Differential Association of Products of Alternative Transcripts of the Candidate Tumor Suppressor *ING1* with the mSin3/HDAC1 Transcriptional Corepressor Complex*

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The candidate tumor suppressor *ING1* was identified in a genetic screen aimed at isolation of human genes whose expression is suppressed in cancer cells. It may function as a negative growth regulator in the p53 signal transduction pathway. However, its molecular mechanism is not clear. The *ING1* locus encodes alternative transcripts of *p47*<sup>ING1a</sup>, *p33*<sup>ING1b</sup>, and *p24*<sup>ING1c</sup>. Here we report differential association of protein products of *ING1* with the mSin3 transcriptional corepressor complex. *p33*<sup>ING1b</sup> associates with Sin3, SAP30, HDAC1, RbAp48, and other proteins, to form large protein complexes, whereas *p24*<sup>ING1c</sup> does not. The ING1 immune complexes are active in deacetylating core histones in vitro, and *p33*<sup>ING1b</sup> is functionally associated with HDAC1-mediated transcriptional repression in transfected cells. Our data provide basis for a *p33*<sup>ING1b</sup>-specific molecular mechanism for the function of the *ING1* locus.

Local acetylation and deacetylation of core histones play an important role in the control of eukaryotic gene expression (1,2). Hyperacetylation of histones increases local accessibility of chromatin templates, enabling subsequent activation or repression of transcription by gene-specific factors, while deacetylation is frequently linked with chromatin condensation and gene silencing. Most histone acetyltransferases and histone deacetylases (HDACs) are enzymes that do not bind DNA directly; instead, they are recruited to chromatin through association with distinct proteins in multiprotein complexes (3). One of the conserved proteins that serve as an organizer for the assembly of histone deacetylases with multiple polypeptides in yeast and mammalian cells is Sin3 (4–7). A biochemically purified mammalian Sin3 complex includes HDAC1 and HDAC2, RbAp48 and RbAp46, SAP30, and SAP18 (6–8). The abundance and relative stability of both Sin3 and HDAC1 proteins have led to the proposal that the “core” Sin3 repressor complexes are pre-assembled and available for recruitment by transient association with gene-specific transcription factors, including Mad, MeCP2, Ikaros, p53, PLZF, nuclear hormone receptors, and yeast Ume6 whose abundance and activities are regulated (3).

The *ING1* (inhibitor of growth 1) gene was recently identified as a candidate tumor suppressor in a genetic screen aimed at isolation of human genes whose expression is suppressed in cancer cells (9). The *ING1* gene was localized to chromosome 13q33–34 (10,11), a region that has been implicated in the progression of various tumors (12). Deregulated expression and mutations of *ING1* gene were found in breast carcinomas (11) and in squamous cell carcinomas (13), respectively. Ectopic expression of the originally isolated *ING1* cDNA or suppression of the *ING1* gene expression by antisense RNA demonstrated that *ING1* is a negative regulator of cell proliferation involved in the p53 growth regulatory pathway (9,14).

It has been subsequently found that the *ING1* gene encodes several differentially initiated and spliced mRNAs, which have common 3’ exon and encode at least two distinct proteins in mouse (15), and possibly three distinct proteins in human cells (*p47*<sup>ING1a</sup>, *p33*<sup>ING1b</sup>, and *p24*<sup>ING1c</sup>) (13,16,17). All the known or anticipated ING1 protein isoforms share an identical C-terminal domain with a conserved PHD finger motif. The PHD finger motif was thought to facilitate DNA binding of proteins otherwise unrelated to ING1 (18), suggesting that ING1 proteins might directly interact with DNA. Significantly, missense mutations were detected within the PHD finger and the nuclear localization motif of ING1 in some head and neck squamous cell carcinomas with allelic loss at the 13q33–34 region, suggesting that the PHD finger and the nuclear function of ING1 is important for its tumor suppressor function (13).

All functional analysis of the biological effects of ectopically expressed ING1 was so far done only with the cDNA encoding *p24*<sup>ING1c</sup> due to the lack of information on the alternative forms of ING1. Owing to a cloning error, the cDNA that suppressed mouse cell growth was incorrectly termed *p33*<sup>ING1b</sup> of ING1. Owing to a cloning error, the cDNA that suppressed mouse cell growth was incorrectly termed *p33*<sup>ING1b</sup> of ING1.
suggests that the shortest of ING1 protein isoforms, the mouse equivalent of human p24\(^{ING1c}\), is required for the activation of p53-responsive genes. In contrast, overexpression of the longer form p37\(^{ING1}\), an equivalent of the human p33\(^{ING1}\) protein, interferes with the activation of p53-dependent promoters when p53 is stabilized after DNA damage (15). It appears that isoforms of ING1 protein may have different roles in growth control and that their unique N-terminal sequences may determine differences in their function.

In a search for mechanisms of function of the ING1 protein, we explored ING1 associated proteins in human cells. We found differential association of p33\(^{ING1b}\) and p24\(^{ING1c}\) with nuclear proteins. p33\(^{ING1}\) resides in a complex of \(-1\)–2 MDa, whereas p24\(^{ING1c}\) does not. Among p33\(^{ING1}\)-associated proteins are known components of the mSin3 transcriptional corepressor complex, including HDAC1. Consistently, p33\(^{ING1}\) is functionally associated with HDAC-dependent transcriptional repression, in reporter gene expression assays in vitro, and in histone deacetylation assays in vitro. We demonstrate that the mSin3-mediated HDAC1-dependent transcriptional repression requires the unique N-terminal 99-amino acid sequence characteristic of the p33\(^{ING1}\) protein, therefore defining a new, p33\(^{ING1}\)-specific mechanism for the function of the ING1 locus.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—Rabbit anti-ING1 antibodies were generated using recombinant His-epitope-tagged human p33\(^{ING1}\) protein prepared from *Escherichia coli*. Goat anti-ING1 antibodies were from Santa Cruz (sc-7566). Mouse monoclonal anti-RbAp48 antibodies were from GeneTex (MS-RBP14-PX1), and rabbit anti-Sin3A antibodies were from Santa Cruz (sc-767). Rabbit anti-HDAC1 and anti-SAP30 antibodies were generous gifts of Dr. Glen Humphreys and Dr. Robert Eisenman, respectively. 

**Purification of the p33\(^{ING1}\) Complexes**—The FLAG epitope-tagged p33\(^{ING1}\) used for mammalian expression was constructed by subcloning the full-length cDNA with the tagged sequence into the pCIN4 expression vector and 106), were transfected by calcium phosphate precipitation on a 10-cm plate essentially as previously described with minor modifications. Five \(\mu\)g of pcDNA-Flag-ING1 expression plasmid with 100 \(\mu\)g of carrier DNA (pGEM-T-11) were used for transfection in each plate. Thirty hours after transfection, the cells were transferred to the same Dulbecco’s modified Eagle’s medium containing 1000 \(\mu\)g/ml G418 (Life Technologies, Inc.) for selection. After 2 months’ selection, single colonies were picked and expanded for Western blot analysis.

The tagged cells were grown in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum and 1000 \(\mu\)g/ml G418, and nuclear extracts were prepared as described previously. Forty milliliters of the nuclear extract prepared from different cell lines was adjusted to 200 mM NaCl and 0.2% Nonidet P-40 by addition of 5 mM NaCl and 10% Nonidet P-40, and incubated with 300 \(\mu\)l of M2-agarose beads (Sigma) at 4 °C for 2 h by rotation. After five washes with BC200 with 0.2% Nonidet P-40, proteins were eluted from beads by incubation at 4 °C for 30 min with 500 \(\mu\)l of BC100 with 0.2% Nonidet P-40 plus 0.2 mg/ml FLAG peptide.

Large scale immunoprecipitation for mass spectrometric analysis was carried out with 10 mg of crude or fractionated nuclear extracts and 100 \(\mu\)g of affinity-purified rabbit anti-ING1 antibodies, with an excess of the purified p33\(^{ING1c}\) antigen as a negative control. Immune complexes were isolated by binding to 100 \(\mu\)l of Sepharose-Protein A beads, washed five times with 100 volumes of NETN buffer, eluted, and separated by SDS-PAGE.

**Identification of Proteins with Mass Spectrometry**—Protein sequencing using mass spectrometry was carried out as described (20). Tryptic peptides that were recovered from in-gel digested protein bands were analyzed using an electrospray ion trap mass spectrometer (LCQ, Finnigan MAT, San Jose, CA) coupled on-line with a capillary high performance liquid chromatography (Magic 2002, Michrom BioResources, Auburn, CA). Data derived from the mass spectrometry/mass spectrometry spectra were used to search a compiled protein database that was composed of the protein data base NR and a six-reading frame translated expressed sequence tag data base to identify the protein using the program PROWIL, which is publicly available on the World Wide Web.

**Immunoprecipitation and Immunoblotting**—Immunoprecipitations were done by incubating 1 mg of HeLa nuclear protein extracts prepared according to the Dignam method, with 5–10 \(\mu\)g of the appropriate antibodies for 2 h at 4 °C, followed by isolation of the immune complexes on Protein A beads (Amersham Pharmacia Biotech.). Immune complexes were washed three times with 1 ml of NETN buffer (50 mM Tris, pH 7.5, 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 0.5 mM sodium orthothiophosphate) prior to SDS-PAGE and immunoblotting. Immunoblottings were done after low voltage protein transfer (30 V, 12 h) from polyacrylamide gels to nitrocellulose in Tris-glycine buffer, pH 8.0, with 5% methanol. Membranes were blocked with 5% milk in Tris-buffered saline with Tween 20 buffer (100 mM Tris, pH 8.0, 150 mM NaCl, 0.05% Tween 20), incubated with 1:200–1:1000 dilution of the primary antibody, washed, and incubated with 1:25000 dilution of horseradish peroxidase-conjugated secondary antibodies. Antibody detection was with ECL (Amersham Pharmacia Biotech.), using horseradish peroxidase-conjugated antibodies from Santa Cruz.

**Histone Deacetylase Assays**—Histone deacetylase activity was measured using acid-soluble histones that were isolated from \(^{[3]H}\)acetate labeled HeLa cells, by a published procedure (21, 22). Immune complexes were incubated for 3 h at 37 °C with 40,000 cpm of \(^{[3]H}\)-labeled histones (2000 cpm/\(\mu\)g) in a total volume of 200 \(\mu\)l of HD buffer (25 mM Tris, pH 7.5, 100 mM NaCl, 2.5% glycerol). Reactions were stopped by addition of 50 \(\mu\)l of STOP buffer (1 M HCl, 0.16 M acetic acid), extracted with two volumes of ethyl acetate, and the supernatant was counted in scintillation fluid. Assays carried out without 10 mM sodium butyrate, as indicated in the figure legends.

**Transcriptional Repression Assays—NIH3T3 cells** (1 \(\times\) 106) were transfected using LipofectAMINE Plus (Life Technologies, Inc.) with a combination of 1 \(\mu\)g of luciferase reporter plasmids 2Py-Luc or 6AP-Luc and 100 \(\mu\)g of the LacZ reporter construct (pCMV-LacZ) to normalize transfection efficiency. One hundred ng of ras expression vector (\(\lambda\)-Hras cdNA cloned in pLXSN vector under the control of the LTR promoter) and 50 ng of each of the test expression vectors FNE2DBD, pCINE2DBD:En, FN-INGE2DBD, and FN-sINGE2DBD (shown in Fig. 4A as 1, 2, 3, and 4, respectively) were cotransfected with the reporter plasmids. Cells were harvested for luciferase and \(\beta\)-galactosidase assays 40 h after transfection. The luciferase and \(\beta\)-galactosidase enzyme activities from the extracts of transfected NIH3T3 cells were measured according the Promega protocols.

Polymerase chain reaction-derived DNA fragments encoding either human p33\(^{ING1}\) or p24\(^{ING1}\) were fused individually to the N terminus of the GAL4 DNA-binding domain (amino acids 1–94). 293T cells (1 \(\times\) 106, in 2.5-cm plate), were transiently cotransfected using GenePORTER (Gene Therapy Systems) with 0.5 \(\mu\)g of appropriate pING1-GAL4 expression vector and 1.5 \(\mu\)g of reporter plasmid. The reporter plasmid carried secreted alkaline phosphatase (SEAP) gene under the control of the constitutively active SV40 early promoter with five Gal4 binding sites. Twenty-four hours after transfection, cells were incubated with 50 ng/ml trichostatin A (TSA), where indicated, and 12 h later cells were harvested and assayed. Expression of the GAL4 fusion proteins was determined by Western blotting with the anti-Gal4 antibody.

**RESULTS**

**p33\(^{ING1}\) Associates with Known Components of the mSin3 Corepressor Complex**—To acquire an insight to mechanisms of ING1 function, we sought to isolate ING1-associated proteins in human cells. We fractionated HeLa nuclear extracted on a DEAE column and immunoprecipitated the endogenous ING1 protein from the 0.2 M KCl fraction, which contains most of the cellular p33\(^{ING1}\) protein. We used mass spectrometry to identify RBP1, Sin3, and HDAC1 along others as ING1-associated proteins (Fig. 1A). To facilitate protein purification and to alleviate the interference of the antibody, we created an H1299-derived cell line, which stably expressed a FLAG-epitope-tagged p33\(^{ING1}\) (19). The FLAG-p33\(^{ING1}\) was overexpressed by \(-10\)-fold compared with the endogenous p33\(^{ING1}\) protein by Western. The recombinant FLAG-p33\(^{ING1}\) protein complex was isolated from nuclear extracts prepared from the stable line using affinity chromatography. Colloidal Coomassie Blue staining of a SDS-PAGE gel containing the p33\(^{ING1}\) complex revealed that \(-10\) polypeptides specifically copurify with the FLAG-p33\(^{ING1}\) on the affinity column (Fig. 1B). More bands that were masked by antibody are clearly detected in the
p33ING1 Associates with the mSin3 Complex

We identified proteins that copurified with p33ING1 in the recombinant p33ING1 complexes by using capillary liquid chromatography electrospray ion trap mass spectrometry. We also identified one novel protein, p42, and p35, which were not reported as components of the mSin3 complex. Rabbit anti-p33, rabbit anti-Sap30, and rabbit anti-HDAC1 antibodies were pre-incubated with an excess of the purified recombinant His-epitope tagged p33ING1 protein with p33ING1 complexes from HeLa extracts. Immunoprecipitations were done as above, except that the anti-p33ING1 antibodies were pre-incubated with an excess of the purified recombinant His-epitope tagged p33ING1 protein. Coelution of p33ING1 with mSin3 complexes in gel filtration analysis. Partially purified HeLa nuclear protein extracts (0.2 mg, 300 mM KCl elution from CM Sepharose) were separated by gel filtration on Superose 6 PC3.2/30 column, in a buffer with 50 mM Tris, pH 7.5, 200 mM KCl, and 0.5 mM dithiothreitol. Proteins from the gel filtration eluates were separated by 10% SDS-PAGE, visualized by staining gels with colloidal Coomassie Blue, and identified by capillary liquid chromatography

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We confirmed the association of Sin3 and HDAC1 with p33ING1 by reciprocal immunoblotting of the endogenous p33ING1, HDAC1, and Sin3 immune complexes from HeLa cells (Fig. 2A). Although the polyclonal rabbit antibodies that we used in these experiments reacted with both p33ING1 and p24ING1 proteins (Fig. 2A, lane 3), due to their identical C-terminal end, only p33ING1 was detected in the Sin3 and HDAC1 immune complexes (Fig. 2A, lanes 1 and 2). Pre-incubation of the anti-ING1 antibodies with an excess of purified recombinant His-tagged p33ING1 protein prevented precipitation of the endogenous p33ING1 and p24ING1 as well as Sin3A and HDAC1 (Fig. 2B), demonstrating specificity of the observed associations. Approximately the same amounts of Sin3A, the Sin3A directly associated protein SAP30 (7) and p33ING1 were present in either Sin3A or ING1 immune complexes of the endogenous proteins, suggesting that p33ING1 is a stoichiometric component of the Sin3A/SAP30 complex in vivo. Moreover, both p33ING1 and Sin3A immune complexes had similar amounts of histone deacetylase HDAC1 and the histone H4-binding protein RbAp48. Gel filtration analysis of partially purified p33ING1 complexes demonstrated that p33ING1,

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Sin3A, HDAC1, and RbAp48 proteins coelute in complexes of an apparent size of 1–2 MDa (Fig. 2C), as reported previously for the Sin3 complex (6). It is not clear whether the previously biochemically purified mSin3 complex is a stable subcomplex of this larger p33ING1b complex. It is possible that extensive column fraction may disrupt weaker associations to yield the stable core mSin3 complex.

To establish that p33ING1b associates with functional HDAC1, we prepared the endogenous ING1 immune complexes from HeLa cells, as presented on Fig. 2A, and assayed them for histone deacetylase activity. We found that ING1 complexes were active in deacetylating 3H-labeled histones in vitro, and this activity was comparable with the activity of HDAC1 immune complexes when assays were performed with similar HDAC1 amounts (Fig. 2D, bars 2 and 4). Addition of 10 mM sodium butyrate, an inhibitor of histone deacetylase activity (24), inhibited the reaction in both HDAC1 and ING1 immune complexes to the level of nonenzymatic 3H-acetyl release (Fig. 2D, bars 3 and 5, compare with bar 1). In contrast, the control immunoprecipitates with various amounts of rabbit preimmune serum alone did not catalyze histone deacetylation (Fig. 2D, bars 6 and 7). Therefore, p33ING1b associates with enzymatically active HDAC1 complexes, suggesting that p33ING1b may act with Sin3 to mediate transcriptional repression by a mechanism that involves targeted recruitment of histone-modifying activity.

The Association with Sin3 Complexes Is Specific to the p33ING1b Isoform and Is Defined by Its Unique N-terminal Sequence—Our analysis of endogenous proteins from HeLa cells suggests that, although we can detect and immunoprecipitate both p33ING1b and p24ING1c isoforms, only p33ING1b associates with Sin3/HDAC1 complexes (Fig. 2). p24ING1c is identical to p33ING1b except for lacking the N-terminal 99 amino acids that are characteristic of the p33ING1b isoform (16), suggesting that the N-terminal fragment of p33ING1b controls its assembly with the Sin3 complex. However, p24ING1c appears to be less abundant than p33ING1b in a variety of cell lines that we tested, and immunodetection of the endogenous p24ING1c in protein extracts was generally poorer or negative (Figs. 2A, lane 5 and 3A), unless the extracts were enriched in p24ING1c by partial purification (Fig. 2C).

To eliminate the possibility that lower abundance of the p24ING1c protein rather than its different protein structure is responsible for the observed differences in the assembly pattern, we analyzed p24ING1c assembly under the condition of its overexpression, using stably transfected HT1080 fibroblasts (14). Quantitative Western blot analysis of nuclear extracts prepared from the transfected and untransfected HT1080 cells demonstrates that the recombinant p24ING1c was at least as abundant as the endogenous p33ING1b (Fig. 3A, compare lanes 4–6 with lanes 1–3). However, overproduction did not force p24ING1c assembly into large protein complexes (Fig. 3B). Identical results were also obtained from stably transfected MCF7 cells. Therefore, the association with Sin3 complexes is specific to the p33ING1b isoform and is defined by its unique N-terminal sequence.

p33ING1b Is Functionally Associated with HDAC1-dependent Transcriptional Repression in Vivo—We used one reporter system to test whether p33ING1b is functionally associated with transcriptional repression in vivo. In this system, the DNA binding domain (DBD) of the transcription factor Ets2 is fused with the mouse p33ING1b and p24ING1c, which are human homologues of p33ING1b and p24ING1c, respectively. The structures of expression constructs are shown schematically in Fig. 4A. A previously described fusion protein containing Ets2 DBD and the repressor domain of the Engrailed protein of Drosophila melanogaster was used as a positive control (construct 2 in Fig. 4A) (25, 26). The reporter plasmid contains the luciferase gene under the control of the minimal promoter of the ras-responsive c-fos gene and oncogene regulatory elements as described previously (27) (Fig. 4B). The reporter (2Py-luc) contains a tandem repeat of a combination of Ets and Ap.1 binding sites from the enhancer of polyoma virus. The 6AP-Luc reporter plasmid containing six tandem AP-1 binding sites was used as a control. Both reporters are ras-responsive if cotransfected with ras-expressing plasmid into NIH 3T3 cells, but 6AP-Luc is insensitive to Ets. Expression plasmids were coexpressed in NIH3T3 cells with activated ras, serving as an activator of Ets-directed transcriptional activation of reporter constructs. Protein levels of the chimeric proteins were normalized using a LacZ gene reporter. The results of luciferase assays are shown in Fig. 4 (C and D). The long form of ING1 fused with Ets2 DBD works as a potent repressor similar to the positive control of the Engrailed repressor domain fused to Ets2 DBD (lanes 5 and 4). In contrast, the short form of ING1 exhibits a moderate repression (lane 6), which is similar to that of the Ets2 DBD alone (lane 3). This moderate repression may be due to competition with the endogenous Ets2 protein. Both ING1 fusion proteins and Ets2 DBD are similarly active in gel mobility shift assays with the oligonucleotide corresponding to the Ets2 DNA binding site (data not shown). The repressor effect is specific to Ets2 since none of the plasmids tested show any effect on the control reporter construct (6AP-luc) lacking Ets2-binding sequences (Fig. 4D).

We obtained similar results using another reporter system, in which the activity of SEAP was used as a reporter of transcription from a constitutively active SV40 promoter that was cloned next to five Gal4 binding sites. In this system, the chimeric GAL-p33ING1b fusion protein also mediates transcriptional repression (Fig. 4E, compare lane 3 and lane 1). The repression is specific to p33ING1b, because the GAL4-p24ING1c fusion protein has little effect on the SV40 promoter. Moreover, treatment of transfected cells with TSA, a specific inhibitor of histone deacetylases, restores the reporter activity, indicating that the transcriptional repression mediated by p33ING1b requires active HDAC. Western blot analysis confirmed that both GAL4-p33ING1b and GAL-p24ING1c fusion proteins were expressed in comparable amounts, regardless of the presence or absence of TSA (Fig. 4E). Therefore, tethering p33ING1b to an artificial promoter in vivo can confer HDAC-dependent transcriptional repression in reporter gene expression systems.
Our finding of differential association of the products of the alternative transcripts of p33\(^{ING1b}\) and p24\(^{ING1c}\) with the mSin3 transcriptional corepressor complex provides a basis for a molecular mechanism of ING1 function as a growth regulator and a candidate tumor suppressor. It also introduces novel aspects into the understanding of the Sin3/HDAC1-mediated transcriptional repression, by identifying new components that might serve as a link to regulation of growth and cell division.

Our data suggest that p33\(^{ING1b}\) is the predominant isoform among ING1 proteins that is associated with the Sin3/HDAC1-mediated transcriptional repression. This is based on the observation that the N-terminal 99 amino acids, which are unique to p33\(^{ING1b}\), are required for: 1) the assembly with Sin3/HDAC1 complexes, and 2) the HDAC1-dependent transcriptional corepressor activity in reporter gene assays. p24\(^{ING1c}\), which is otherwise identical to p33\(^{ING1b}\) except for missing the N-terminal domain, does not seem to interact with the Sin3/HDAC1 complexes even when overproduced. The p47\(^{ING1a}\) isoform has a unique N-terminal fragment that is distinct from that of p33\(^{ING1b}\). Although in this study we did not rigorously examine the assembly of p47\(^{ING1a}\), the putative endogenous p47\(^{ING1a}\) protein that we can detect with the affinity purified polyclonal anti-ING1 antibodies does not coelute with the Sin3/HDAC1 complexes in gel filtration experiments. Moreover, p47\(^{ING1a}\) cannot be immunoprecipitated by Sin3 and HDAC1 (data not shown). This suggests that p47\(^{ING1a}\) may associate with different protein partners, but the nature and roles of the p47\(^{ING1a}\) assembly remain to be determined.

The cooperation of ING1 with p53 was the first mechanism that was proposed to account for the growth suppressor function of ING1 (14). Recent analysis of the ING1 isoforms in mouse suggests that the equivalent of the human p24\(^{ING1c}\) homologue is required for the activation of p53-dependent promoters. In contrast, the mouse equivalent of the human p33\(^{ING1b}\) isoform interferes with the activation of the p53-dependent responses (15). This result is consistent with our finding that p33\(^{ING1b}\) functions in transcriptional repression, not activation. Moreover, Sin3-mediated HDAC1 activity was recently indicated in the repression of p53-responsive genes (15).

It will be important to test whether p33\(^{ING1b}\) plays a role in the negative regulation of the p53-responsive genes in cooperation with Sin3/HDAC1. A model for the function of the ING1 locus can be envisaged from our data and previous studies that the interplay of the ING1 isoforms in collaboration with p53 sets the transcriptional program that determines cell proliferation or arrest. We propose that p33\(^{ING1b}\) together with the mSin3 corepressor machinery represses p53-responsive genes that halt cell cycle progression and that p24\(^{ING1c}\) serves as an antagonist to relieve this repression. The relative ratio of p33\(^{ING1b}\) and p24\(^{ING1c}\) thus may determine the proliferative potential of the cell.

Work from a number of laboratories demonstrated that Sin3 serves as scaffold protein for the assembly of multiprotein complexes, which target histone deacetylase activities to selected genes by interacting with specific transcription factors. These complexes facilitate transcriptional repression through a mechanism of induction of local rearrangements of the chromatin structure (3). In contrast to Sin3 and HDAC1, which are relatively stable proteins and do not seem to be cell cycle-dependent protein.
regulated, ING1 is cell cycle-regulated. p24ING1 accumulates in cells that are quiescence or senescence and overexpression of p24ING1 in primary fibroblasts arrests cells in G1 phase of the cell cycle (28). p33ING1 also accumulates in quiescence, but induction of cell division by addition of mitogens leads to rapid decline of the p33ING1 protein. In light of our data presented in this paper, these observations suggest that p33ING1 may serve as a regulatory subunit of the mSin3 complex and together with mSin3 might be involved in repression of some essential cell cycle regulatory genes. The identities of those genes that among possible candidates are genes that are regulated by the Rb/E2F pathway through an interaction of RBP1 with Rb. This intriguing possibility agrees with the observation that HDAC1 interacts with the Rb protein and that the HDAC1 activity is required for full transcriptional repression of some of the Rb-regulated genes (29,30). Therefore, our data presented here may also link Sin3/HDAC1 to cell cycle regulation through the association with p33ING1.

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