Molecular Biology of the Cell
Vol. 8, 2421–2436, December 1997

SET1, A Yeast Member of the *Trithorax* Family, Functions in Transcriptional Silencing and Diverse Cellular Processes

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Submitted January 28, 1997; Accepted September 3, 1997
Monitoring Editor: David Botstein

The *trithorax* gene family contains members implicated in the control of transcription, development, chromosome structure, and human leukemia. A feature shared by some family members, and by other proteins that function in chromatin-mediated transcriptional regulation, is the presence of a 130- to 140-amino acid motif dubbed the SET or Tromo domain. Here we present analysis of *SET1*, a yeast member of the *trithorax* gene family that was identified by sequence inspection to encode a 1080-amino acid protein with a C-terminal SET domain. In addition to its SET domain, which is 40–50% identical to those previously characterized, *SET1* also shares dispersed but significant similarity to *Drosophila* and human *trithorax* homologues. To understand *SET1* function(s), we created a null mutant. Mutant strains, although viable, are defective in transcriptional silencing of the silent mating-type loci and telomeres. The telomeric silencing defect is rescued not only by full-length episomal *SET1* but also by the conserved SET domain of *SET1. set1* mutant strains display other phenotypes including morphological abnormalities, stationary phase defects, and growth and sporulation defects. Candidate genes that may interact with *SET1* include those with functions in transcription, growth, and cell cycle control. These data suggest that yeast *SET1*, like its SET domain counterparts in other organisms, functions in diverse biological processes including transcription and chromatin structure.

INTRODUCTION

Transcription is regulated not only by RNA polymerases and specific gene activators, but also by elements that modulate chromatin structure to establish and maintain distinct transcriptional states. For example, the SWI/SNF proteins function in a large, multisubunit complex that is required for transcriptional enhancement by gene-specific activator proteins (Winston and Carlson, 1992; Peterson and Tamkun, 1995). SWI/SNF homologues regulate such diverse transcriptional activators as GAL4 in yeast (Côté *et al.*, 1994), mammalian steroid receptors (Yoshinaga *et al.*, 1992), and *Drosophila ftz* (Peterson and Herskowitz, 1992). The SWI/SNF complex is widely conserved, as are other distinct macromolecular complexes responsible for remodeling chromatin (Carlson and Laurent, 1994; Cairns *et al.*, 1996). The idea that many different chromatin regulators may be broadly conserved is underscored by the discovery of the SET domain genes, an emerging, well-conserved gene family encoding proteins with chromatin-based transcriptional activities.

The SET (Tschiersh *et al.*, 1994) or Tromo (Stassen *et al.*, 1995) domain is a 130- to 140-amino acid motif that was first recognized as a common element encoded in a number of *Drosophila* genes, including *trithorax (trx), Enhancer of zeste (E(z))* and *Su(var)3–9* (Jones and Gelbart, 1993; Tschiersh *et al.*, 1994). Additional SET domain-containing proteins have since been uncovered in organisms ranging from fungi to plants and mammals (Stassen *et al.*, 1995; Laible *et al.*, 1997; Tripoulas *et al.*, 1996; Goodrich *et al.*, 1997). In the SET domain family as a whole, sequence similarity is usu-

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Table 1. Yeast strains and plasmids

Strain number	Genotype	Origin
a227 (LPY24)	*MAT*a *lys1-1*	I. Herskowitz
RS927 (LPY253)	W303-1a, except *hml::TRP1*	R. Sternglanz
RS928 (LPY254)	RS927, except *MATα*	R. Sternglanz
UCC1001 (LPY917)	*MAT*a *ade2-101 his3Δ-200 leu2Δ1 trp1Δ1 lys2-801 TELadh4::URA3*	D. Gottschling
W303-1a (LPY5)	*MAT*a *ade2-1 can 1-100 his3-11,15 leu2,3,112 trp1-1 ura3-1*	R. Rothstein
LPY1297	UCC1001 *set1::HIS3*	This study
LPY1621	W303-1a *set1::LEU2* transformed with pLP399	This study
LPY2159	UCC1001, except *sir3::HIS3*	This study
LPY2456	*MATα ade2-101 his3Δ-200 leu2Δ1 trp1Δ1 lys2-801 set1::HIS3 TELadh4::URA3 DIA5-1 ADE2@VR* transformed with pRS315	This study
LPY2457	LPY2456, transformed with pLP344	This study
LPY2458	LPY2456, transformed with pLP346	This study
LPY2460	LPY2456, transformed with pLP354	This study
LPY2461	UCC1001, transformed with pLP559	This study
LPY2462	LPY2456, transformed with pLP559	This study
LPY2463	UCC1001, transformed with pLP354	This study
LPY2546	as RS928, except *set1::HIS3*	This study

ally confined to the conserved SET domain (ranging between 40–50% amino acid identity; Figure 1), although some family members, such as *Drosophila trx* and the human gene *ALL-1/HRX/MLL*, which is associated with human acute leukemias, may be highly homologous throughout their coding regions (Stassen *et al*., 1995; Laible *et al*., 1997). Genetic analyses in *Drosophila* have revealed that SET proteins can have antagonistic functions. For example, both *E(z)* and *trx* possess SET domains, yet *E(z)* is a homeotic gene repressor, whereas *trx*-group genes function as homeotic gene activators. Because the functions of other SET proteins remain poorly understood, the role of the SET domain in chromatin-mediated transcriptional regulation is not yet clear.

We identified *SET1* as the *Saccharomyces cerevisiae* gene encoding the yeast protein most closely related to SET domain proteins of multicellular organisms. To understand functionally conserved elements of chromatin-mediated gene regulation, we analyzed *SET1* and its mutant phenotypes. The *SET1* gene is not essential for viability, but when mutated reveals a role in many aspects of growth and developmental regulation. In particular, *set1*Δ mutants show transcriptional derepression of normally silenced loci, have competitive growth disadvantages, are sporulation defective, and lose viability in Go. To uncover those genes affected by loss of *SET1* function, we performed a screen to identify *SET1* transcriptional targets. The targets identified substantiate the roles for *SET1* suggested by our phenotypic analyses. These studies, in concert with recent data demonstrating the broad functional conservation of SET proteins (Laible *et al*., 1997), point to roles for SET proteins in many aspects of cell growth and development.

MATERIALS AND METHODS

Reagents

5-fluoorooritic acid (5-FOA) was from Toronto Biochemicals (Toronto, Ontario, Canada). Anti-glutathione-*S*-transferase (GST) antibody was from Pharmacia LKB (Uppsala, Sweden), and secondary antibodies were from Promega (Madison, WI). Unless indicated, other reagents were from Sigma Chemical (St. Louis, MO) or Difco (Detroit, MI).

Yeast Strains, Media, and Culture Conditions

Genotypes of strains are presented in Table 1. Standard genetic methods were used for yeast grown at 30°C in standard rich or selective media with a variety of carbon sources (Rose *et al*., 1989). Yeast transformations were performed using a lithium acetate protocol (Gietz and Woods, 1994).

Telomeric Silencing Assays

Assays to evaluate expression of telomere-proximal reporter genes were performed essentially as described (Gottschling *et al*., 1990). Cultures grown to saturation were transferred to 0.5-ml 96-well dilution plates, serially diluted fivefold and transferred to test plates using a pin replicator. The accuracy of this technique was determined to be ±5% by plating twofold dilutions of cultures at identical optical densities on YPD and counting colonies formed after 2 d.

Primers for Polymerase Chain Reaction (PCR) and Mutagenesis

Oligonucleotide primers (5′–3′) used in these studies were as follows:

HR1 (pLP244 forward library primer): CTAATCGCATTATCATCCTA

HR2 (pLP244 reverse library primer): ATAGGCGTATCACGAGGCCC

SKOP (SET1 5′ deletion primer): CCTTATTTGAATCTTTATAAGAGGTCTCTGCGTTTAGACTCTTGGCCTCCTCTAG

SKOT (SET1 3′ deletion primer): ATCAGGAAGCTCCAAACAAATCAA TGTATCGCTAGTTCTCGTTCAGAATGACACG

SET1CHK (to confirm deletion): CTGGACACTTGCGATTTCTAGC

HISCHK (to confirm deletion): TACATATTAAGTAATACACT

SET15′ (to clone SET1 into pTRP): GCCTCGAGATGTCAAATTACTATAGAAGA

SET1domain5′ (to clone SET domain into pTRP): GCCTCGAGATGGATTTGCAGAATGCTATC

SET 13′ (to clone SET1 into pTRP): GCGAGCTCTCAAGAAACCTTTACAATTAC

XBA1 (upstream primer to create G950S substitution fragment): AAGTTTCATCCTCTAGA

XBA2 (Mutagenic primer for G950S substitution fragment): TTTCCTTTG CTGCGATAGAGTGAGATCTCCTACTTTGAA

PCR-mediated Deletion of SET1

A null mutation of *SET1* was created using a PCR product as described (Baudin *et al.*, 1993). In this strategy, the entire open reading frame (ORF) is replaced by a selectable marker so that none of the gene-of-interest's coding sequences remain. The upstream primer (SKOP) contains 33 base pairs (bp) complementary to the 5′ upstream sequence of the *SET1* AUG. The 17 3′ bp of this primer are complementary to *HIS3*. Similarly, the downstream primer (SKOT) contained 33 bp of sequence directly following the *SET1* stop codon, and the 17 bp at the 3′ end of this primer were complementary to the 3′ end of *HIS3*. Ten micrograms of the PCR product were used to transform UCC1001 (LPY917). For each transformation, gene replacement of the *SET1* locus was confirmed in multiple HIS^+ isolates by genomic blot and PCR.

Plasmid Construction

Plasmid constructs were assembled using standard techniques (Sambrook *et al.*, 1989). Details of individual plasmid constructions are presented below. The *SET1* locus was cloned from phage lysates of ATCC Lambda clone PM-2226 (reference identification number 70357) as an *Msc* I–*Nru* I fragment into the *Sma*I site of pLP271 (Bonneaud *et al.*, 1991) to generate pLP237. A *Sal*I--*Sac*I fragment of *SET1*-GST (pLP 399) was subcloned into the bacterial expression vector pRSET-B (Invitrogen, San Diego, CA) (pLP147) to generate pLP563. *SET1-GST* (pLP399) was constructed by cloning a 3.5 kilobase (kb) *Sal*I–*Sac*I fragment of *SET1* in-frame with the GST portion of pEG(KT) (Mitchell *et al.*, 1993). *SET1*-135 (pLP562) was constructed by subcloning a 3.8-kb *Kpn*I–*Hin*dIII genomic fragment from pLP237 containing *SET1* into Yep351 (Hill *et al.*, 1986) digested with *Kpn*I and *Hin*dIII. *SET1*-CEN (pLP343) was constructed using the same *Kpn*I–*Hin*dIII fragment cloned into pRS315 (Sikorski and Hieter, 1989). pTRP-*SET1* pLP560 was constructed by PCR amplification of the entire coding sequence of *SET1* using primers containing a 5′ *Xho*I site (SET15′) and a 3′ primer containing a *Sac*I site (SET13′). The PCR product was digested with both enzymes and cloned into pTRP (Ramer *et al.*, 1992) digested with both *Xho*I and *Sac*I. pTRP-*SET1* (pLP559) was constructed using a similar PCR strategy as described for pTRP-*SET1*, except the 5′ primer (SET domain 5′) lay 450 bp upstream of the *SET1* transcription stop. The primer also contained a *Xho*I site and an AUG codon. The SET domain was amplified using this 5′ primer and the 3′ primer used for pTRP-*SET1*, digested with both enzymes, and subcloned into pTRP (pLP354).

SET domain mutants were prepared as follows: A deletion of the SET domain (pLP344) was constructed by digestion of pLP343 with *Xba*I and religation. This deletion results in a frame-shift mutation followed by a premature termination, which leads to synthesis of a mutant set1p missing the entire SET domain and 23 upsteam amino acids. We refer to this construct as the SET domain deletion. A point mutant of glycine^{951} to serine was constructed by using an upstream primer (XBA1) that spanned 5′ *Xba*I site and a second mutagenic primer (XBA2) that spanned the 3′ *Xba*I site followed by PCR. The resulting amplified product was digested with *Xba*I and ligated into pLP343 that had been digested with *Xba*I. Plasmids containing the insertion were sequenced across the *Xba*I–*Xba*I interval to confirm they carried only the intended mutation.

Expression of subcloned genes was evaluated by immunoblots of cell extracts prepared from yeast transformants probed with appropriate antisera.

Genomic DNA and RNA Blot Analysis

Yeast DNA and RNA were prepared from logarithmically growing cells (Rose *et al.*, 1989). For genomic Southern blots, digested genomic DNA was resolved on 0.8% tris-acetate-EDTA (TAE) agarose gels, soaked in 0.25 M HCl, 1.5 M NaCl/0.5 M NaOH, and 1 M ammonium acetate/0.05 M NaOH. Transfer to nitrocellulose was performed without additional buffer. Membranes were baked for 1 h at 80°C, hybridized, and washed. RNA gels were run using the 3-*N*-(morpholino)propanesulfonic acid-formaldehyde protocol with constant buffer recirculation (Sambrook *et al.*, 1989) except that the formaldehyde concentration was reduced 10-fold to 0.22 M. Radiolabeled probes were prepared by random priming with ^{32}P-dCTP or by "hot" PCR (Taylor, 1991). The telomeric $C_{1-3}A$ probe (derived from pYLPV, a gift of V. Zakian) was used to probe *Xho*I-digested genomic DNA. Probes derived from the DNA-target site screen were prepared by PCR amplification using primers that flank the 5′ (HR1) and 3′ (HR2) sides of the cloning site of pLP244. RNA levels were quantitated using the public domain NIH Image v.1.6 program (developed at the U.S. National Institutes of Health and available at http://rsb.info.gov/NIH-Image/). Briefly, the 'analyze' function was used to measure the total density of a fixed area that contained the band of interest from an autoradiogram. Background density values from an identically sized area were subtracted from experimental values that were normalized against an *ACT1* signal from the same lane.

Cytological Techniques

Logarithmically growing cells (UCC1001 and LPY1297) were prepared for flow cytometry as described (Weiss and Winey, 1996). The same samples were used for budding index determination. 4,6-Diamidino-2-phenylindole (DAPI) staining of UCC1001 and LPY1297 was performed on both log phase and saturated cultures by fixing cells in 30% methanol:70% acetone on dry ice for at least 10 min, washing once in water, incubating in DAPI (0.05 mg/ml; Boehringer Mannheim, Indianapolis, IN), followed by three to four washes in water.

Electron microscopy was performed using a high-pressure freezing/freeze substitution procedure (Ding *et al.*, 1993). Strains LPY917 and LPY1297 were grown to an OD_{600} of 0.5, concentrated by vacuum filtration, frozen by high pressure freezing, and freeze-substituted in acetone containing 2% OsO_4 and 0.05% uranyl acetate for 4 d with a stepwise increase in temperature from [minus190°C to 20°C before embedding in Epon-Araldite. Chemical fixation of cells involved sequential treatment with 1% potassium permanganate and 1.5% uranyl acetate followed by dehydration in acetone and embedding in Epon-Araldite. Sections were poststained with 1.0% lead citrate and 1.5% uranyl acetate (Glauert, 1975). Thin (60 nm) sections were cut and viewed on a CM10 electron microscope (Philips Electronic Instruments, Mahwah, NJ).

Coculture Analysis

Isogenic strains that differed only in whether they were *SET1*, *set1Δ::HIS3 or sri3Δ::HIS3*, were grown to mid-log phase and mixtures of (UCC1001 and LPY1297) or (UCC1001 and LPY2159) were prepared. Inoculum size was determined by hemocytometer and spectrophotometric quantitation. Cultures were incubated for up to 14 d at 30°C. Aliquots were removed at intervals beginning at 0 h and plated on YPD plates. After 2 days, plates were replicated to

his$^-$ plates to determine the proportion of His$^+$ cells present in the culture.

Antigen Production

The *E. coli* expression host *BL21* (Studier *et al*., 1990) was transformed with the *SET1* expression construct pLP563. Five-milliliter overnight cultures were grown at 37°C, then diluted 1:100 to inoculate 1-l cultures containing 60 μg/ml carbenicillin. Expression was induced when the cultures reached an OD_{595} of 0.4 by addition of isopropyl-1-thio-β-D-galactoside (IPTG) to a final concentration of 0.1 mM. Induction was continued for 2 h at 37°C at which point the cells were harvested and inclusion bodies prepared according to Lin and Cheng (1991). Inclusion bodies were resolved on 6% SDS-PAGE, transferred to nitrocellulose, and stained briefly with Ponceau S. The rSet1p band was excised, rinsed in water, dried, and then dissolved in dimethylsulfoxide. This material was mixed with Freund's adjuvant (complete 1×, incomplete 5×) with 50 μg protein used for each of six rat immunizations. Protocols for immunization and serum collection were as described (Harlow and Lane, 1988).

Preparation and Analysis of Yeast Protein Extracts

Yeast protein extracts were prepared either using a glass bead disruption procedure (Rose *et al*., 1989) in 1/2× phosphate-buffered saline with protease inhibitors (Soybean trypsin inhibitor, phenylmethylsulfonyl fluoride, L-1-tosylamide-2-phenylethylchloromethyl, pepstatin A, Pefabloc (Boehringer-Mannheim), aprotinin, and leupeptin at 10 μg/ml), or by two passages through a French Pressure Cell Press (American Instrument Co., Silver Spring, MD) at 900 pounds per square inch at 4°C. In each case, before disruption, cells were washed in ice-cold purified water and then resuspended in 1/20 of their original volume in 1/2 × phosphate-buffered saline + protease inhibitors. Protein samples were resuspended in an equal volume of 5× sample buffer (Laemmli, 1970); 62.5 mM Tris, pH 6.8, 2.0% SDS, 10% glycerol, and 5% 2-mercaptoethanol), separated by electrophoresis through a 10–15% SDS-polyacrylamide gradient gel, and electroblotted. Transfer was performed in Towbin buffer containing 20% methanol (Harlow and Lane, 1988) onto either 0.2- μm nitrocellulose (Schleicher & Schuell, Keene, NH) or 0.45-μm Immobilon membranes (Millipore, Bedford, MA). Prior to antibody incubation, blots were blocked in Tris-buffered saline with 0.05% Tween 20 and 5% nonfat dry milk (Harlow and Lane, 1988). Primary antibodies were used at a dilution of 1:5000; alkaline phosphatase-conjugated secondary antibodies were used at 1:10,000. Antibody incubations were performed for 1–2 h at room temperature in Tris-buffered saline with 0.5% Tween 20 and developed using nitro blue tetrazolium (NZT) and 5-bromo-6-chloro-3-indolyl phosphate (BCIP) as substrates.

DNA Target Library Construction and Screen

A yeast DNA-binding site library was prepared in the vector pBM2389 (gift of M. Johnston), that contains a *Bam*HI cloning site directly upstream of a promoter-defective *HIS3* gene (Liu *et al*., 1993). Genomic DNA was prepared from strain W303–1a (LPY5) using a double-CsCl banding procedure (Wach *et al*., 1994). Purified DNA was then subjected to partial *Sau* 3A digestion. Optimal digestion conditions to recover fragments in the 100- to 1000-bp range were determined empirically, size selected by electrophoresis through 2% TAE agarose gels, electroeluted, and concentrated with a Microcon 100 centrifugal filter (Amicon, Danvers, MA). Fragments were ligated into the *Bam*HI site of pBM2389 and transformed into electrocompetent *E. coli* (DH5α). Random PCR sampling of 40 plasmids demonstrated that the library contains 60% recombinants with an average insert size of 500 bp. Library DNA was prepared and used to transform LPY1621 that contained *SET1*-GST (pLP399) as the sole source of *SET1*. Ura$^+$/Trp$^+$ transformants were replica plated to ura-, trp-, his- plates to identify *HIS+* colonies. To identify those His$^+$ colonies that were *SET1*-dependent, these plates were replicated to 1) ura-, trp-, and his-galactose; 2) ura-, trp-, and his-glucose; and 3) 5-FOA/trp- and his-galactose medium. Only those colonies that grew on the first selection were analyzed further. These *SET1*-dependent strains were cured of the *SET1*-containing plasmid by growth on 5-FOA. Once cured, the *TRP1* library plasmids were recovered from yeast (Rose *et al*., 1989) with the following modifications: after extraction by vortexing cells with phenol/chloroform and glass beads, the aqueous phase was bound to silica, washed, and the DNA eluted in water. Recovered plasmid DNA was used to retransform LPY1621 followed by the same regimen of screening as described above. Plasmids that passed the second round of screening were subjected to single-pass DNA sequencing. Sequence information was compared with data available in GenBank and the *Saccharomyces* Genome Database, SGD (Cherry *et al*., 1996).

Prenyltransferase Assays

Prenyltransferase assays (Gomez *et al*., 1993) were performed as follows: 80 μl reaction mixtures containing 40 μg of protein extracts in 50 mM Tris-HCl pH 8, 10 mM $MgCl_2$, 5 mM dithiothreitol, 5 μM $ZnCl_2$ and 0.2 μm ^{3}H-farnesyl pyrophosphate (DuPont-NEN, specific activity 1.5 GBq/.050 mCi) were incubated at 37°C. Time points were taken at 0, 3, 6 and 15 min by spotting 20 μl of reaction mixture onto filter paper and precipitating incorporated counts with 10% trichloroacetic acid. Control samples were prepared as above with the addition of EDTA to a final concentration of 5 mM. Protein concentrations were determined by the Bradford dye-binding assay (Bio-Rad, Richmond, CA) using bovine serum albumin as a standard. Samples were normalized to a concentration of 20 mg/ml.

RESULTS

SET1 Is a Yeast Member of the Trithorax Gene Family

Inspection of an ORF on yeast chromosome VIII revealed significant similarity to *Drosophila trx* and its human homolog *ALL-1/HRX/MLL*. The overall BLASTP (Altschul *et al*., 1990) values were at least 10^{-28}, in numerous short stretches throughout all three genes. The most significant similarity was in a carboxy-terminal region that is 40–50% identical to a domain diagnostic for all members of the *trx* gene family (Stassen *et al*., 1995). We named the yeast gene *SET1* in recognition of the conserved region, dubbed the SET or tromo domain (Tschiersh *et al*., 1994; Stassen *et al*., 1995) that is found in diverse proteins, including transcriptional repressors and activators as well as proteins that possess both repressing and activating functions (Figure 1). Although *SET1* is the yeast gene with greatest similarity to other members of the SET family, there are five other *S. cerevisiae SET* genes, one of which is more similar to the *Enhancer of Zeste* gene family and two of which contain PHD fingers (Aasland *et al*., 1995; T. Hesman and M. Johnston, R. Aasland and A.F. Stewart, personal communication).

To assess the phenotypes of yeast cells lacking *SET1* function, we constructed a complete deletion of the *SET1* gene, replacing the entire chromosomal ORF with *HIS3* (Baudin *et al*., 1993). The resulting *set1*Δ

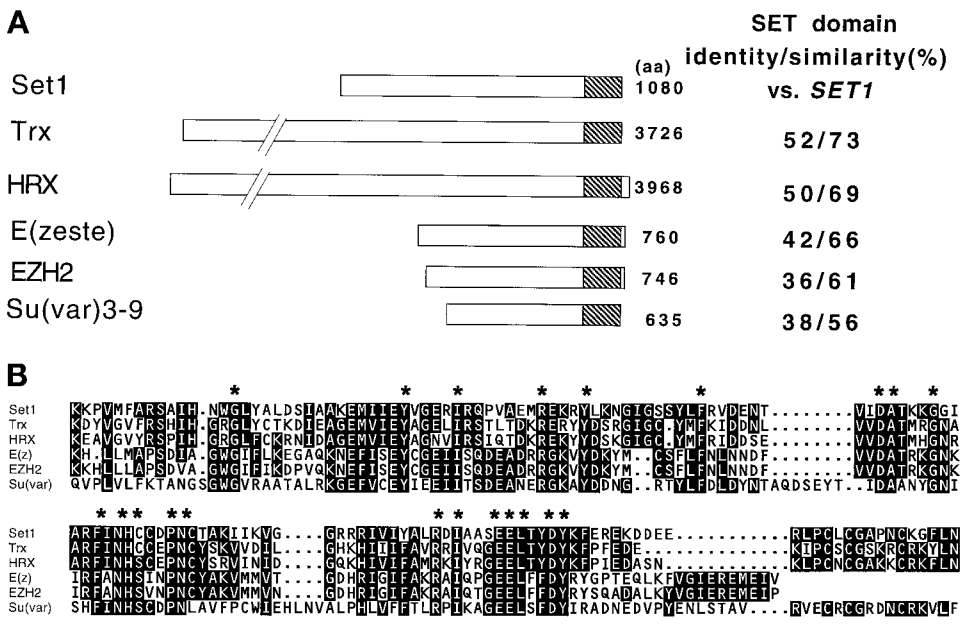

Figure 1. *SET1* is a yeast member of the *trithorax* gene family. (A) The *trithorax* gene family contains more than 30 members ranging from plants to humans (Stassen *et al.*, 1995). Each member contains a region of ~130–140 conserved residues (shaded) termed the SET domain (Tschiersh *et al.*, 1994). Outside this domain the proteins share little extensive sequence similarity and vary widely in size, from 55–450 kDa. Six SET domain proteins [yeast Set1, *Drosophila* trx, E(zeste) and Su(var)3–9, and human ALL-1/HRX/MLL and EZH2], including members outside the *trx* family are depicted here. HRX has been selected to emphasize that ALL-1/HRX/MLL is the human homologue of Trithorax. Number of amino acid residues is indicated at right. The percent amino acid identity and similarity of the *Drosophila* and human SET domain proteins are compared with Set1p. (B) An alignment of the SET domains (shaded inpanel A) of the proteins cartooned above. To emphasize the strong homology between these domains, only those residues that are identical in at least half of the domains shown are highlighted. Residues that are conserved among all six proteins are starred (*). Position of the G951S substitution allele (see Figure 6B and text) originally identified as the *trx*^{Z11} mutation (Mortin *et al.*, 1992; Stassen *et al.*, 1995) is the first starred G. This alignment, for Set1p beginning at amino acid 937, was generated using the GCG Pileup program with standard options and is very similar to that of Stassen *et al.*, (1995), except that the human EZH2 sequence (Laible *et al.*, 1997) has also been added.

mutants were viable, yet displayed several phenotypes, each of which suggested possible roles for the wild-type *SET1* gene product.

set1 Mutants Have Morphological, Developmental, and Growth Defects

We compared *set1* mutants to isogenic wild-type strains by phase contrast microscopy, DAPI staining, and electron microscopy. The *set1* mutant cells were distinguishable from wild-type cells with each method. Cultures of *set1* mutant cells contained a high proportion of oddly shaped cells, frequently containing several buds and large protrusions (Figure 2A). The size of *set1* cells is also more variable than wild-type cells. DAPI staining of *set1* cells revealed more diffuse nuclear staining and increased cytoplasmic staining as well as multiple buds with no discernible DAPI-stained chromatin (Figure 2A). Ultrastructurally, *set1* cells differed from wild type in that the outer mannoprotein layer of the cell wall was thinner than that of isogenic wild-type strains (Figure 2B). Perhaps consistent with these cell wall differences, *set1*Δ strains flocculate severely when grown in liquid medium.

set1 mutant colonies are initially smaller than those formed by wild-type cells at all temperatures tested (Figure 3A). They do, however, reach a comparable size after 5 d. We asked whether the smaller colonies seen in *set1* mutants were due to smaller individual size or different cell cycle properties of the mutants. To test these ideas, logarithmically growing *set1* and *SET1* cultures were analyzed by flow cytometry to measure DNA content (with propidium iodide) and cell volume (as reflected by light scattering). Although the cell sizes of both strains were comparable, we observed a greater proportion of cells with G_2 DNA content in the *set1* mutant cultures (Figure 3B). This modest G_2 bias was corroborated by budding indices of these cultures which revealed a 15–20% increase in large budded and multi-budded cells in the *set1* cultures (Figure 3C). When we compared the growth of *set1* mutant and *SET1* controls in liquid culture, the doubling times were very similar. The *set1* mutants did, however, take longer in exiting lag phase. Because of this slight delay in entering log phase, we asked whether the *set1* mutants had defects in stationary phase viability. To test this possibility, three separate cultures of *set1* and *SET1* cells were grown for 14 d at 30°C such that they had entered a deep stationary arrest (Werner-Washburne *et al.*, 1993). Equal numbers of cells from each culture were plated onto rich medium, and the number of colonies formed was compared. Results from this experiment showed that *set1* mutants are compromised in their ability to recover from stationary phase, exhibiting only 34% average viability (ranging from 23% to 41% in three separate experiments) compared with *SET1* cultures. In expo-

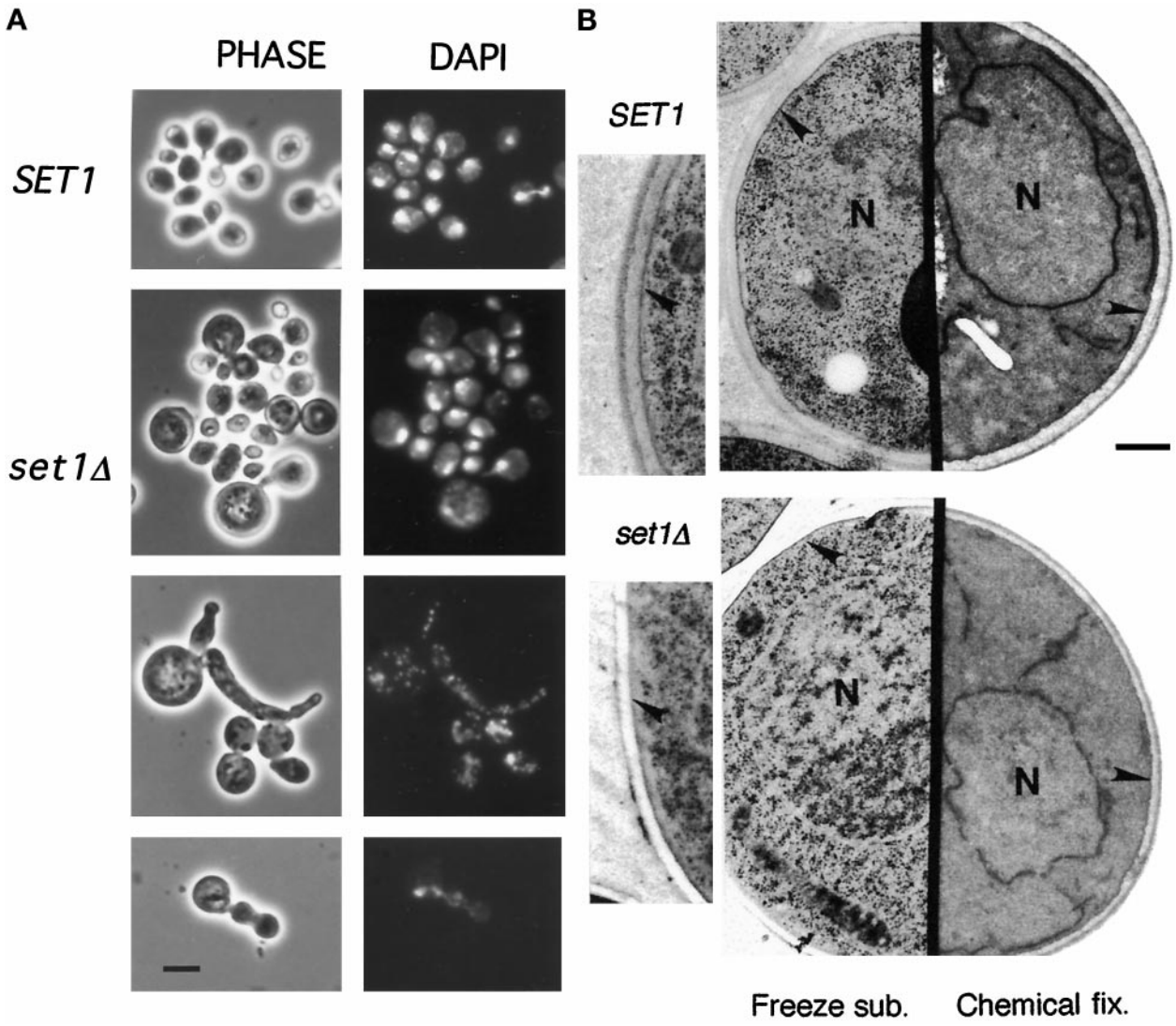

Figure 2. *set1* mutants have morphological defects. (A) *SET1* (UCC1001) and *set1* (LPY1297) strains were examined by phase contrast microscopy and by fluorescence microscopy for DAPI staining. Phase contrast images reveal that *set1* mutants are distinguished by large, abnormally shaped buds, multiple buds on individual cells, and odd cell shapes. The DNA distribution in *set1* mutants is also perturbed, as revealed by DAPI staining. In the mutants, DAPI-stained chromatin is often unequally distributed between mother and daughter cells, and some buds appear to lack discrete nuclear DNA. Bar, 5 μm. (B) Electron microscopy of *set1* mutant cells reveals additional morphological defects. *set1* cells (LPY1297) prepared by high-pressure freeze substitution reveal cell walls that are 10–20% thinner than those of comparably grown wild-type cells (UCC1001, compare panels at left). Chemical fixation of these two strains show similar, although less extreme, differences in cell wall morphology. In addition, the *set1* mutants display subtly altered organization of cytoplasmic membranes and cytoplasmic organelles. Bar, 1 μm.

nentially growing cultures, there were no differences between wild-type and *set1* cells. This loss of viability upon extended culture could reflect defects in either entry or exit from G_o.

Individually, the growth phenotypes described above are modest, yet cumulatively they may be significant. To determine the relevance of these defects we employed a coculture assay in which two different strains of cells are incubated together. In this manner, subtle growth differences may be amplified and the relative fitness of a mutant more easily assessed (Basson *et al.*, 1987; Smith *et al.*, 1995). Equal numbers of cells of logarithmically growing *SET1*, *set1::HIS3*, and *sir3::HIS3* strains were mixed to produce three *SET1-set1* and three *SET1-sir3* replicate cultures. With the exception of the *HIS3* marked mutation all three strains are isogenic. The *sir3* strain was included as a control for growth differences that might be conferred by *HIS3* as well as to control for growth differences intrinsic to strains with silencing defects (see below).

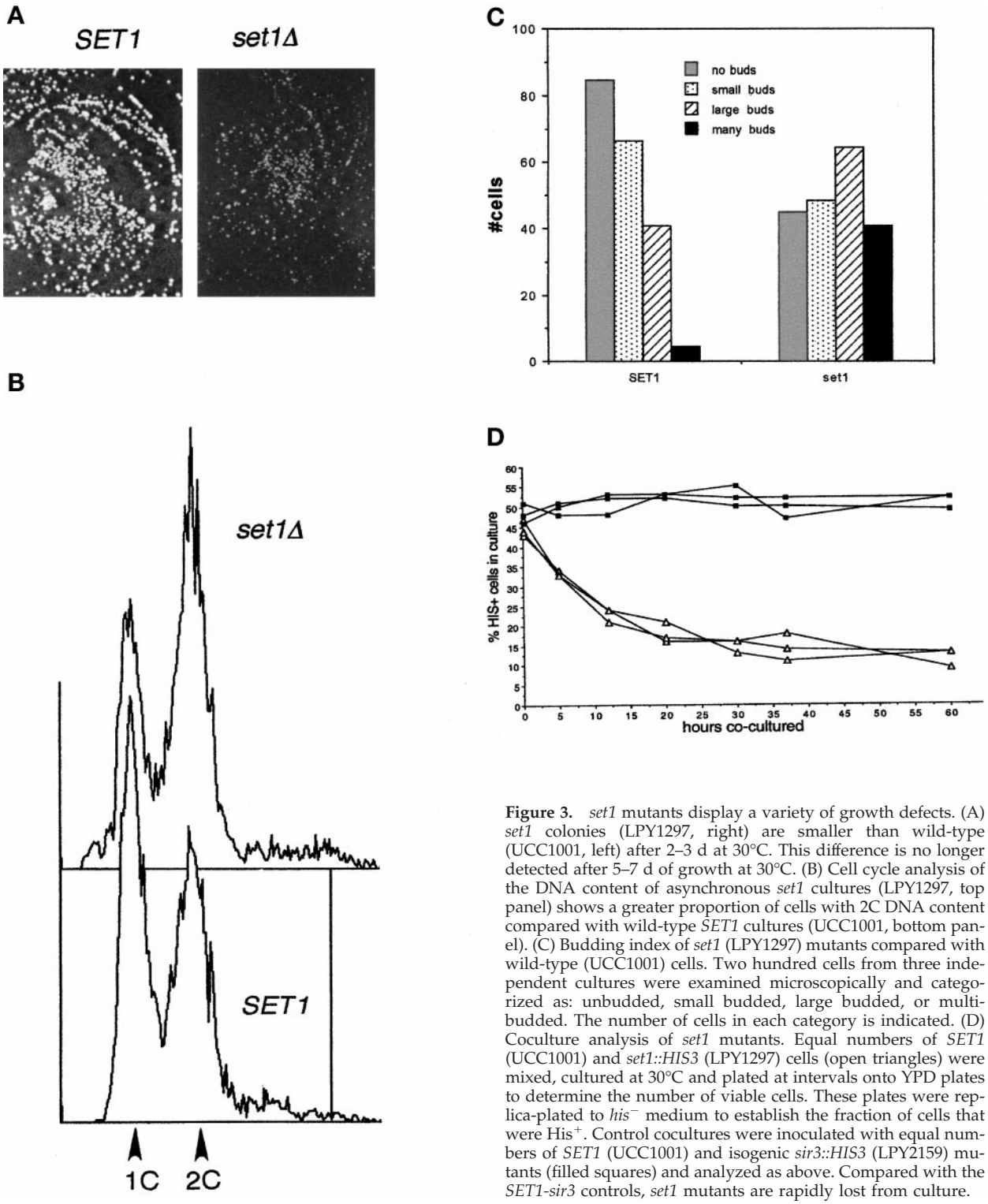

Figure 3. *set1* mutants display a variety of growth defects. (A) *set1* colonies (LPY1297, right) are smaller than wild-type (UCC1001, left) after 2–3 d at 30°C. This difference is no longer detected after 5–7 d of growth at 30°C. (B) Cell cycle analysis of the DNA content of asynchronous *set1* cultures (LPY1297, top panel) shows a greater proportion of cells with 2C DNA content compared with wild-type *SET1* cultures (UCC1001, bottom panel). (C) Budding index of *set1* (LPY1297) mutants compared with wild-type (UCC1001) cells. Two hundred cells from three independent cultures were examined microscopically and categorized as: unbudded, small budded, large budded, or multibudded. The number of cells in each category is indicated. (D) Coculture analysis of *set1* mutants. Equal numbers of *SET1* (UCC1001) and *set1::HIS3* (LPY1297) cells (open triangles) were mixed, cultured at 30°C and plated at intervals onto YPD plates to determine the number of viable cells. These plates were replica-plated to *his*$^-$ medium to establish the fraction of cells that were His$^+$. Control cocultures were inoculated with equal numbers of *SET1* (UCC1001) and isogenic *sir3::HIS3* (LPY2159) mutants (filled squares) and analyzed as above. Compared with the *SET1-sir3* controls, *set1* mutants are rapidly lost from culture.

At intervals after inoculation, equivalent numbers of cells from the cocultures were plated onto rich medium to measure the total viable cell number then replicated to his$^-$ plates to evaluate the proportion of mutant cells in the culture. For the three *SET1-sir3* cocultures, 50% of the cells were His$^+$ throughout the

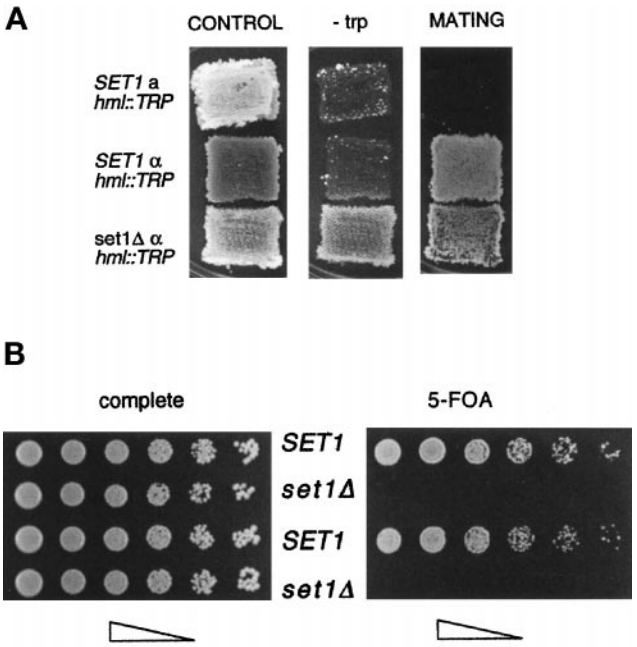

Figure 4. *set1* mutants are defective in silencing. (A) *SET1* was deleted in a strain containing a *TRP1* reporter gene at *HML*. Compared with wild-type strains, the *set1* mutant grows well in the absence of tryptophan and shows slightly decreased mating, demonstrating derepression of the *HM* silent mating-type loci. The strains shown are RS928 (*MAT*α *SET1*), RS927 (*MAT*a *SET1*), and LPY2546 (*MAT*α *set1*Δ). The *MAT*a mating tester lawn used was a227. (B) *SET1* was deleted in a strain containing a *URA3* telomeric reporter gene. Using the 5-FOA assay described in MATERIALS AND METHODS, *URA3* expression was evaluated for two wild-type (UCC1001) and two *set1* mutant (LPY1297) cultures. Growth is comparable on complete medium, but *set1* strains are unable to grow on medium containing 5-FOA, demonstrating that the normally silenced telomeric *URA3* reporter gene is transcribed.

course of the experiment, indicating no significant growth advantages or disadvantages in the *sir3* strains (Figure 3D). In contrast, the *SET1-set1* cocultures revealed a sharp decline in fitness to 35% at 5 h (h only 10–15% of the viable cells were His^+. This proportion did not change for 30 additional hours, indicating that a subset of the *set1* mutants was stably maintained in the mixed culture. The rapid loss of viability of *set1* cells observed in this assay is consistent with multiple mutant growth and morphological defects.

In the course of performing crosses with *set1* mutants, we observed that *set1/set1* homozygous diploids did not sporulate. In 1000 diploid cells from sporulation medium examined microscopically, no tetrads were observed. The sporulation efficiency of *SET1/set1* heterozygotes was also compromised, achieving only 15–25% that of wild-type *SET1/SET1* diploids. Episomal *SET1* restored sporulation competence to homozygous mutants, confirming that the sporulation defect is due to the *set1* mutation. Together, the growth and sporulation defects of *set1* mutants suggest that *SET1* functions in multiple developmental and growth processes.

set1 Mutants Have Silencing and Telomeric Defects

Because members of the SET-domain gene family function in chromatin-mediated transcriptional regulation (Tschiersh *et al.*, 1994), we asked whether *SET1* played a similar role in transcriptional control in yeast. We first examined the expression of a *TRP1* reporter gene located at the normally transcriptionally silenced *HML* locus. In *SET1* strains carrying this reporter no growth is observed on trp^- plates, whereas growth is seen in an otherwise isogenic *set1* mutant strain. In quantitative assays we observed that on average only 1% of *SET1* strains had any Trp^+ papillating colonies, whereas the *set1* mutants on average had 13% Trp^+ colonies. Furthermore, *set1* mutants show modestly reduced mating proficiency compared with wild-type strains (Figure 4A). These two phenotypes, decreased mating efficiency and expression of a normally repressed reporter gene, demonstrate that *HML* silencing is disrupted in *set1* mutants.

Because many properties of *HM* silencing are shared by telomere-proximal reporter genes (reviewed in Laurenson and Rine, 1992), we asked whether silencing was perturbed at telomeres in *set1* mutants. By analyzing telomeric silencing, we could examine either positive or negative silencing influences of *SET1* function because telomeric silencing is a metastable phenomenon in which some cells express the reporter gene whereas others repress it (Gottschling *et al.*, 1990). We employed a sensitive assay capable of detecting effects on transcriptional silencing at telomeres where a *URA3* reporter gene is placed adjacent to the telomere on the left arm of chromosome VII (Gottschling *et al.*, 1990). In wild-type yeast approximately 30–50% of the cells express *URA3* and, therefore, cannot form colonies on 5-FOA, a suicide substrate that kills cells expressing *URA3* (Boeke *et al.*, 1987). In contrast, cells repressing *URA3* are able to form colonies on 5-FOA. By diluting cultures onto control and 5-FOA plates, a quantitative assessment of telomeric silencing is obtained.

When *set1* mutants are analyzed using this assay, the cells are completely sensitive to 5-FOA, indicating complete (>100,000 fold) derepression of *URA3* (Figure 4B). This dramatic derepression was dependent on the presence of wild-type *PPR1*, the trans-activator of *URA3* expression (our unpublished observations), and thus is subject to the same control of activated expression as previously demonstrated for telomeric silencing (Aparicio and Gottschling, 1994). Plasmid-borne *SET1* restored silencing, demonstrating that the telomeric derepression was due to the *set1* mutation. A second telomeric reporter gene, *ADE2*, was also com-

pletely derepressed in *set1* strains, demonstrating that the *set1* effect is not gene specific.

Mutations in several yeast genes that disrupt telomeric silencing also decrease the length of telomeres (Palladino *et al.*, 1993). For example, *sir3* and *sir4* mutants, in which telomeric silencing is disrupted, have telomeric repeats that are 50–100 bp shorter than wild type. Because *set1* mutants share some of the silencing phenotypes of these mutants, we asked whether telomere structure was similarly affected by comparing the length of telomeres in *SET1* and *set1* mutants. In *set1* strains, telomeres were reproducibly 50 bp shorter than wild type (Figure 5). These data suggest that *SET1*, like *SIR3* and *SIR4*, is involved in both transcriptional silencing and chromosome structure, as reflected by alterations in telomere length.

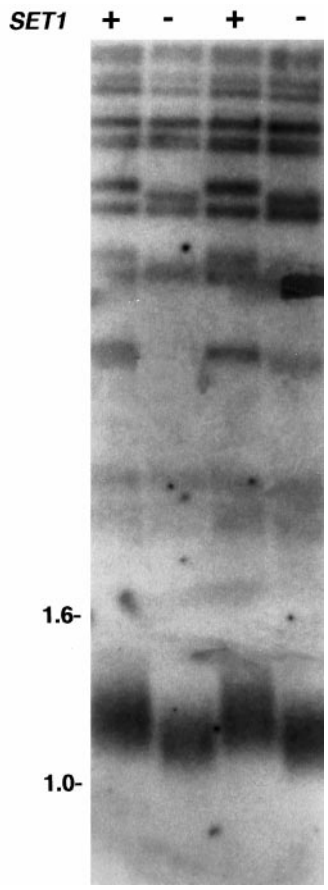

Figure 5. *SET1* strains have shortened telomeres. Genomic DNA was prepared and analyzed by *Xho*I digestion and genomic blotting, probed with a 350-bp telomere-specific probe that contains $C_{1-3}A$ telomeric repeats and a portion of the Y′ subtelomeric repeat. Telomeric DNA was compared for two independent cultures of *SET1* (UCC1001) and *set1* (LPY1297) cells. Telomeric repeat DNA for both *set1* isolates is approximately 50 bp shorter than *SET1* strains (arrowhead). Analysis of multiple independent *set1* isolates reveals that the decrease in telomeric repeat length is a consistent feature of the mutant strains.

The SET Domain of SET1 Is Sufficient for Telomeric Silencing

The silencing defects observed for the *set1* mutant suggested that *SET1*, like other SET family members, functioned in chromatin-mediated transcriptional regulation. Because the primary feature shared by all members of the SET family is the C-terminal SET domain, we asked whether this alone could effect transcriptional silencing. To examine the role of the isolated SET domain, *set1* mutants were transformed with a plasmid in which a fragment encoding only the carboxy-terminal conserved SET domain was placed under control of a galactose-inducible promoter. Induced expression of this limited portion of *SET1*, comprising 13% of the wild-type protein, effectively rescued the telomeric silencing defect in *set1* mutants (Figure 6A). This result demonstrates that the conserved SET domain of *SET1* has the capacity to promote telomeric silencing. Expression of the SET domain was confirmed by immunoblot analysis using a rat antiserum raised against recombinant Set1p, which revealed the presence of a plasmid-dependent immunoreactive doublet of ~13–14 kDa (Figure 6C). The nature of this doublet is not yet understood but may be due to posttranslational modification or processing of the SET fragment. Its presence is strictly correlated with the SET domain plasmid. In addition to the Set1p-specific bands, several nonspecific cross-reactive species are observed. These bands are variable in both their presence and intensity. For all immunoblots, a sample from a null allele was routinely included so that Set1p-specific material could be identified unambiguously.

Expression of *SET1* in *sir* mutants did not restore silencing, demonstrating that the SET domain function does not generally bypass other essential elements of silenced chromatin, but rather may act within the context of *SIR*-promoted silencing (our unpublished observations). When the SET domain was expressed in *SET1* strains, a modest but variable inhibitory effect was observed. This modest effect on wild-type strains raises the possibility that in the context of the wild-type protein, expression of the SET domain alone has the capacity to interfere with chromatin structures normally required for telomeric silencing.

Because the experiments above suggest a central role for the SET domain in telomeric silencing functions, we next asked whether mutations within the SET domain perturbed silencing. Accordingly, we analyzed the effects of two different mutations. The first was an amino acid substitution of glycine at amino acid 951 to serine (G951S). We chose this substitution because this particular glycine is completely conserved in all SET domain proteins (Stassen *et al.*, 1995; and see Figure 1B) and furthermore, in *Drosophila trx* this mutation causes embryonic lethality (Mortin *et al.*, 1992). A second mutation tested removed the *SET1* SET domain (amino acids 937-1080) and 23 amino acids upstream of the SET domain. This deletion includes $Glycine^{951}$. *set1* null mutants expressing either plasmid-borne mutant gene as the sole source of *SET1* function were assayed for telomeric silencing. The G951S point mutant supported minimal but reproducible growth on 5-FOA, and the deletion mutation

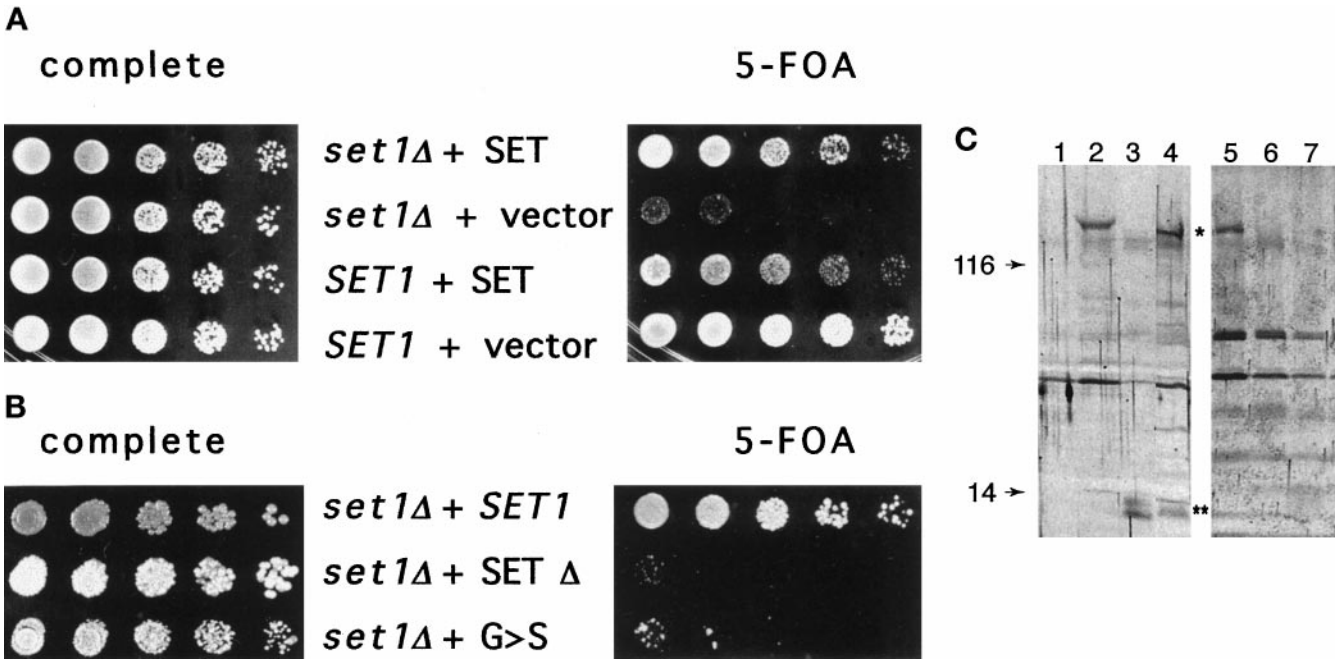

Figure 6. The SET domain functions in telomeric silencing. (A) The SET domain alone rescues the *set1* telomeric silencing defect. *SET1* and *set1* mutant strains were transformed with plasmids containing the 130 amino acid SET domain (LPY2461, LPY2462) or a control vector (LPY2463, LPY2460). When telomeric silencing of a *URA3* reporter gene was evaluated using the 5-FOA sensitivity assay, expression of the SET domain alone restored silencing to the *set1* mutants. (B) Telomeric silencing assays were performed with *set1* mutants bearing either wild-type *SET1* (LPY2456), a deletion encompassing the SET domain (LPY2457), or a *set1* point mutation that alters glycine^{951} to serine (LPY2458). Strains bearing the point mutation show minimal growth on 5-FOA and the SET deletion mutant is completely unable to grow on 5-FOA, demonstrating that mutation of the SET domain reduces or destroys telomeric silencing, depending on the mutation. (C) Immunoblot analysis of protein extracts from a *set1* mutant strain transformed with pTRP vector alone (lane 1, LPY2460) do not contain the 135-kDa band seen in the same *set1* strain transformed with pTRP bearing the full-length *SET1* gene (lane 2, asterisk, LPY2459). The *set1* strain transformed with a plasmid encoding the SET domain alone shows an immunoreactive doublet of 13–14 kDa (lane 3, double asterisk, LPY2462). A *SET1* strain with the SET-domain-only plasmid has immunoreactive species at 135 kDa and the 13- to the 14-kDa doublet (lane 4, LPY2461). The *set1* mutant strains transformed with wild-type SET1 (lane 5, LPY2456), the *set1-G951S* allele (lane 6, LPY2458), or the *set1* SET domain deletion allele (lane 7, LPY2457) are shown. Normal Set1 protein is observed in lane 5, whereas little or no immunoreactive material is seen with the mutant constructs. Additional nonspecific bands of variable molecular mass and intensity are seen in lanes 1–7. Molecular mass markers in kilodaltons are shown at left.

showed no growth on 5-FOA (Figure 6B). These results demonstrate that neither mutant form of Set1p was notably functional in telomeric silencing. The observation that the point mutant displays a small degree of telomeric silencing raises the possibility that this mutant may possess a low level of *SET1* function. Analysis of Set1p expression in these strains shows that although plasmid-encoded Set1p is readily detected, *set1* mutants with a plasmid bearing the G951S allele express a faint, faster migrating immunoreactive band (Figure 6C). No comparable immunoreactive material is observed in *set1* strains with a plasmid encoding *SET1* with the deletion encompassing the SET domain. This immunoblot profile reflects the silencing phenotypes of these strains (Figure 6B). The severe reduction or absence of mutant set1 protein is consistent with the observation that similar mutations in *Drosophila* behave as loss-of-function alleles (Mortin *et al.*, 1992), potentially due to loss of Trx protein. Neither *set1* mutant had any dominant interfering effect on telomeric silencing in the presence of wild-type *SET1.* Taken together, these data show that the presence of an intact SET domain is required for telomeric silencing.

A Screen to Identify DNA Targets of SET1 Activity

SET domain proteins in *Drosophila* are known to regulate the activity of multiple target genes (Stassen *et al.*, 1995). Given the diverse phenotypes of yeast *set1* mutants, it seemed likely that Set1p might regulate the activity of other yeast genes. To identify potential target genes that may be positively regulated by *SET1* activity, we used a DNA-binding site selection screen (Liu *et al.*, 1993) that employs two plasmids. The first is a 2μ "activator" plasmid containing *SET1* downstream of a galactose-inducible promoter as well as a *URA3* marker, allowing for both positive and negative selection. The second "reporter" plasmid contains a *TRP1* selectable marker as well as random DNA frag-

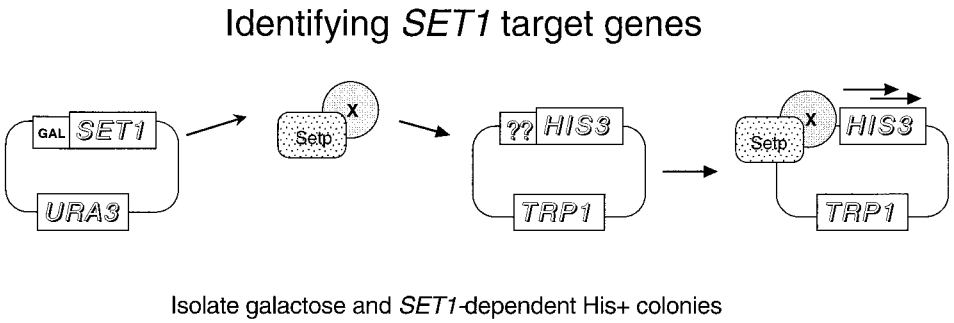

Figure 7. A screen to identify *SET1*-DNA targets. A one-hybrid screen (Liu *et al.*, 1993) was performed to identify potential *SET1* targets. A library of yeast DNA fragments was prepared from genomic DNA of W303–1a, subcloned into the *HIS3* reporter plasmid pBM2389 (pLP244), and transformed into LPY1621, a *set1::LEU2* deletion strain bearing an episomal, galactose-inducible GST-Set1 fusion protein as the sole source of *SET1* function. Trp^+ transformants that were His^+ only in the presence of galactose were selected for further analysis.

ments cloned upstream of an inactive, promoter-less *HIS3* gene. His^+ transformants are selected to identify plasmids bearing potential binding sites for the protein of interest (Figure 7). This strategy has been used successfully in the search for mammalian and *Drosophila* target genes of several different regulatory molecules (Wilson *et al.*, 1991; Mastick *et al.*, 1995; Mak *et al.*, 1996). For yeast regulators, the screen has the inherent advantage that the protein of interest need not bind DNA directly because any required cofactors for indirect binding through complex formation may be present endogenously.

The screen was conducted by first constructing a library of random small (500 bp average) yeast genomic DNA fragments cloned upstream of the defective *HIS3* gene. This library was transformed into a *set1* parental strain (LPY1621) with a chromosomal deletion of *SET1* and a galactose-inducible *SET1* gene on the activator *URA3* plasmid. Two hundred thousand initial transformants were obtained, nearly 5,000 of which were His^+. One hundred eight of the His^+ colonies were *SET1*-dependent (i.e., transformants were His^+ only when grown on medium containing galactose as the carbon source, and only if the *SET1-URA3* plasmid was present; see Figure 7). The library plasmids from these strains were recovered, passaged through *E. coli*, and retransformed into the parental yeast strain. Seventy plasmids passed *SET1* dependence tests a second time. Partial sequence of the plasmids was obtained and used to search the *S. cerevisiae* genomic database. Excluding redundant isolates and uninterpretable sequences, we identified 22 independent DNA fragments in this screen that were subjected to further analysis. The fragments ranged in size from 240 bp to 1800 bp and were found in both 5′ and 3′ regions as well as within the ORFs of potential target genes. Sequences were identified flanking and containing genes involved in transcriptional regulation, meiosis, sporulation, and growth and cell cycle control, as well as several previously uncharacterized genes. A selection of these genes is shown in Table 2. Many of these genes will be explored in detail in future analyses. To test the feasibility of this target–selection approach as a method to uncover genes that functionally interact with *SET1*, we further examined one of the genes identified.

RAM2 Is a Target of SET1 Function

As a first approach to determine whether genes linked to or encoded by *SET1*-interacting DNA fragments were affected by *SET1* function, we analyzed transcript levels of several of these genes. Blots to examine RNAs of *SET1* wild-type and *set1* mutant cells were probed with radiolabeled fragments of DNAs obtained in the screen. Of 12 fragments examined, seven showed significantly reduced transcript levels. Two examples are shown in Figure 8A. The levels of *HAS1* (a previously uncharacterized 'DEAD-'box helicase that was isolated nine separate times in the screen) and *RAM2* RNAs were decreased 5.7- and 4.5-fold, respectively when *set1* mutants were compared with isogenic *SET1* strains. These data provide evidence that genes identified as *SET1* targets may indeed be subject to transcriptional regulation by *SET1*.

RAM2, isolated twice in the screen, was of special interest. It encodes an essential subunit of a heteromeric prenyltransferase complex encoded by *RAM1* and *RAM2* (He *et al.*, 1991). This enzyme prenylates Ras1p and a-factor as well as other yeast substrates (Goodman *et al.*, 1990). Because *RAM2* transcription is reduced in *set1* mutants, we asked whether Ram2p activity was similarly affected. Prenyltransferase activity was assayed (Gomez *et al.*, 1993) by comparing the ability of crude extracts of *SET1* and *set1* mutant cells to transfer ^{3}H-farnesyl pyrophosphate into trichloroacetic acid-precipitatable counts. Results from this experiment revealed that overall farnesyltransferase activity was reduced 3–5 fold in *set1* mutant strains (Figure 8B). Although these assays do not measure

Table 2. Candidate Set1p-target loci identified from the *Saccharomyces* genome database[a]

Gene (alias)	Description or protein information
ARG2	Acetylglutamate synthase, catalyzes the first step in the ornithine biosynthetic pathway
ATS1	α-Tubulin suppressor-1, similar to the mammalian gene, *RCC1*
CCR4	Carbon catabolite repressor, transcriptional regulator for some glucose-repressed genes
DNA2	DNA replication helicase
DOG2	2-Deoxyglucose-6-phosphate-phosphatase
FSP2	Flocculent specific protein with similarity to a-glucosidase (maltase)
HAL5	Protein kinase homolog; mutant is pH and salt sensitive
HAS1	Essential, putative DEAD box helicase (our unpublished results)
HEM13	Coproporphyrinogen III oxidase; oxygen repressed, functions in sixth step of heme biosynthesis
HMG1	3-Hydroxy-3-methylglutaryl-coenzyme A reductase isozyme
MYO4	Unconventional class V myosin heavy chain
PCL1(HCS26)	G1/S-specific cyclin that can interact with the kinase Pho85
RAM2	CAAX-Farnesyl transferase, α-subunit
RFC3	Subunit 3 of replication factor C: processivity factor for DNA polymerases delta and epsilon
SIN3(RPD1,SDI1,UME4)	transcriptional regulator identified in many screens to negative and positive effects on individual gene's expression
SOL3	Putative oxidoreductase
SNC1	Synaptobrevin (SNARE) homologue present on post-Golgi vesicles
SPT10	Global transcriptional repressor of core promoter activity; the magnitude of transcriptional regulation at multiple loci
STP1	Involved in pre-tRNA splicing
SWI1(ADR6)	Component of the SWI/SNF transcription complex
TPK1(PKA1,SRA3)	Putative catalytic subunit of cAMP-dependent protein kinase
VAS1	Valyl-tRNA synthetase
YBR061c	Hypothetical gene product is homologous to *E. coli* fstJ, a bacterial cell division protein.

[a] Library plasmid inserts were sequenced and compared to the yeast genome data base. In several cases two genes or gene fragments were on the same insert. Nine putative ORFs for which there is no further information are not shown. Annotations for gene descriptions and protein information are from the *Saccharomyces* Genome Database (Cherry *et al*., 1996).

RAM-encoded enzyme activity exclusively [there are two other known yeast prenyl- and geranyl-geranyl transferases (Gomez *et al*., 1993)] these experiments suggest that Ram2p activity, in addition to its transcription, is reduced in *set1* mutants.

DISCUSSION

SET1 encodes a yeast member of the SET domain protein family that functions in diverse aspects of cell morphology, growth control, and chromatin-mediated transcriptional silencing. In particular, *set1* mutants derepress silencing of genes at telomeres and the *HML* silent mating-type locus. Expression of the *SET1*-conserved SET domain alone is sufficient to restore telomeric silencing, demonstrating functional significance of this protein motif. A genetic screen to uncover DNA targets of Set1p activity yielded 22 potential interacting sequences, many of which lie adjacent to or within genes involved in transcriptional regulation, growth and cell cycle control, and meiosis. In addition, some sequences are near predicted ORFs of undetermined function. Preliminary analysis of one Set1p-interacting gene, *RAM2*, shows that both its transcription and associated enzymatic activity are attenuated in *set1* mutants. Together these observations suggest that *SET1* is involved in multiple cellular processes and that these roles may be mediated at the level of transcriptional regulation.

Phenotypes of set1 Mutants Suggest Roles for Set1p in Both Silencing and Activating Transcription

In *set1* mutants, transcriptional silencing at the *HML* silent mating-type locus is defective, as evidenced by expression of a normally repressed *TRP1* reporter gene and modest decreases in mating. This effect is not locus-specific because in *set1* mutants, telomeric silencing is completely abrogated, resulting in greater than 100,000-fold increase in expression of telomere-proximal reporter genes. Two potential explanations for these results are that Set1p interacts with other components of the silencing machinery, or alternatively, that *SET1* regulates transcription of silencing genes. The latter does not appear to be generally the case because transcript levels of the silencing genes *SIR3* and *SIR4* are unaffected in *set1* strains (our unpublished observations). Furthermore, none of the genes previously identified to function in silencing were uncovered in our binding site screen. We favor the explanation that Set1p is itself a component of chromatin that has the capacity to regulate transcrip-

tion both positively and negatively. Indeed, recent analysis of transcriptional complexes in yeast supports the existence of several distinct macromolecular complexes, each of which affects transcription in ways that are still being defined (Denis *et al.*, 1994; Cairns *et al.*, 1996; Peterson, 1996). It will be important to determine whether Set1p is a component of any of these chromatin complexes.

The DNA target site screen was performed to identify target sequences for Set1p and, by extension, perhaps for other SET domain proteins. Such information will also allow us to determine in future experiments whether these proteins bind DNA directly or in concert with other proteins. A number of potential *SET1* target genes were uncovered in our Set1p-binding site screen. We began analysis of Set1p interactions by examining *RAM2*, one of the interacting genes identified in the screen. Our data show that *RAM2* transcription is decreased in *set1* mutants, but that transcription of this essential gene is not fully dependent on *SET1*. Indeed, *set1* mutants display a significant decrease in overall farnesyltransferase activity, suggesting that *SET1* functions in achieving maximal expression of *RAM2*. Our analysis of *RAM2* thus provides validation for the target site screen as an experimental approach for analyzing Set1p target genes and will guide future analysis of the other targets identified. It will also be important to extend and modify this approach to identify targets that may be negatively regulated by *SET1*.

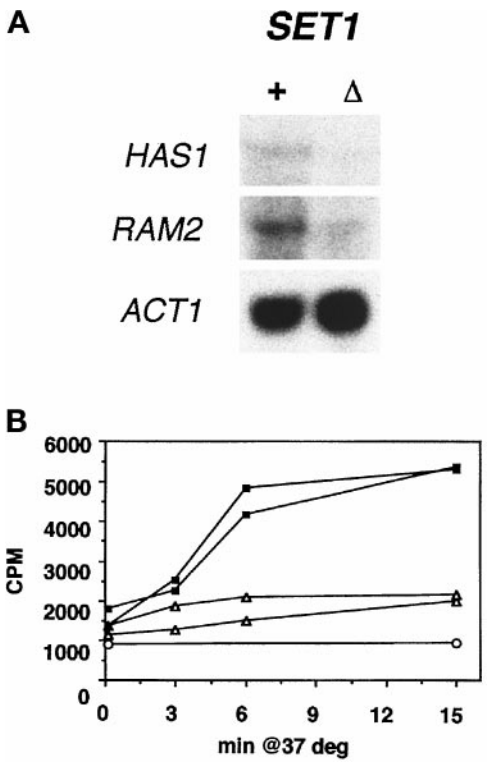

Figure 8. Analysis of *RAM2*, a candidate *SET1*-interacting gene. (A) Transcription of *RAM2* and *HAS1* was analyzed in *SET1* (UCC1001) and *set1* (LPY1297) strains by hybridizing blots containing RNA from each strain with probes prepared from radioactively labeled DNA fragments obtained in the target screen. *ACT1* served as a loading control. *RAM2* and *HAS1* transcript levels were diminished in *set1* mutant RNA, suggesting that these genes are transcriptional targets of Set1p. (B) Prenyltransferase assay of protein extracts prepared from *SET1* (UCC1001) and *set1* (LPY1297) mutants. Clarified extracts from duplicate cultures of both strains were analyzed for incorporation of ^{3}H-farnesylpyrophosphate into trichloroacetic acid-precipitable counts as a function of time. Filled squares indicate *SET1* extracts, open triangles indicate *set1* extracts, and open circles indicate control extracts from *SET1* cells plus 5 mM EDTA.

The SET Protein Domain Can Function in Telomeric Silencing

The conserved SET domain found in *SET1* and other *trithorax* family members appears fundamental for the activities of these genes (Stassen *et al.*, 1995), possibly by allowing them to bind to DNA. We tested whether the SET domain of *SET1* was important for telomeric silencing in the following ways: 1) an invariant glycine within the SET domain was mutagenized to serine, 2) a deletion encompassing the SET domain was constructed, and 3) the SET domain alone was expressed in a *set1* null mutant. The G951S mutant is analogous to the *Drosophila* embryonic lethal *trithorax*^{Z11} allele (Mortin *et al.*, 1992; Stassen *et al.*, 1995). In both the missense and deletion mutants of the SET domain, telomeric silencing was abolished. Conversely, when the SET domain alone was expressed in *set1* null mutants, telomeric silencing was restored to wild-type levels, even though this domain comprises only 13% of the full-length Set1 protein. These results extend recent data demonstrating that heterologous mammalian SET proteins can promote telomeric silencing in both *Drosophila* and *Saccharomyces* (Laible *et al.*, 1997) by suggesting that the SET domain itself may serve as a primary unit of function.

Set1p Shares Mechanistic Features of Other SET Domain Proteins

The SET domain is conserved in proteins with potentially diverse functions (Stassen *et al.*, 1995). The best studied SET domain genes, such as those from *Drosophila*, play key roles in both embryonic and adult development (Ingham, 1981; Breen and Harte, 1993). In fact, much of our understanding of the roles of these genes, including *trx*, has been gleaned from the study of early development (Singh, 1994). In *Drosophila* and mammals, SET domain genes, in concert with other members of the *trithorax*- and *Polycomb*- group

genes, play crucial roles in setting the initial patterns of gene expression during early embryogenesis (Schumacher and Magnuson, 1997).

The well-studied *Drosophila* SET domain protein, Trx, regulates many target genes to maintain their expression within specific temporal and spatial boundaries (Castelli-Gair and Garcia-Bellido, 1990; Breen and Harte, 1993; Chinwalla *et al.*, 1995). Trx positively regulates multiple homeotic genes within both the *BTX* and *ANT-C* complexes, including the gene *engrailed*. Trx binds to the polytene region where *engrailed* and 60 additional genes lie, suggesting that *trx* may regulate the expression of its target genes by binding to their regulatory regions, (Castelli-Gair and Garcia-Bellido, 1990; Chinwalla *et al.*, 1995) most likely as a member of a complex of chromatin proteins. These and other observations suggest that *trx*, like *SET1*, is involved in the regulation of multiple biological pathways. Consistent with this idea, the targets uncovered in our Set1p target screen suggest that *SET1* interacts with a large number of yeast genes. Future studies may allow us to define a Set1p DNA-binding element within the relatively small (240 bp-1.8 kb) target sequence fragments identified in our screen. By comparison, to date the smallest *trx*-responsive sequence identified is defined by 8.2-kb and 10.0-kb regions adjoining the *Drosophila* loci *sex combs reduced* and *antennapedia* genes (Gindhart and Kaufman, 1995). Thus, the approach we present may be useful comparatively and in future refinement of sequences through which other SET proteins may act.

How might SET domain genes affect transcriptional regulation of such a wide range of potential target genes? Although we observed that Set1p is a nuclear protein (not shown) and that it appears to interact with several target genes, the mechanism by which it acts is not known. Recent evidence suggests that the Trithorax family of proteins acts by remodeling chromatin (Peterson and Tamkun, 1995). In yeast, silencing involves interactions between protein complexes and DNA, presumably enhancing chromatin condensation and thereby blocking access of transcriptional enzymes. In an opposing manner, access to chromatin appears to be facilitated by the SWI/SNF protein complex (Peterson, 1996). Members of the phylogenetically conserved SWI/SNF complex are required for transcription of many diversely regulated genes (Winston and Carlson, 1992; Peterson and Tamkun, 1995). Biochemical analysis of purified yeast and mammalian SWI/SNF complexes demonstrates that they may function by disrupting nucleosome structure (Peterson, 1996). Both repressive and activating complexes may interact, possibly by competing for the same target genes. A screen for suppressors and enhancers of *Pc* and *ANT-C* mutants recovered several *trx* alleles as well as alleles of *brahma*, a *Drosophila* homologue of *SNF2*, one of the key *SWI/SNF* subunits, providing additional evidence that *Pc* and *trx-G* complexes interact (Kennison and Tamkun, 1988). More recent experiments have shown that *Drosophila* snr1, a transcriptional regulator, is homologous to yeast Snf5p (Dingwall *et al.*, 1995), further supporting the idea that chromatin-remodeling activities are conserved. These precedents are critical in linking the transcriptional regulatory activities of *trx* with the known chromatin remodeling activities of the SWI/SNF complex.

It is apparent that SET domain proteins are able to act as transcriptional repressors, transcriptional activators, or both. Our results with Set1p are consistent with it being a transcriptional repressor at normally silent loci in yeast, whereas the target site screen supports the idea that Set1p may be an activator of transcription. A straightforward but as yet untested explanation to unify these observations is that *SET1*, by analogy with the transcriptional regulator *RAP1* (Shore, 1995), acts in a locus- and context-specific manner to either up-regulate or down-regulate transcription. Further experiments designed to test potential dual activities of *SET1* will clarify these possibilities. This issue is key for understanding functions of the human *trx* homolog, *ALL-1/HRX/MLL*, which is involved in many acute leukemias associated with chromosomal translocations (reviewed in Rabbitts, 1994) and at least one solid tumor (Baffa *et al.*, 1995). It has been variously argued that human disease may result from dominant-negative or neomorphic effects of translocation chimeras or from loss of function of the normal gene (Gu *et al.*, 1992; Tkachuk *et al.*, 1992; Zeleznik-Le *et al.*, 1994; Baffa *et al.*, 1995; Schichman *et al.*, 1995; Fidanza *et al.*, 1996). Because domain analyses suggest that *ALL-1/HRX/MLL* may have activation and repression domains that are separated in some chromosomal translocations associated with leukemias (Zeleznik-Le *et al.*, 1994), it will be especially important to identify normal targets of the gene's activity. It is likely that at least some of the genes regulated by *SET1* may also be targets for the human and *Drosophila* genes. Understanding regulation by these SET domain proteins is thus likely to lead to deeper understanding of hematopoietic differentiation and other complex developmental programs that are influenced by chromatin-mediated gene regulation.

ACKNOWLEDGMENTS

We thank D. Gottschling, M. Johnston, R. Sternglanz, and V. Zakian for strains and plasmids; E. Stone and M. Winey for experimental advice; G. Hertz and G. Stormo for help with computational analyses; and E. Stone, J. Heilig, J. Duffy, M. Winey, G. Hertz, and G. Stormo for helpful comments on the manuscript. D. Lorenz and V. Lombillo provided assistance with graphics, and M. Morphew of the Boulder Laboratory for 3-Dimensional Fine Structure (supported by National Institutes of Health grant RR-00592) helped in all aspects of electron microscopic analyses. C.N. was supported by a postdoctoral fellowship from the American Cancer Society and funds from the Cancer League of Colorado. E.R. received Under-

graduate Research Assistance Program support from the Howard Hughes Medical Institute and the Cancer Student Assistantship Program of the University of Colorado Cancer Center. L.P. is a Pew Scholar in the Biomedical Sciences and a National Science Foundation New Young Investigator and gratefully acknowledges laboratory support from those programs. Request reprints from L.P.; request experimental materials from C.N.

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