demonstrated for other factors, such as Brinker, Hex1, and ZEB (20–22). In the case of Brinker, the two domains employ different co-repressor molecules to mediate transcriptional repression. These domains can function independently or cooperatively to repress dpp-responsive genes (22). Whether a similar paradigm applies to Sall1 remains to be established.

The role of the mouse Sall1 N terminus in transcriptional repression has a potential implication for patients with human SALL1 mutations. A hotspot for mutations that cause Townes-Brocks Syndrome birth defects occurs in the region before the first set of double zinc fingers (6, 23). Some of these mutations cause premature stop codons and, if a truncated protein were expressed from these mutated alleles, that protein would encode only the N-terminal domain. Because the region of mouse Sall1 required for repression (amino acids 1–130) are 99% identical to human SALL1, the experiments presented here suggest that this truncated protein would be an extremely powerful transcriptional repressor. The repression domain would be unthethered from the DNA-binding region of SALL1 but, according to our results, could still interact with repression complexes. This unregulated repressor could be a major factor in the phenotype of Townes-Brocks Syndrome possibly by conferring dominant negative activity as a nonspecific transcriptional regulator.

Because murine Sall1 can repress transcription close to and at a distance from the promoter and the HDAC-inhibitor TSA can alleviate some of the repression, HDAC complexes may mediate transcriptional inhibition by Sall1. This hypothesis is further substantiated by the fact that the same Sall1 constructs that repress, the full-length protein and the first 435 N-terminal amino acids can also interact with HDAC complex components. However, since Sall1 repression is only partially relieved by HDAC inhibitors, it is likely that deacetylase enzyme activity is augmented by other repression mechanisms to mediate strong inhibition of gene transcription in the Sall1 repressor complex. It also remains to be determined which, if any, of the HDAC complex components interact with Sall1 directly. The protein directly responsible for Sall1 association with HDAC complexes requires the first 76 amino acids of Sall1 for binding and could be HDAC itself. Regardless of the identity of the direct Sall1-binding protein(s), the strict association between strong repression and HDAC complex interaction suggests that a large multiprotein complex is responsible for Sall1 repression of target genes. Such a model is similar to that proposed for other transcription factors, such as the zinc finger protein Ikars (16). However, while Ikars-containing complexes display both ATP-dependent chromatin remodeling and histone deacetylation activities, we were unable to detect the NuRD-associated DNA-dependent ATPase Mi2 in a complex with Sall1. Thus, either Sall1 does not recruit chromatin remodeling ATPase activity or alternatively it recruits a chromatin-remodeling complex independent of NuRD, such as SWI/SNF. It is also possible that Sall1 assembles a unique HDAC-containing complex related to NuRD, similar to that identified for nuclear receptors in matrix-associated deacetylase bodies (24). A definitive characterization of the active Sall1 repression complex will require analysis using an endogenous target gene.

The N-terminal region of Sall1 that also contains the repression domain is required for cellular localization of Sall1 in discrete, punctate nuclear foci. Transcriptional modulators are often found in this type of localization pattern and have been described as components of nuclear subdomains termed splicing factor speckles, coiled bodies, PML oncopgenic domains (reviewed in Ref. 25), or matrix-associated deacetylase bodies (24). Localization of the transcriptional repressors Ikaros and the Kruppel-associated box-containing zinc finger proteins to heterochromatin is implicated as a mechanism of heritable gene silencing and these proteins also display a punctate nuclear staining pattern (26–30). Thus, considerable evidence indicates that discrete nuclear subdomains may be used to concentrate repressor complexes. Our data strongly suggest that localization to punctate nuclear foci is important for Sall1 to function as a potent repressor. While we have determined that Sall1 does not co-localize with SC-35 (data not shown), a protein that is found in a speckled nuclear subdomain enriched for splicing factors, it remains to be determined if Sall1 foci correspond to the subdomains described for repressor complexes such as PML or matrix-associated deacetylase bodies.

In summary, we provide the first evidence that mouse Sall1 is a direct transcriptional repressor. This repression is dependent, at least in part, on HDAC activity and likely involves a large multiprotein complex that includes factors associated with NuRD. The extreme N-terminal domain mediates repression and is necessary for Sall1 localization to nuclear foci that are likely essential for repression function. It will be important to identify in vivo target genes regulated by Sall1 to determine the biochemical mechanism of Sall1 repression.

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Note Added in Proof—During submission of this manuscript, human SALL1 was also shown to repress transcription and localize to pericentric heterochromatin foci by another group (Netzer, C., Rieger, L. B., Brevo, A., Zhang, C.-D., Hinze, M., Kohlhase, J., and Bohlander, S. L. (2001) Hum. Mol. Genet. 10, 3017–3024).
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