Sth1p, a *Saccharomyces cerevisiae* Snf2p/Swi2p Homolog, Is an Essential ATPase in RSC and Differs From Snf/Swi in Its Interactions With Histones and Chromatin-Associated Proteins

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ABSTRACT

The essential Sth1p is the protein most closely related to the conserved Snf2p/Swi2p in *Saccharomyces cerevisiae*. Sth1p purified from yeast has a DNA-stimulated ATPase activity required for its function *in vivo*. The finding that Sth1p is a component of a multiprotein complex capable of ATP-dependent *r*emodeling of the *s*tructure of *c*hromatin (RSC) *in vitro*, suggests that it provides RSC with ATP hydrolysis activity. Three *sth1* temperature-sensitive mutations map to the highly conserved ATPase/helicase domain and have cell cycle and non-cell cycle phenotypes, suggesting multiple essential roles for Sth1p. The Sth1p bromodomain is required for wild-type function; deletion mutants lacking portions of this region are thermosensitive and arrest with highly elongated buds and 2C DNA content, indicating perturbation of a unique function. The pleiotropic growth defects of *sth1-ts* mutants imply a requirement for Sth1p in a general cellular process that affects several metabolic pathways. Significantly, an *sth1-ts* allele is synthetically sick or lethal with previously identified mutations in histones and chromatin assembly genes that suppress *snf*/*swi*, suggesting that RSC interacts differently with chromatin than Snf/Swi. These results provide a framework for understanding the ATP-dependent RSC function in modeling chromatin and its connection to the cell cycle.

EUKARYOTIC DNA is organized into nucleosomes and higher-order chromatin structure that inhibit initiation of transcription by RNA polymerase II *in vitro* and *in vivo* (Grunstein 1990; Kornberg and Lorch 1992). Conversion of genes from transcriptionally repressed to transcriptionally active states often occurs with changes in nucleosome positioning *in vivo* by replication-independent mechanisms (Croston *et al.* 1991; Felsenfeld 1996; Wolffe 1996). This structural remodeling of chromatin can be accomplished by several cellular mechanisms, including modification of histones, the binding of gene-specific transcriptional factors, and the activity of protein complexes capable of reconfiguring nucleosomes. Several eukaryotic multiprotein complexes have been implicated in such chromatin-remodeling processes, including the *Saccharomyces cerevisiae* Snf/Swi (Winston and Carlson 1992; Carlson and Laurent 1994; Peterson and Tamkun 1995; Kingston *et al.* 1996), mammalian BRG1 and hbrm (Imbalzano *et al.* 1994; Kwon *et al.* 1994; Wang *et al.* 1996a,b), and Drosophila NURF, CHRAC, ACF, and BRM complexes (Dingwall *et al.* 1995; Tsukiyama *et al.* 1995; Ito *et al.* 1997; Mizuguchi *et al.* 1997; Varga-Weisz *et al.* 1997).

The yeast *SNF* and *SWI* genes were identified originally as mutants defective in the expression of specific genes (Neigeborn and Carlson 1984; Stern *et al.* 1984) and have since been implicated in the transcription of several differently regulated promoters (Abrams *et al.* 1986; O'Hara *et al.* 1988; Estruch and Carlson 1990; Laurent *et al.* 1990; Happel *et al.* 1991; Peterson and Herskowitz 1992), although they are not required for transcription of all genes. The Snf/Swi proteins were also shown to assist activation by several sequence-specific transcriptional activators expressed in *S. cerevisiae* (Laurent and Carlson 1992; Peterson and Herskowitz 1992; Yoshinaga *et al.* 1992). The yeast Snf/Swi complex is composed of 11 proteins and has an apparent mass of 2 MD (Cairns *et al.* 1994; Peterson *et al.* 1994). Genetic evidence provided the first clue that the Snf/Swi proteins function in transcriptional activation by relieving chromatin-mediated repression. Growth and transcriptional defects of *snf*/*swi* mutants are suppressed by mutations in genes encoding histones H2A and H2B (Hirschhorn *et al.* 1992), H3, H4 (Prelich and Winston 1993; Kruger *et al.* 1995), nonhistone chromatin assembly factors, including Spt4p, Spt5p, and Spt6p (Neigeborn *et al.* 1987; Sternberg

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et al. 1987; Kruger and Herskowitz 1991; Swanson and Winston 1992; Hirschhorn *et al.* 1995; Bortvin and Winston 1996), and an HMG1-like protein (Kruger and Herskowitz 1991). The chromatin structure of the *SUC2* promoter is also altered in *snf2*, *snf5*, and *swi1* mutants (Hirschhorn *et al.* 1992; Matallana *et al.* 1992; Gavin and Simpson 1997; Wu and Winston 1997). Biochemical evidence for a direct role in nucleosome remodeling was provided by experiments in which purified yeast Snf/Swi complex was shown to disrupt histone-DNA contacts enabling activators to bind to nucleosomal sites *in vitro* in an ATP-dependent manner (Côté *et al.* 1994).

The yeast Snf2p protein contains motifs similar to those of DNA-dependent ATPases and DNA helicases (Gorbalenya *et al.* 1989; Laurent *et al.* 1992; Henikoff 1993) and is the prototypal member of a large protein superfamily. Representatives of the superfamily include prokaryotic and eukaryotic proteins that participate in a variety of nuclear processes, including transcription, chromosome segregation, and DNA repair and recombination (see Carlson and Laurent 1994). Phylogenetic analysis of proteins within this superfamily generates several subfamilies believed to have similar functions, one of which includes in addition to Snf2p, the *S. cerevisiae* Sth1p, *Drosophila melanogaster* brm, and human hbrm and BRG1 (brm/SWI2 related gene) proteins (Eisen *et al.* 1995). Importantly, recombinant Snf2p has a DNA-dependent ATPase activity (Laurent *et al.* 1993), and purified yeast Snf/Swi is capable of a similar ATP hydrolysis activity (Cairns *et al.* 1994; Peterson *et al.* 1994). Moreover, the nucleosome restructuring activities of the yeast Snf/Swi and human hSWI/SNF complexes require the Snf2p and BRG1 ATPases, respectively (Côté *et al.* 1994; Kwon *et al.* 1994), and the Drosophila brm protein in the BRM complex and the Drosophila ISW1 protein present in the NURF, ACF, and CHRAC ATP-dependent nucleosome remodeling factors are likewise believed to contribute the necessary ATP hydrolysis activity (Tsukiyama *et al.* 1995; Tsukiyama and Wu 1995; Ito *et al.* 1997; Varga-Weisz *et al.* 1997). Snf2p and its relatives therefore provide several independent chromatin-modeling complexes with the ATP-dependent enzymatic activity required to rearrange nucleosomal DNA.

In addition to transcription by RNA polymerase II, dynamic chromatin-remodeling activities are important in several other basic cellular processes that have been conserved during eukaryotic evolution, any of which might require multiprotein complexes with activities similar to those of the Snf/Swi, BRG1, hbrm, Brm, NURF, ACF, or CHRAC complexes. These processes include transcription by RNA polymerases I and III, the replication, repair, and recombination of DNA, epigenetic regulation, retroelement integration, and the assembly and maintenance of stable mitotic chromosomes. Interestingly, the human counterparts of Snf/Swi have been shown to associate with nuclear matrix or scaffold (Reyes *et al.* 1997). Moreover, in addition to their roles in remodeling chromatin, ACF and CHRAC functions are also necessary for the assembly and proper spacing of nucleosomes, implying roles in DNA replication (Ito *et al.* 1997; Varga-Weisz *et al.* 1997).

We have initiated studies with yeast Sth1p/Nps1p (Laurent *et al.* 1992; Tsuchiya *et al.* 1992), the most closely related protein to Snf2p in the yeast genome. Sth1p shares several regions of homology with Snf2p including a 518-amino-acid ATPase/helicase-related domain, a 75-amino-acid bromodomain of unknown function, which is present in the Snf2p subfamily proteins and in several other proteins (Haynes *et al.* 1992; Laurent *et al.* 1992; Tamkun *et al.* 1992; Jeanmougin *et al.* 1997) and four shorter regions flanking the ATPase domain (see Figure 4A). The ATPase/helicase-related domains of Snf2p and Sth1p are interchangeable (Laurent *et al.* 1993). There are, however, functional differences between *SNF2* and *STH1*. *STH1* is essential for mitotic growth of yeast cells, while *SNF2* is not. Increased gene dosage of either *SNF2* or *STH1* fails to compensate for loss of the other and a DNA-bound LexA-Snf2p hybrid protein activates transcription of a target gene dramatically, whereas a LexA-Sth1p fusion protein activates transcription only very weakly (Laurent *et al.* 1992). In addition, *STH1* depletion experiments suggest a role for Sth1p in mitotic cell cycle progression (Tsuchiya *et al.* 1992).

Significantly, Sth1p was shown to be a component of an abundant 16-protein complex with the ability to *r*emodel the *s*tructure of *c*hromatin (RSC) *in vitro* (Cairns *et al.* 1996). RSC shares several features with the Snf/Swi complex. First, RSC and Snf/Swi alter nucleosome structure *in vitro*, as measured by changes in DNAse I digestion patterns. Second, both remodeling activities require ATPase activities that are stimulated equally well by single-stranded, double-stranded, or nucleosomal DNA. Finally, at least four subunits of RSC are essential homologs of the Snf2p, Snf5p, Swi3p, and Swp73p components of Snf/Swi (Cairns *et al.* 1996; Cao *et al.* 1997). However, in contrast to Snf/Swi, RSC is essential and abundant, suggesting that the two complexes carry out distinct functions.

Here we take biochemical and genetic approaches to elucidate the essential function(s) of the yeast Sth1p. We show that an Sth1p fusion protein expressed in yeast has DNA-stimulated ATPase activity, which is abolished by mutation of the nucleoside triphosphate (NTP)-binding site, the same mutation that previously eliminated *STH1* function *in vivo*. To study the phenotype caused by loss of *STH1* gene function, three temperature-sensitive mutations were isolated. All mutations map to the highly conserved ATPase/helicase-related domain: one allele causes cells to arrest in G_2/M, and the other two cause cells to arrest asynchronously, suggesting additional essential roles of Sth1p in the cell. We also demonstrate that the C-terminal bromodomain motif of Sth1p is required for wild-type *STH1* function. The aberrant

morphology of cells lacking portions of the bromodomain suggests that these sequences are required for a discrete function. Finally, genetic interactions between an *sth1* mutation and previously identified mutations affecting nucleosome structure suggest that RSC contacts chromatin in a manner that is different from that of Snf/Swi.

MATERIALS AND METHODS

Strains, genetic methods, and media: *Saccharomyces cerevisiae* strains are listed in Table 1. The *Escherichia coli* strains were XL1-Blue (Stratagene, La Jolla, CA) and GM48 (F^- *thr leu thi lacY gelK gelT ara fhuA tsx dam dcm supE44*). Rich (YPD), synthetic complete (SC), and sporulation media were prepared as described previously (Rose *et al.* 1990). YPgal, YPsuc, YPraf, YPeth/gly, and SCgal media were 2% in galactose, sucrose, raffinose, ethanol and glycerol, and galactose, respectively. For the analysis of temperature-sensitive strains, logarithmic-phase cultures growing at 30° (the permissive temperature) were shifted to 37° (the fully restrictive temperature). Yeast strains were transformed by the lithium acetate procedure (Ito *et al.* 1983). Standard genetic procedures of crossing, sporulation, and tetrad analysis were followed (Rose *et al.* 1990).

Plasmids: All plasmids were constructed by standard techniques (Ausubel *et al.* 1988) and are listed in Table 2.

Preparation of GST-Sth1p fusion proteins for ATPase assays: The pDJ84, pDJ84KR, pDJ85, and pDJ86 plasmids (Table 2) expressed GST-$Sth1_{288\text{-}1359}$, GST-$Sth1K501R_{288\text{-}1359}$, GST-$Sth1_{288\text{-}1014}$, and GST-$Sth1K501R_{288\text{-}1014}$ fusion proteins, respectively, from the *GAL1,10* promoter in yeast strain BLY36. Cells

TABLE 1

List of *S. cerevisiae* strains used

Strain[a]	Genotype
BLY1[b]	*MATα his3-Δ200 ura3-52 lys2-801 SUC2*
BLY18	*MAT**a**/MATα his3-Δ200/his3-Δ200 ura3-52/ura3-52 ADE2/ade2-101 LYS2/lys2-801*
BLY29[c]	*MAT**a**/MATα his3-11,15/his3-11,15 ura3-1/ura3-1 ade2-1/ade2-1 leu2-3, 112/leu2-3, 112 trp1-1/trp1-1 can1-100/can1-100*
BLY30	*MAT**a**/MATα sth1-Δ2::HIS3/STH1 his3-11,15/his3-11,15 ura3-1/ura3-1 ade2-1/ade2-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 can1-100/can1-100*
BLRY30-1	*MATα sth1-Δ2::HIS3 his3-11,15 ura3-1 ade2-1 leu2-3,112 trp1-1 can1-100* pDJ67(*CEN6 ARS1 URA3 STH1*)
BLY36[d]	*MATα his3Δ200 ura3-52 leu2-Δ1 trp1Δ63*
BLY46	*MAT**a** his3-11,15 ura3-1 leu2-3,112 trp-1 ade2-1 can1-100*
BLY47	*MATα sth1-1ts his3-11,15 ura3-1 leu2-3,112 trp-1-1 ade2-1 can1-100*
BLY48	*MATα sth1-2ts his3-Δ200 ura3-52 lys2-801 SUC2*
BLY49	*MAT**a** sth1-3ts his3-Δ200 ura3-52 ade2-101*
BLY49-2D	*MATα sth1-3ts his3-Δ200 ura3-52 ade2-101*
BLY76	*MAT**a**his3-Δ200 ura3-52 ade2-101 lys2-801*
BLY81	*MAT**a**/MATα sth1-Δ3::HIS3/STH1 his3-Δ200/his3-Δ200 ura3-52/ura3-52ADE2/ade2-101 LYS2/lys2-801*
BLY85[e]	*MATα Δhta1-htb1::URA3 his3-Δ200 ura3-52*
BLY88	*MAT**a** ssn20-1 ura3-52 lys2-801*
BLRY116	*MAT**a** sth1-Δ3::HIS3 his3-Δ200 ura3-52 ade2-101* pIN11(*CEN6 ARSH4 URA3 sth1Δ1349*)
BLRY117	*MAT**a** sth1-Δ3::HIS3 his3-Δ200 ura3-52 ade2-101 lys2-801* pDJ66(*CEN6 ARSH4 URA3 sth1Δ1338*)
BLRY118	*MATα sth1-Δ3::HIS3 his3-Δ200 ura3-52 ade2-101* pIN14(*CEN6 ARSH4 URA3 sth1Δ1274-1335*)
BLRY119	*MATα sth1-Δ3::HIS3 his3-Δ200 ura3-52 ade2-101* pIN15(*CEN6 ARSH4 UTA3 sth1Δ1274*)
BLRY134	*MATα sth1-Δ3::HIS3 his3-Δ200 ura3-52 ade2-101 lys2-801* $psth1^{S806L}$(*CEN6 ARSH4 URA3 sth1*^{S806L})
BLRY135	*MATα sth1-Δ3::HIS3 his3-Δ200 ura3-52 ade2-101 lys2-801* $psth1^{T881M}$(*CEN6 ARSH4 URA3 sth1*^{T881M})
BLRY136	*MAT**a** sth1-Δ3::HIS3 his3-Δ200 ura3-52 ade2-101 lys2-801* $psth1^{S806L,\ T881M}$ (*CEN6 ARSH4 URA3 sth1-3ts*)
BLRY139	*MAT**a** sth1-Δ3::HIS3 his3-Δ200 ura3-52 ade2-101 lys2-801* pDJ67(*CEN6 ARSH4 URA3 STH1*)
BLY150	*MAT**a** sth1-3ts ssn20-1 his3-Δ200 ura3-52 ade2-101 lys2-801*
BLY151	*MATα sth1-3ts Δhta1-htb1::URA3 his3-Δ200 ura3-52 ade2-101*
BLY157[f]	*MATα hht2Δ::URA3 ura3-52 leu2*$^-$ *HO-lacZ*
BLRY181	*MAT**a** sth1-Δ3::HIS3 his3-Δ200 ura3-52 ade2-101 lys2-801* pDJ2ts(*CEN6 ARSH4 URA3 sth1*^{P646L})
BLY184	*MAT**a** sth1-3ts hht2Δ::URA3 ura3-52 ade2-101 HO-lacZ*
BLY271	*MAT**a** hhf1Δ::HIS3 hhf2Δ::LEU2 his3-Δ200 ura3-52 leu2 trp1 ade2-101* pUK499(*URA3* pHHF2)
BLY272	*MATα sth1-3ts hhf1Δ::HIS3 hhf2Δ::LEU2 his3-Δ200 ura3-52 leu2 trp1 ade2-101* pUK499(*URA3* pHHF2)
BLY273	*MAT**a** hhf1Δ::HIS3 hhf2Δ::LEU2 his3-Δ200 ura3-52 leu2 trp1 ade2-101* pCP327(*URA3 hhf2-8*)
BLY274	*MAT**a** sth1-3ts hhf1Δ::HIS3 hhf2Δ::LEU2 his3-Δ200 ura3-52 leu2 trp1 ade2-101* pCP327(*URA3 hhf2-8*)
BLY275	*MAT**a** sth1-3ts hht2Δ::URA3 ura3-52 ade2-101 his3-Δ200*

[a] All strains have the S288C genetic background except for BLY29, BLY30, BLRY30-1, BLY46, and BLY47, which are derived from W303.

[b] BLY1 and BLY88 are the same as strains MCY829 and MCY2484, respectively, obtained from M. Carlson.

[c] BLY29 is the same as W303, obtained from R. Rothstein.

[d] BLY36 is the same as FY250, obtained from F. Winston.

[e] BLY85 is the same as DN106, obtained from M. A. Osley.

[f] BLY157 is the same as WK48-20a, obtained from I. Herskowitz.

TABLE 2

List of plasmids used

Plasmid	Description	Source or reference
pBL50	$Sth1_{1\text{-}1359}$ in YEp24 (2 μm, *URA3*, Ap^r)	Laurent *et al.* (1992)
pDJ63	*sth1-Δ2::HIS3* in pUC19 (deletion of amino acids 1 to 1299)	This study
pDJ63-2	*sth1-Δ3::HIS3* in pUC19 (deletion of amino acids 249 to 882)	This study
p376-1-12	*sth1-ts* in pRS314	This study
p14-1-7	*sth1-ts* in pRS314	This study
p137-1-10	*sth1-3ts* in pRS314	This study
psth1-1ts	*sth1-1ts* in pRS306	This study
psth1-2ts	*sth1-2ts* in pRS306	This study
psth1-3ts	*sth1-3ts* in pRS306	This study
pDJ2ts	*sth1-2ts* in PRS316	This study
pDJ64	$Sth1_{1\text{-}1359}$ in pRS314	This study
pDJ66	$Sth1_{1\text{-}1337}$ in pRS316	This study
pDJ67	$Sth1_{1\text{-}1359}$ in pRS316	This study
pDJ84	GST-Sth $1_{288\text{-}1359}$ in pEG(KT)	This study
pDJ84KR	GST-Sth $1_{288\text{-}1359}$-K501R in pEG(KT)	This study
pDJ85	GST-$Sth1_{288\text{-}1014}$ in pEG(KT)	This study
pDJ86	GST-$Sth1_{288\text{-}1014}$-K501R in pEG(KT)	This study
pDJ87	GST-Sth1-$1_{288\text{-}1014}$ in pEG(KT)	This study
pDJ88	GST-Sth1-$2_{288\text{-}1014}$ in pEG(KT)	This study
pDJ89	GST-Sth1-$3_{288\text{-}1014}$ in pEG(KT)	This study
pIN11	*Stu*I-*Spe*I fragment generated by PCR amplification of pDJ67 primed by OLStuI (5′-GGCCAGAAGCGCT-3′) and OLSpeI (5′-CGACTAGTCACTCATTTAA-3′) replaced the *Stu*I-*Spe*I fragment of pDJ67 ($Sth1_{1\text{-}1348}$ in pRS316)	This study
pIN12	*Stu*I-*Spe*I fragment of pDJ64 ligated to the *Bam*HI-*Nhe*I fragment of pDJ64 and the *Stu*I-*Bam*HI fragment generated by PCR amplification of pDJ67 primed by OLStuI and OLBamHI (5′-CGGGATCCTGGATGGGAGTC-3′) ($Sth1_{\Delta 1274\text{-}1335}$)	This study
pIN14	*Eco*RI-*Xba*I of pIN12 replaced *Eco*RI-*Xba*I of pDJ67 ($Sth1_{\Delta 1274\text{-}1335}$ in pRS316)	This study
pIN15	*Stu*I-*Bam*HI fragment generated by PCR amplification of pDJ67 primed by OLStuI and OLBamHI replaced the *Stu*I-*Bam*HI fragment of pDJ67 ($Sth1_{1\text{-}1273}$ in pRS316)	This study
$psth1^{S806L}$	$Sth1_{1\text{-}1359}$-S806L in pRS316	This study
$psth1^{T881M}$	$Sth1_{1\text{-}1359}$-T881M in pRS316	This study
$psth1^{S806L,T881M}$	$Sth1_{1\text{-}1359}$-S806L, T881M in pRS316	This study
pEG(KT)	$P_{GAL1,10}$, GST, 2 μm, *URA3*, Ap^r	Mitchell *et al.* (1993)
pRS306	YIp derivative (*URA3*, Ap^r)	Sikorski and Hieter (1989)
pRS314	*CEN6*, *ARSH4*, *TRP1*, Ap^r	Sikorski and Hieter (1989)
pRS316	*CEN6*, *ARSH4*, *URA3*, Ap^r	Sikorski and Hieter (1989)
CP204	*HHF2*, *CEN*, *URA3*	Kruger *et al.* (1995)
CP206	*HHT2*, *CEN*, *URA3*	Kruger *et al.* (1995)
2-pRS313	*hht2-2*, *CEN*, *HIS3*	This work
3-pRS313	*hht2-3*, *CEN*, *HIS3*	This work
CP238	*hht2-2*, *CEN*, *URA3*	Kruger *et al.* (1995)
CP239	*hht2-3*, *CEN*, *URA3*	Kruger *et al.* (1995)
CP327	*hhf2-8*, *CEN*, *URA3*	Kruger *et al.* (1995)
FC204	*CLN3*, *CEN*, *URA3*	F. Cross
CLB2pic19	*CLB2*	B. Futcher

[a] $P_{GAL1,10}$, the *GAL1,10* promoter.

were grown in SC media lacking uracil and expression of the fusion proteins was induced by addition of 4% galactose. Exponentially growing cells at 30° were harvested by centrifugation, washed with sorbitol buffer [0.3 m sorbitol, 0.1 m NaCl, 5 mm $MgCl_2$, and 10 mm Tris-HCl (pH 7.4)] and resuspended in lysis buffer (sorbitol buffer containing 2 mm phenylmethylsulfonyl fluoride, 0.1 mm leupeptin, 0.1 mm aprotinin, and 0.001 mm pepstatin A). All steps were performed at 4° ac-

cording to the protocol described by Mitchell *et al.* (1993). Cells were broken by vortexing with glass beads, and protein concentration determined using a colorimetric assay (Bio-Rad, Richmond, CA) with bovine serum albumin as a standard. A 50% (w/v) slurry of glutathione agarose beads (Sigma, St. Louis) was added to protein extracts at a ratio of 10 μl slurry per milligram of protein. The solution was incubated for 30 min at 4° on a nutator. Proteins adsorbed to the beads were washed three times with sorbitol buffer including 1% NP-40 and once with sorbitol buffer alone. Adsorbed GST fusion proteins were released from the beads by elution buffer [10 mm glutathione, 50 mm Tris-HCl (pH 7.5), 5% glycerol, 5 mm DTT] twice at room temperature for 2 min. Beads were centrifuged at 8160 × *g* for 1 min in a microcentrifuge and the supernatants were collected and pooled. Proteins were analyzed by 6% SDS-polyacrylmide gel electrophoresis. As judged by Tris-Glycine polyacrylamide gel electrophoresis, the eluted GST-Sth1p fusion proteins constituted >85% of the total protein.

ATPase assay: The hydrolysis of ATP was measured as the release of inorganic phosphate, $^{32}P_i$, from [γ-^{32}P]ATP. The standard assay mixture (20 μl) contained 4 μl of reaction buffer A [50 mm $MgCl_2$, 250 mm NaCl, 25% glycerol, 0.1 mg/ml bovine serum albumin, 5 mm dithiothreitol, 5 mm ATP, 100 mm HEPES (pH 7.0)], 1 μCi of [γ-^{32}P]ATP (6000 Ci/mmol; Amersham Corp., Arlington Heights, IL), 9 μl of GST-Sth1p fusion protein (or an equal volume of the vector-only control) immobilized on agarose beads in sorbitol buffer or 9 μl of GST-Sth1p fusion protein in elution buffer (or an equal volume of the eluate from the vector-only control), and 6 μl of H_2O or nucleic acid.

After 30 min at 37°, the reaction was terminated by the addition of 1 μl of 0.5 m EDTA. A sample (1 μl) of the reaction mixture was then spotted onto a poly(ethyleneimine)-cellulose thin-layer chromatography plate (J. T. Baker, Phillipsburg, NJ or Selecto Scientific), and ascending chromatography was carried out in 0.8 m LiCl and 0.8 m acetic acid. The amount of $^{32}P_i$ formed was quantitated using a phosphorImager (Molecular Dynamics, Sunnyvale, CA) and normalized to the total amount of radioactivity applied to each lane. Double-stranded (ds) DNA was prepared from pBluescript KS− (Stratagene). Partially purified nucleosomal TRP1-ARS1-derived plasmid DNA (Roth *et al.* 1990) was also tested. Correctly positioned nucleosomes on the chromatin episome were confirmed by micrococcal nuclease digestion and indirect end-label analysis (B. C. Laurent, unpublished results). Alternatively, the hydrolysis of ATP was measured by a colorimetric assay (Lanzetta *et al.* 1979).

Disruption of the chromosomal *STH1* locus: Diploid strains BLY29 and BLY18 were transformed to histidine independence with the 2.3-kb *Eco*RI-*Xba*I fragment of pDJ63 and the 4.1-kb *Eco*RI-*Bam*HI fragment of pDJ63-2 to replace the wild-type *STH1* genes with *sth1-Δ2::HIS3* and *sth1-Δ3::HIS3* alleles, creating BLY30 and BLY81, respectively. The correct structures of gene replacements were confirmed by Southern blot analysis.

Isolation of temperature-sensitive alleles of *STH1*: The centromere-containing plasmid pDJ64 (*TRP1*) carrying the wild-type *STH1* gene was mutagenized *in vitro* by incubating 12 μg of plasmid DNA in a solution of 1 m hydroxylamine (Sigma), 50 mm sodium pyrophosphate (pH 7.0), 100 mm NaCl, and 2 mm EDTA for 1 hr at 75° as described (Sikorski and Boeke 1991). The solution was extracted twice with phenol:chloroform:isoamylalcohol (25:24:1) and precipitated twice with ethanol, and the excess hydroxylamine removed by gel filtration through a small Sephadex G-50 column. Mutagenized plasmid DNA in 10 mm Tris-HCl (pH 8.0), 1 mm EDTA was then used directly to transform BLRY30-1, a yeast strain carrying a deletion of the chromosomal *STH1* gene and a copy of the wild-type *STH1* gene on a second centromere-containing plasmid, pDJ67 (*URA3;* Sikorski and Boeke 1991). Approximately 6000 transformants grown selectively for both plasmids were replicated to supplemented synthetic complete (SC) medium containing 5-fluoroorotic acid to counterselect against the plasmid carrying the wild-type *STH1* gene. Transformants (2000) carrying only the mutagenized plasmid were screened for conditional growth (lethality at 37°). A total of 300 transformants showing a temperature-sensitive (Ts^-) phenotype were retested for conditional growth on nonselective (YPD) plates to eliminate cells bearing mutations in the plasmid-borne selectable marker gene. Forty-six mutants were conditional for growth also on YPD plates. Plasmid DNA was recovered from 10 of these colonies. Restriction fragment swapping experiments confirmed that three of the mutations are located within *STH1*. Plasmids p376-1-12, p14-1-7, and p137-1-10 from these temperature-sensitive strains were digested with *Nhe*I and *Xba*I to recover a 5.3-kb fragment containing the *sth1-1ts*, *sth1-2ts*, and *sth1-3ts* mutant alleles, respectively. These fragments were cloned into the integrating vector pRS306 (*URA3*), creating psth1-1ts, psth1-2ts, and psth1-3ts, which were then used to replace the wild-type *STH1* locus by transformation and subsequent growth on SC 5-fluoroorotic acid plates. Temperature-sensitive and Ura^- colonies were analyzed by genetic complementation to confirm integration of the *sth1-ts* alleles; pDJ67 (*STH1 CEN6*) and pBL50 (*STH1* 2 μm) complemented the growth defect at 37°. Genetic crosses to an isogenic *STH1* strain confirmed that all the mutations are recessive. p14-1-7 was digested with *Eco*RI and *Sma*I and the recovered 6.4-kb fragment was cloned into pRS316 to create pDJ2ts.

The locations of each mutation were determined by interchanging fragments of the wild-type *STH1* gene with homologous fragments of the mutant allele. The 1.1-kb *Bgl*II-*Age*I fragments of p376-1-12 and p14-1-7 and the 1-kb *Age*I-*Stu*I fragment of p137-1-10 were subcloned into pUC19 and sequenced by the dideoxy-chain termination method (Sanger *et al.* 1977) with a Sequenase 2.0 kit (United States Biochemical, Cleveland). As confirmation, "hot start" PCR amplification (Chou *et al.* 1992) using ampliwax PCR gems (Perkin Elmer, Norwalk, CT) was first performed directly on genomic DNA isolated from the *sth1-1ts* and *sth1-3ts* strains or from psth1-1ts, psth1-2ts, and psth1-3ts with primers MK011 (5′-TCAAT TACGAGGTTTAGAATGGATG-3′) and MK012 (5′-TTTGT CCAATTCTGTGAGCTCTATC-3′). The PCR products were purified by Wizard PCR preps (Promega, Madison, WI) and then analyzed directly by thermal cycle sequencing using SequiTherm DNA polymerase (Epicentre Technologies, Madison, WI) and primers MK011, MK012, and MK016 (5′-ATGC TCAATCGAAGTTATCATTCAC-3′) to detect the *sth1-1ts*, *sth1-3ts*, and *sth1-2ts* lesions, respectively. Allele-specific PCR amplification (Ault *et al.* 1993) of genomic DNA from BLY48 (*sth1-2ts*) using primers MK013 (5′-CGTTAATTCTAGTTTCTCT TGGGTACC-3′) and MK030 (5′-GCACTGTTGAACTTTGTT TTGC**T**-3′; the C to T substitution mutation is set boldface) confirmed the presence of the *sth1-2ts* mutation. The nucleotide changes and predicted amino acid changes are as follows: *sth1-1ts* is C-1514 to T, S505F; *sth1-2ts*, C-1937 to T, P646L; and *sth1-3ts*, C-2417 to T and C-2642 to T, S806L and T881M, respectively.

Budding morphology: To determine the mitotic index, cells grown to midlog phase at 30° were diluted to a density of 4 × 10^6 cells/ml into YPD medium prewarmed to 37°. We found that the phenotype of cells diluted first into prewarmed YPD and then shifted to 37° was more dramatic than that of cells shifted directly to 37°. At several time points, aliquots were removed, fixed with 3.7% formaldehyde in PBS [100 mm NaCl

(80 mm Na_2HPO_4, 20 mm NaH_2PO_4, pH 7.3)], and the number of single, small-budded, and large-budded cells was determined microscopically.

DNA flow cytometry: Early- to midlog phase cultures of temperature-sensitive and isogenic wild-type strains grown in YPD medium with 2% glucose at 30° were split and diluted into prewarmed 30° or 37° YPD media to concentrations of approximately 4×10^6 cells/ml. At various time intervals, approximately 2×10^7 cells were removed, sonicated briefly, and fixed in 70% ethanol at room temperature for 12 hr. After two washes in 50 mm Tris-HCl (pH 7.8), fixed cells were resuspended in 1 ml of the same buffer containing 2 mg/ml RNAse A and incubated at 37° for 12 hr on a nutator. Cells were pelleted, resuspended in 0.5 ml of 55 mm HCl containing 5 mg/ml pepsin (Sigma), and incubated at 37° for 30 min. Cells were washed once in 1 ml of TMN buffer [200 mm Tris-HCl (pH 7.5), 211 mm NaCl, and 78 mm $MgCl_2$] and then resuspended in TMN buffer containing 50 μg/ml propidium iodide (Sigma). The fluorescence intensities of stained cells were measured after 10–30 min on a Becton Dickinson (San Jose, CA) 900 FACScan machine.

RNA analysis: Cells were grown in YPD liquid media to a concentration of $0.5–1.0 \times 10^8$ cells/ml, and total RNA was isolated using RNeasy spin columns according to the manufacturer's protocol (QIAGEN Inc., Chatsworth, CA) and quantitated by measuring absorbence at 260 nm. RNAs (20 μg) were size fractionated in a 1.5% agarose gel containing formaldehyde and transferred to a GeneScreen membrane (DuPont/New England Nuclear, Boston) and UV cross-linked. The filter was successively hybridized to ^{32}P-labeled probes prepared by PCR amplification of CLB2pic19, genomic (S288C) DNA, or FC204 primed by the primer pairs CLB2-1 (5′-GGCGTT GGATCAAGCACTGAGGTAG-3′) and CLB2-2 (5′-GCCCCT CTTCTCATTCATGCAAGGTC-3′), CSE4-1 (5′-GAAGATCA CTCAGTAACGTCAACAGGC-3′) and CSE4-2 (5′-GCGGAGA GCACTTGTCGAACC3′), and CLN3-1 (5′-ATGGCCATATTG AAGGATACC-3′) and CLN3-2 (5′-CAGCGAGTTTTCTTG AGGTTGCTAC-3′) to generate PCR fragments of *CLB2*, *CSE4*, and *CLN3*, respectively. All probes were labeled by random priming. Filters were hybridized in 5× SSC, 50 mm sodium phosphate (pH 7.0), 1× Denhardt's solution, 0.1% SDS, 100 μg/ml denatured salmon sperm DNA, and 5% formamide at 42° for 12–16 hr, followed by three 15-min washes with 0.1× SSC and 0.1% SDS at 50°.

Immunofluorescence microscopy: Cells grown to early- or midlog phase were fixed with formaldehyde for 2 hr and processed for indirect immunofluorescence microscopy as described (Pringle *et al.* 1991). Microtubule structures were observed by staining with YOL1/34 rat monoclonal anti-yeast α-tubulin primary antibody (Sera Lab) diluted 1:100 and fluorescein isothiocyanate-conjugated goat anti-rat immunoglobulin G secondary antibody (Sigma) diluted 1:100. DNA was visualized by staining with 4′,6-diamidino-2-phenylindole (DAPI). Epifluorescence and Nomarski differential interference contrast photomicrography were performed with a Nikon Labophoto-II photomicroscope.

RESULTS

Sth1p has DNA-stimulated ATPase activity: To determine whether Sth1p has ATPase activity, the conserved C-terminal 1072 amino acids of the protein including the central 518-amino-acid ATPase/helicase-related domain (Laurent *et al.* 1992, 1993) were expressed in yeast. As a negative control, an analogous mutant fusion protein containing a single amino acid substitution (K501R) within the NTP-binding motif (Walker *et al.* 1982), which abolishes *STH1* function *in vivo* (Laurent *et al.* 1992), was also expressed.

The wild-type protein preparation exhibited ATPase activity with a specific activity of 355 pmol of inorganic phosphate (P_i) released per microgram per minute in the absence of added nucleic acid, an activity that was sixfold higher than that of the mutant protein or the GST moiety alone (Figure 1). The hydrolysis activities associated with GST and GST-Sth1K501R preparations are probably due to the presence of copurifying contaminants. Partially purified nucleosomal DNA and dsDNA stimulated the ATPase activity modestly (Figure 1). The ATPase activity of GST-Sth1p in the absence of added DNA suggests that this preparation contains nucleic acid. Prior treatment of a preparation of GST-Sth1p with DNase I did not significantly reduce the ATPase activity, nor did addition of dsDNA stimulate GST-Sth1p activity following DNAse I treatment (data not shown), although we cannot rule out the possibility that DNA bound by GST-Sth1p is inaccessible to DNase I. The specific activity of the Sth1p ATPase, 124 pmol P_i/pmol protein/min in the presence of DNA, is comparable to that of the purified Snf/Swi (Cairns *et al.* 1994; Côté *et al.* 1994) and RSC (Cairns *et al.* 1996) complexes, with an optimal NaCl or KCl concentration of 50–150 mm, an optimal $MgCl_2$ concentration of 1–2 mm, and an optimal pH range of pH 6.0–8.0. The finding that none of the other putative components of RSC has homology to DNA-dependent ATPases (B. R. Cairns,

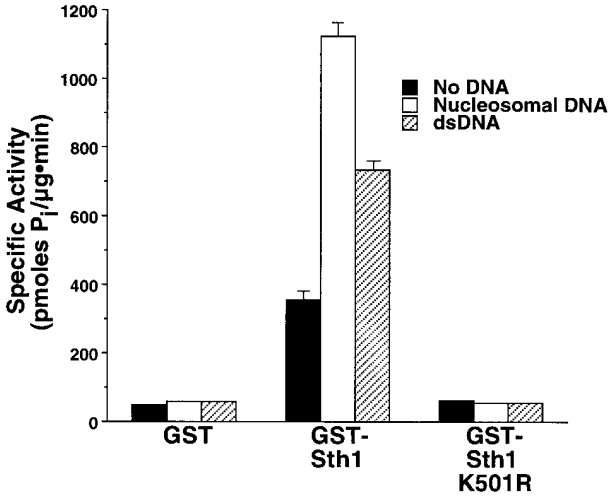

Figure 1.—The GST-Sth1p fusion protein has DNA-stimulated ATPase activity. GST, GST-Sth1, and GST-Sth1K501R (0.08 μg of each) proteins expressed in yeast were assayed for ATPase activity in the absence and in the presence of added nucleic acid (see materials and methods). Incubation was for 30 min. Reaction mixtures contained no nucleic acid, partially purified nucleosomal DNA (1 μg), or dsDNA (1 μg). The hydrolysis of ATP was linear with time up to 60 min (data not shown). A background hydrolysis of 13 pmol/μl of assay mixture was subtracted from all samples. Error bars were calculated from three separate assays.

personal communication) together with the similarities of the biochemical properties of the Sth1p ATPase to those of purified RSC, strongly suggest that Sth1p provides RSC with the ATP hydrolysis activity.

In addition, a GST-Sth1p fusion protein expressing the ATPase-related domain alone possessed ATPase activity comparable to that of GST-Sth1 (data not shown), suggesting that the C-terminal 346 amino acids, including the bromodomain, are dispensable for ATP hydrolysis activity *in vitro*. In summary, the *in vitro* biochemical data agree with the genetic evidence that the K501R mutation abolishes *STH1* function *in vivo* (Laurent *et al.* 1992), indicating that the ATPase activity is required for RSC function *in vivo*.

Temperature-sensitive mutations in *STH1* map to the conserved ATPase/helicase-related region: To characterize the phenotype of cells defective in *STH1*, temperature-sensitive mutations were generated by random mutagenesis of the cloned gene. Three independent temperature-sensitive *sth1* mutations were identified in a screen of ~2000 transformants using the plasmid shuffle technique (Sikorski and Boeke 1991). The integrated alleles segregated 2+ : 2− for growth at 37° in crosses to a wild-type strain. All of the mutations are recessive and were complemented by plasmids containing the wild-type *STH1* gene.

The three mutants, *sth1-1ts*, *sth1-2ts*, and *sth1-3ts*, displayed a range of different growth phenotypes at permissive (30°) and nonpermissive (37°) temperatures relative to the isogenic wild-type strains (Figure 2A). None of these *sth1-ts* mutants appears to show a first cycle arrest and the *sth1-2ts* and *sth1-3ts* alleles are more severe than the *sth1-1ts* allele (Figure 2A). Significantly, all of the mutations alter amino acids in the highly conserved ATPase/helicase-related domain and these residues lie within or between the signature helicase motifs (Figure 2, B and C; Gorbalenya *et al.* 1989; Bork and Koonin 1993; Henikoff 1993). Each of the amino acids mutated is highly conserved among all members of the Snf2 protein subfamily, further indicating the functional importance of conserved sequences within the ATPase-related domain.

sth1-1ts and *sth1-3ts* mutants incubated at 37° expressed Sth1p levels that were similar to those at 30°, and *sth1-2ts* mutants expressed Sth1p levels that were only three- to fivefold reduced compared to Sth1p levels at 30° (data not shown). Sth1p levels in the three *sth1-ts* mutants grown at 30° were comparable to those in wild-type cells grown at 30° or 37°. These results suggest that the arrest phenotypes of *sth1-ts* mutants reflect temperature-dependent conformational changes induced at the nonpermissive temperature, rather than proteolysis of the mutant proteins.

The Sth1-1, Sth1-2, and Sth1-3 proteins have wild-type ATPase activity *in vitro*: Because each of the *sth1-ts* alleles alters amino acids within the conserved ATPase/helicase-related domain, which alone is sufficient for ATP hydrolysis *in vitro*, we tested whether the mutations affect ATP hydrolysis activity. The specific activities of partially purified preparations of GST-ATPase/helicase-related domain fusion proteins expressing the S505F, P646L, S806L, and T881M substitutions were 120, 115, and 166 pmol P_i/pmol protein/min, respectively, comparable to that of the wild-type GST-Sth1p (124 pmol P_i/pmol protein/min). This result indicates that these mutations do not affect the ability of Sth1p to hydrolyze ATP *in vitro* and suggests further that this domain carries out other functions in addition to ATP hydrolysis.

***sth1-ts* mutants exhibit cell cycle and non-cell cycle phenotypes:** To determine whether *sth1-ts* mutants show morphological abnormalities, cultures of logarithmically growing *sth1-1*, *sth1-2*, and *sth1-3* temperature-sensitive mutants were diluted into prewarmed 30° or 37° YPD and examined microscopically at several time intervals. One of the mutants, *sth1-3ts*, exhibited a dramatic increase in the number of large-budded cells when grown at the nonpermissive temperature. At least 75% of *sth1-3ts* cells arrested with a budded phenotype and of these, 60% were large budded (Figure 3A), suggesting a block in mitosis specifically at the G2/M transition. These large-budded cells exhibited a heterogenous morphology: approximately half contained a divided nucleus, and half contained nuclei that were bilobed and extended across the bud neck (Figure 3B), characteristic of cells in G_2 or M phase. This suggests that in a large fraction of *sth1-3ts* mutant cells the DNA was replicated (the nucleus had divided) but that cell division had not occurred. Indirect immunofluorescence microscopy of anti-α-tubulin-stained *sth1-3ts* mutant cells showed shortened intranuclear microtubule spindles compared to *sth1-3ts* cells grown at 30° (Figure 3B) or to wild-type cells at 30° or 37° (data not shown), indicating that poleward elongation of microtubule spindles does not occur in *sth1-3ts* cells. Examination of the DNA content of *sth1-3ts* cells by flow cytometry confirmed a perturbation of the cell cycle distribution (Figure 3C). Together, these results suggest that *STH1* is required for progression of cells through the cell cycle, specifically through the G_2/M phase transition.

To compare the phenotype exerted by the *sth1-3ts* allele to the phenotype caused by an *sth1* null allele in an isogenic background (S288C), we transformed diploid strain BLY81 (*sth1-Δ3*/*STH1 ura3*/*ura3*) with a *URA3* centromere-derived plasmid carrying the wild-type *STH1* gene expressed from the inducible *GAL10* promoter. Following 12 hr of growth in 2% glucose, 73% of an *sth1-Δ3* haploid strain carrying the *GAL10-STH1* plasmid for viability arrested with a budded phenotype (data not shown), a bud morphology bias very similar to what we observed following a shift of the conditional *sth1-3ts* mutant to the nonpermissive temperature. A previously described *nps1*/*sth1* deletion allele also resulted in G_2 arrest, in *STH1* depletion experiments of similar design (Tsuchiya *et al.* 1992). This

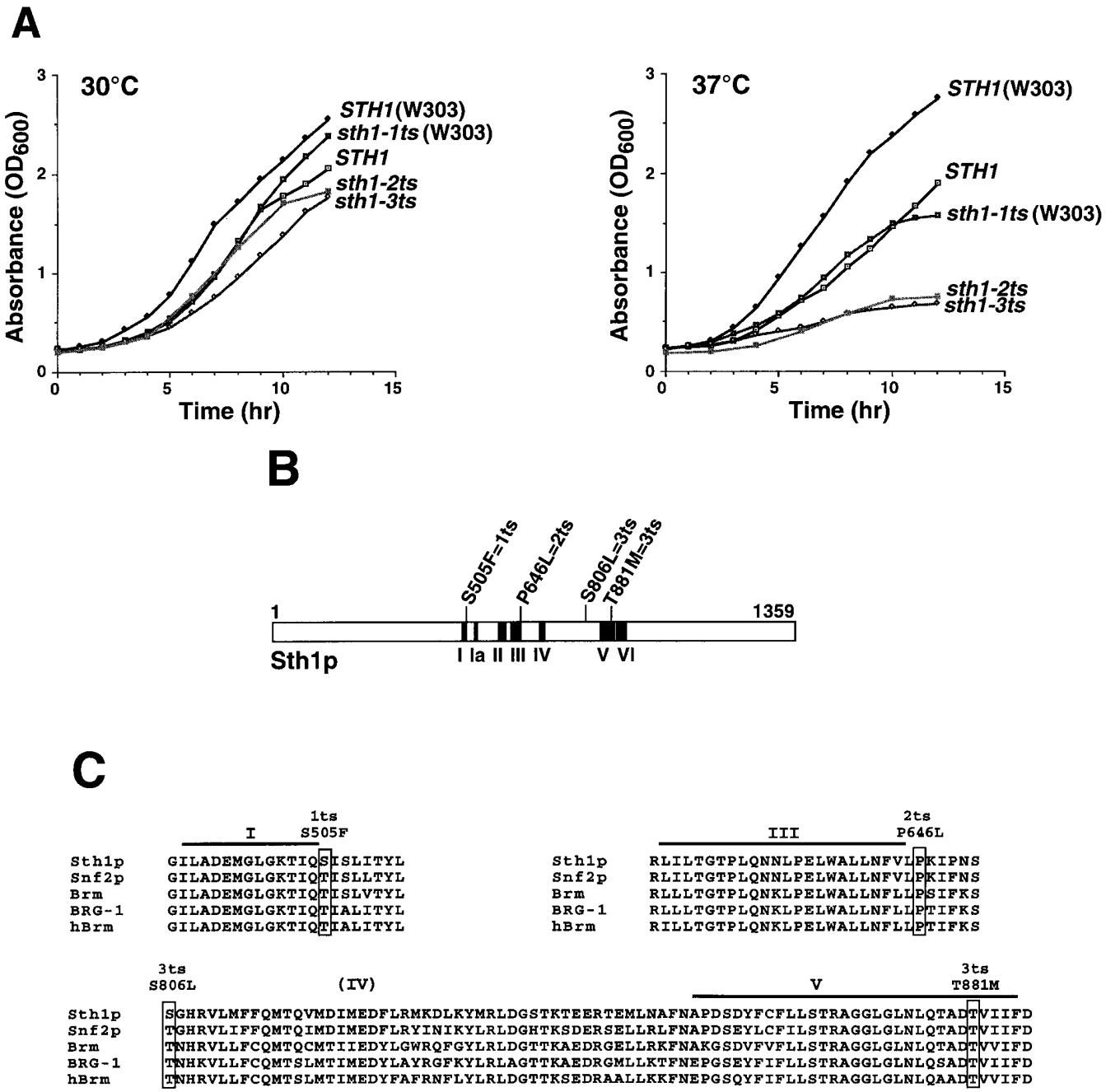

Figure 2.—Identification of *sth1* temperature-sensitive mutations. (A) Growth phenotypes of the *sth1-ts* mutants at 30° and 37°. Log-phase cultures of *STH1*(W303) (BLY46), *STH1* (BLY76), *sth1-1ts*(W303) (BLY47), *sth1-2ts* (BLY48), and *sth1-3ts* (BLY49) strains grown in YPD media at 30° were shifted to 37°. Aliquots were removed at regular intervals and optical density at 600 nm measured. OD_{600} values are plotted *vs.* time of incubation. BLY46 and BLY47, W303 genetic background; all other strains are derived from the S288C background. (B) Schematic representation of the Sth1 protein. The signature helicase motifs (I–VI as defined by Bork and Koonin (1993) and the positions of mutations and the amino acid changes are indicated. (C) The alignment of amino acid sequences from three of the highly conserved regions of *S. cerevisiae* Sth1p and Snf2p, *D. melanogaster* brm, and human BRG1 and hbrm proteins. Sequences that correspond to consensus motifs identified in families that include DNA helicases are overlined by a solid line. (IV), a sequence motif highly conserved among the proteins most closely related to Sth1p located between consensus motifs IV and V. The amino acids mutated in each of the *sth1-ts* alleles are boxed. Alignments were by the Clustal unweighted pair group method using the PAM250 residue weight table of MEGALIGN (DNASTAR).

result indicates that the phenotype caused by the *sth1-3ts* mutation is indistinguishable from that caused by an *sth1* null mutation in cells of the S288C genetic background.

In contrast to the *sth1-3ts* allele, the *sth1-1ts* and *sth1-2ts* mutations arrested cells at random positions throughout the cell cycle and flow cytometric analysis of the arrested mutants confirmed the random cell type

distribution (data not shown). The similar growth defects of *sth1-3ts* and *sth1-2ts* mutants argue against the lesser penetrance of the temperature-sensitive defect in *sth1-2ts* mutants. In addition, the primary defects of the *sth1-1ts* and *sth1-2ts* mutations do not appear to affect synthesis of the gene product. We favor the model that the *sth1-1ts* and *sth1-2ts* mutations are defective in additional essential function(s) of Sth1p required throughout the cell cycle, although it is possible that Sth1p has instead a single important cellular function at G_2/M and that the *sth1-1ts* and *sth1-2ts* are leaky hypomorphic alleles.

The *sth1*^{S806L} and *sth1*^{T881M} mutations function synergistically: To further analyze the nature of the *sth1-3ts* temperature-sensitive growth defects and to determine the relative contributions of each point substitution to the phenotype, we constructed centromere (*URA3*) plasmids bearing each of the two *sth1-3ts* point mutations, designated psth1^{S806L} and psth1^{T881M}, and a plasmid containing both of the mutations, psth1^{S806L,T881M}. Diploid strain BLY81 (*sth1-Δ3/STH1 ura3/ura3*) was transformed with each of these plasmids, and Ura$^+$ transformants were sporulated and subjected to tetrad dissection to recover *sth1-Δ3* haploid strains expressing these plasmids. *sth1*^{T881M} cells were moderately temperature-sensitive (Ts$^-$) for growth, but much less so than *sth1*^{S806L,T881M} cells (Figure 3D), which had a terminal arrest morphology similar to that of arrested *sth1-3ts* cells. Significantly, *sth1*^{S806L} cells grew as well as cells carrying the wild-type *STH1* gene at both temperatures (Figure 3D). These results suggest that the two mutations function synergistically to confer the growth defects of *sth1-3ts* cells.

The bromodomain of Sth1p is required for wild-type function: The presence of several highly conserved sequences suggests that Sth1p contains discrete functional domains. In addition to the ATPase/helicase-related domain, the evolutionarily conserved bromodomain at the C terminus is a candidate for one such domain. The functional importance of the bromodomain, which extends from amino acids 1274 to 1359, including a 22-amino-acid extension predicted to form a third α-helix, here designated α3 and recently called helix C (Jeanmougin *et al.* 1997; Figure 4A), was investigated by transforming diploid strain BLY81 (*sth1-Δ3/STH1 ura3/ura3*) with centromere (*URA3*) plasmids pDJ66, pIN11, pIN14, and pIN15 carrying the *sth1Δ1338*, *sth1Δ1349*, *sth1Δ1274-1335*, and *sth1Δ1274* deletion alleles, respectively (Figure 4A). Ura$^+$ transformants were sporulated and subjected to tetrad analysis to recover *sth1-Δ3* spore clones carrying *sth1* or *STH1* plasmids for viability. All *sth1* segregants were thermosensitive for growth on YPD, although the mutants displayed a range of temperature sensitivity (Figure 4, A and B). Segregants carrying *sth1Δ1338* and *sth1Δ1349* were extremely thermosensitive and failed to grow at 37°, and those carrying *sth1Δ1274-1335* exhibited moderate Ts$^-$ growth defects. *sth1Δ1274* segregants displayed Ts$^-$ defects intermediate between those of *sth1Δ1338*/*sth1Δ1349* and *sth1Δ-1274-1335* (Figure 4, A and B).

To understand the nature of the Ts$^-$ growth defects, cells were analyzed microscopically for morphological differences after a shift to 37°. *sth1-Δ3* cells carrying *sth1Δ1338* or *sth1Δ1349* exhibited an extremely tight growth arrest and exhibited an unusual morphological defect. Eighty-one percent of the arrested *sth1Δ1338* cells have a budded phenotype: 11% are small budded, 39% are large budded, and 31% have unusual, highly elongated buds (Figure 4C). Approximately one half of the population of elongated cells is multiply budded at 37° (see Figure 4C). DAPI staining revealed that the majority of cells with elongated buds contained stained nuclei at the mother bud neck (Figure 4C), indicating a delay in nuclear division. *sth1Δ1349* cells displayed a similar, although less pronounced terminal arrest morphology (data not shown). *sth1* cells carrying *sth1Δ1338* or *sth1Δ1349* grown permissively were larger than *sth1* cells expressing wild-type Sth1p but did not contain abnormal elongated buds (Figure 4C; data not shown). Importantly, despite the thermosensitivity of cells lacking Sth1p amino acids 1274 to 1335 or 1274 to 1359, these cells do not arrest their growth at the nonpermissive temperature and exhibit nearly wild-type budding morphologies, with no elongated or multiple buds (data not shown).

To test the possibility that the arrest of *sth1Δ1338* and *sth1Δ1349* cells reflects a block in the cell cycle at G_2 or M phases, these cells were examined by flow cytometry at several time points after a shift from 30° to 37°. The majority of *sth1Δ* cells carrying either *sth1Δ1338* or *sth1Δ1349* contained a 2C DNA content (Figure 4D). Even at the permissive temperature, a significant proportion of the mutants were large budded and had G_2/M DNA contents (Figure 4, C and D), indicating a delay in mitosis. Thus, these *sth1* cells undergo DNA replication and nuclear migration to the mother bud neck, consistent with arrest in the cell division cycle at the G_2/M boundary.

Levels of the C-terminally truncated Sth1 proteins detected in *sth1* cells carrying *sth1Δ1338*, *sth1Δ1349*, *sth1Δ1274-1335*, or *sth1Δ1274* grown at 30° or 37° were comparable to those of the full-length Sth1p, as judged by immunoblotting using anti-Sth1p antibody (data not shown). Together, these results indicate that the bromodomain is required for wild-type function. In other experiments, we showed that the *sth1Δ1338* allele on a high copy plasmid introduced into a wild-type strain does not confer a dominant negative phenotype (data not shown). The morphological differences of cells expressing *sth1Δ1338* or *sth1Δ1349*, which lack all or part of the predicted α3 helix, respectively, compared to those expressing *sth1Δ1274-1335* or *sth1Δ1274*, which lack either two or all three of the predicted bromodomain α-helices, respectively, further suggest a critical

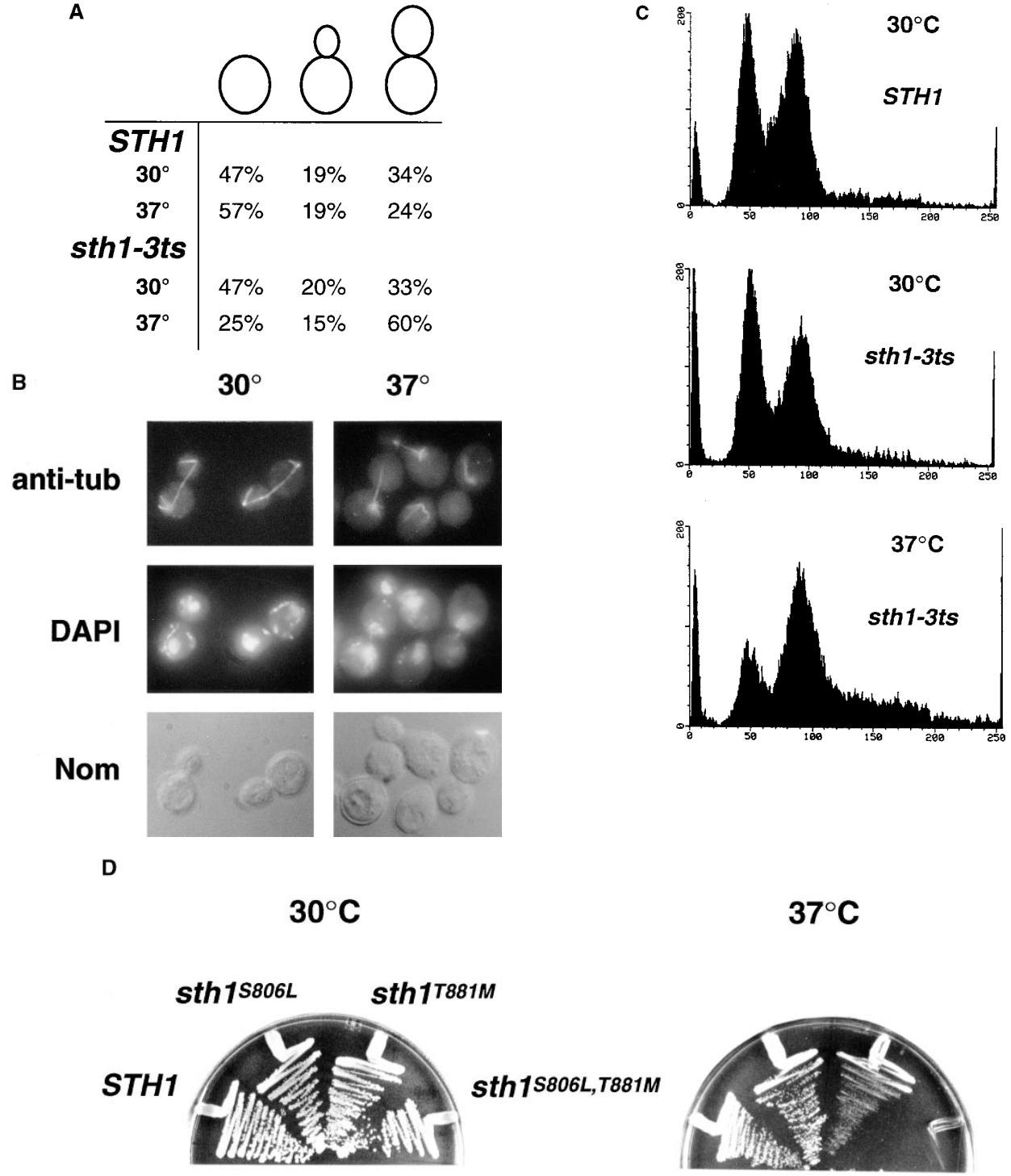

Figure 3.—Altered cell cycle distribution in *sth1-3ts* mutant cells. (A) The budding morphology of *sth1-3ts* mutant cells. The distribution of single, small-budded, and large-budded cells is compared for *sth1-3ts* cells and wild-type cells grown at 30° and 37°. Logarithmically growing *sth1-3ts* and wild-type cells (for comparison) were split and diluted to a concentration of 4×10^6 cells/ml and maintained in prewarmed 30° or 37° YPD media. Aliquots of cells were removed at regular time intervals (shown here, 9 hr for *sth1-3ts* cells grown at 30° and 37°; 6 hr for *STH1* cells grown at both 30° and 37°) for analysis. Similar results were obtained in at least four different experiments scoring approximately 200 to 400 cells of each sample. Strains used were BLY49 (*sth1-3ts*) and BLY76 (*STH1*). (B) Aberrant morphology of *sth1-3ts* mutant cells. *sth1-3ts* strain (BLY49) was grown in YPD at 30° or 37°, as described above. Cells were examined by Nomarski differential interference contrast micrography (Nom), DAPI staining of DNA (DAPI), and indirect immunofluorescent staining of microtubules (anti-tub). A field of *sth1-3ts* cells grown at 30° with fully elongated spindles is shown for comparison. *sth1-3ts* cells grown at 37° are larger than cells grown at 30°. Nomarski optics, DAPI, and microtubule staining of isogenic wild-type strain BLY76 grown at 30° or 37° were indistinguishable from BLY49 grown at 30° (data not shown). Magnification, ×100 (all panels). (C) Flow cytometric analysis of *sth1-3ts* and wild-type cells grown at 30° or 37°. Cells were treated exactly as in A. Aliquots from cultures were removed at the same time points as in A and analyzed

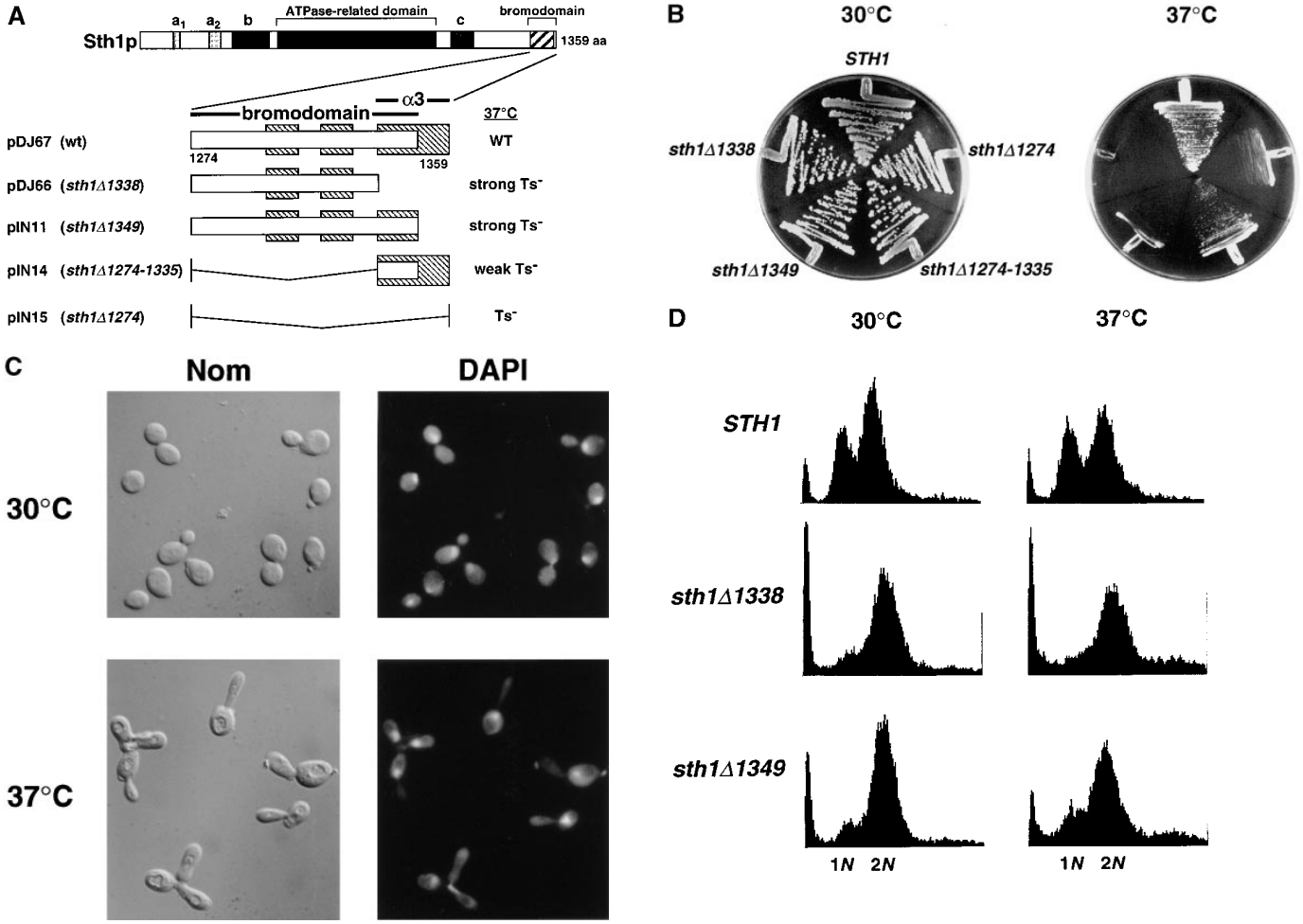

Figure 4.—The bromodomain of Sth1p is required for function. (A) A schematic diagram of the Sth1 protein is shown. Highly conserved regions shared by the Sth1p, Snf2p, brm, BRG1, and hbrm proteins are boxed and labeled a_1, a_2, b, ATPase-related domain, c, and bromodomain. a_1 includes residues 108–127; a_2, residues 225–263; b, residues 291–420; ATPase-related domain, residues 447–966; c, residues 1021–1106; and the bromodomain, as defined originally, includes residues 1274–1348 (Tamkun *et al.* 1992), and with the α3 helix extension, includes residues 1274–1359. Growth on YPD plates of *sth1-Δ3* cells expressing wild-type Sth1p from the centromere plasmid pDJ67 or C-terminally truncated Sth1 proteins from centromere plasmids pDJ66, pIN11, pIN14, and pIN15 was compared at 37° and scored, in decreasing ability to grow on YPD, as WT > weak Ts^- > Ts^- > strong Ts^-. The structures of the encoded Sth1 proteins are shown for the region between amino acids 1274 and 1359, and *sth1* deletion alleles are indicated within parentheses. α3, the C terminus of three α helices (denoted by cross-hatched boxes) predicted by the bromodomain motif, including amino acids 1338–1359. (B) Diploid strain BLY81 (*sth1-Δ3/STH1 ura3/ura3*) was transformed with pDJ67 (*STH1*), pDJ66 (*sth1Δ1338*), pIN11 (*sth1Δ1349*), pIN14 (*sth1Δ1274-1335*), or pIN15 (*sth1Δ1274*) to uracil prototrophy, and the transformants sporulated and subjected to tetrad dissection. *sth1* spore clones carrying the various plasmids for viability were streaked to single colonies on YPD plates and incubated at 30° and 37°. *sth1* cells carrying the deletion plasmids grew slower than those carrying wild-type *STH1* even at 30°; the 30° and 37° plates were photographed after 2 days of growth. Strains used were BLRY139 (*STH1*), BLRY117 (*sth1Δ1338*), BLRY116 (*sth1Δ1349*), BLRY118 (*sth1Δ1274-1335*), and BLRY119 (*sth1Δ1274*). (C) Aberrant morphology of *sth1* cells carrying carboxy-terminal deletions of Sth1p. Log-phase cultures of *sth1-Δ3* cells expressing pDJ66 (BLRY117) were grown in YPD at 30°, shifted to 37° for 6 hr, and the cells examined by Nomarski differential interference contrast micrography (Nom) and DAPI staining of DNA. (D) Flow cytometric analysis of *sth1-Δ3* cells expressing pDJ67 (*STH1*), pDJ66 (*sth1Δ1338*), or pIN11 (*sth1Δ1349*) grown at 30° or 37°. Cells containing wild-type or temperature-sensitive deletion alleles of *STH1* were analyzed 6 hr after being shifted from 30° to 37°. The number of cells, depicted on the vertical axis, is plotted *vs.* the fluorescent intensity of emitted light on the horizontal axis. Strains used were BLRY139 (*STH1*), BLRY117 (*sth1Δ1338*), and BLRY116 (*sth1Δ1349*).

by flow cytometry. The number of cells, depicted on the vertical axis (arbitrary units 0 to 200), is plotted *vs.* the fluorescent intensity of emitted light (proportional to the amount of DNA per cell) on the horizontal axis (arbitrary units 0 to 250). Strains used were *STH1* (BLY76) and *sth1-3ts* (BLY49). (D) The *sth1-3ts* point substitutions function synergistically. *sth1Δ* cells carrying centromere plasmids expressing $sth1^{S806L}$, $sth1^{T881M}$, $sth1^{S806L,T881M}$, or wild-type *STH1* were streaked to single colonies on YPD plates and compared for growth at permissive (30°) or nonpermissive (37°) temperatures. The strains used were BLRY139 (*STH1*), BLRY134 ($sth1^{S806L}$), BLRY135 ($sth1^{T881M}$), and BLRY136 ($sth1^{S806L,T881M}$).

role for the α3 helix in cells that also express the remaining sequences of the bromodomain.

Mitotic DNA damage checkpoint is not activated by the *sth1-3ts* or *sth1Δ1338* alleles: One hypothesis to explain the G_2/M arrest in strains with either the *sth1-3ts* or *sth1Δ1338* mutations is that lesions in Sth1p activate one or more cell cycle checkpoints. To test whether the arrest in either the *sth1-3ts* or *sth1Δ1338* cells requires the *RAD9* checkpoint, we compared the growth of *sth1-3ts* or *sth1Δ1338* single mutants with *sth1-3ts* or *sth1Δ1338* cells from which *RAD9* had been deleted, following a shift to 37°. The *RAD9* checkpoint is activated by damaged and incompletely replicated DNA (Weinert and Hartwell 1988). For example, *RAD9* is required for the G_2 arrest of cells with mutations in the genes coding for DNA ligase, DNA polymerase α, and DNA polymerase γ, and *rad9* cells carrying mutations of any of these genes exhibit greatly reduced viability (Weinert *et al.* 1994). The *rad9Δ* mutation did not alleviate the cell cycle arrest conferred by either of the *sth1-ts* alleles (data not shown). In addition, the viability of the *sth1-3ts rad9Δ* or *sth1Δ1338 rad9Δ* double mutants was comparable to that of the *sth1-3ts* or *sth1Δ1338* mutant (data not shown), indicating that the arrest of *sth1-3ts* or *sth1Δ1338* and the *RAD9* checkpoint were independent. We also tested whether mutation of a second G_2/M DNA damage and replication repair checkpoint gene, *RAD53*/*MEC2*, which encodes a protein kinase signal transducer believed to function downstream of Rad9p (Weinert *et al.* 1994; Elledge 1996), is required for the *sth1-3ts* arrest. The *mec2-1* allele also failed to alleviate the G_2/M arrest caused by *sth1-3ts* (data not shown) and did not enhance the lethality of *sth1-3ts* cells. These results indicate that the *RAD9* and *MEC2* genes necessary for monitoring DNA metabolism are not required for the *sth1-3ts* or *sth1Δ1338* arrest.

Transcription of genes required for cell cycle progression through G_2/M upon inactivation of *STH1*: Despite the inability of a LexA-Sth1p fusion protein to activate gene expression (Laurent *et al.* 1992), other roles for Sth1p in transcription are still possible. One explanation for the G_2/M arrest of *sth1-3ts* mutants shifted to the nonpermissive temperature is that *STH1* is required specifically for transcription of a gene or set of genes necessary for cell cycle progression. For example, temperature-sensitive mutations in $yTAF_{II}145$ that cause a G_1 arrest were shown to abolish transcription of the G_1/S cyclin genes *CLB5* and *CLB6* (Walker *et al.* 1997). To test whether Sth1p is required for expression of G_2/M-specific genes, we analyzed RNA levels of two important genes required for progression through the G_2/M transition, *CLB2* and *CSE4*, upon inactivation of *STH1*. *CLB2* encodes a B-type cyclin important for progression from G_2 into M and is transcribed only during G_2/M (Surana *et al.* 1991), and *CSE4* encodes a chromatin-associated protein necessary for the segregation of chromosomes, whose inactivation arrests cells in mitosis

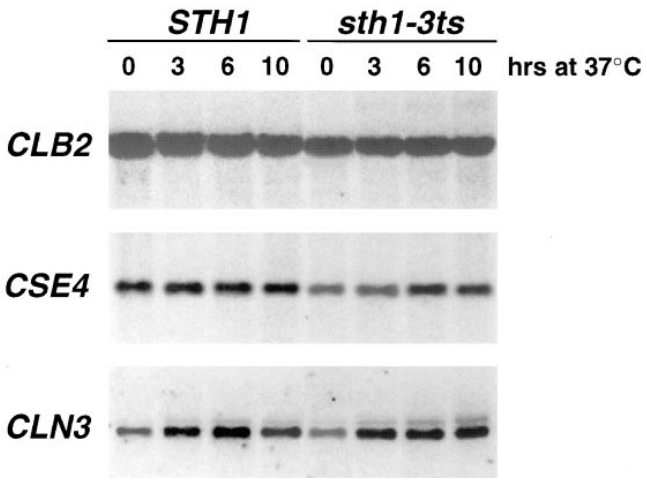

Figure 5.—Transcription of two genes required for progression through the G_2/M transition, *CLB2* and *CSE4*, is unaffected with inactivation of *STH1*. Total RNA was isolated from strains BLY1 (*STH1*) or BLY49 (*sth1-3ts*) following temperature shift and analyzed for transcription of the indicated genes by Northern blot analysis. *CLN3* RNA serves as a control for loading and transfer. The identity of the RNA that hybridizes weakly to the *CLN3* probe and migrates slightly slower than the *CLN3* RNA is unknown.

(Stoler *et al.* 1995). Transcription levels of *CLB2* and *CSE4*, and as a control, *CLN3*, which encodes a G_1 cyclin that is expressed constitutively (Nash *et al.* 1988), were unaffected in the *sth1-3ts* mutant at 3, 6, or 10 hr following a shift to the nonpermissive temperature (Figure 5). Thus, the G_2/M arrest in *sth1-3ts* cells cannot be explained by a specific defect in the transcription of either *CLB2* or *CSE4*.

***STH1* is required for nonglucose carbon source utilization:** To gain insight into the genetic targets of Sth1p, the growth phenotypes of *sth1-ts* mutants on fermentable and nonfermentable carbon sources other than glucose were tested at semipermissive temperatures (Figure 6). The mutants grew nearly as well as isogenic wild-type strains on glucose, but showed a variety of growth defects on other carbon sources (Figure 6). *sth1-1ts* mutants were defective for growth on ethanol + glycerol and significantly defective for growth on raffinose, sucrose, and galactose (Figure 6). *sth1-2ts* mutants were defective for growth on raffinose, but capable of some growth on sucrose, galactose, and ethanol + glycerol (Figure 6). *sth1-3ts* mutants were defective for growth on all nonglucose carbon sources tested, further distinguishing the three *sth1-ts* mutants. These pleiotropic defects suggest that Sth1p functions in a general cellular process affecting several metabolic pathways.

One explanation for the broad mutant phenotype is that Sth1p functions in some general aspect of transcription, like the Snf/Swi proteins, despite the finding that *STH1* function was not required for expression of the G_2/M-specific transcripts, *CLB2* or *CSE4*. Therefore, expression of the *SUC2* and *GAL10* promoters, under glu-

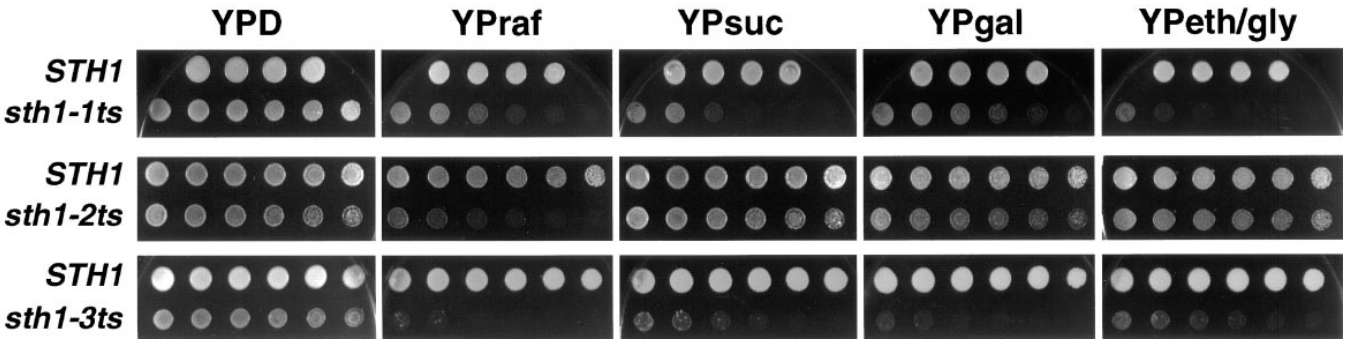

Figure 6.—The *sth1-ts* mutants show altered growth phenotypes on media containing nonglucose carbon sources. Twofold serial dilutions of log-phase *sth1-ts* and isogenic wild-type cells were plated onto rich medium 2% in glucose (YPD), raffinose (YPraf), sucrose (YPsuc), galactose (YPgal), or ethanol + glycerol (YPeth/gly) to determine growth at semipermissive temperatures (36.5° for *sth1-1ts* and 35° for *sth1-2ts* and *sth1-3ts*). Growth of the isogenic wild-type (*STH1*) cells is shown in the upper row of each panel. The plates were photographed after 2 to 4 days of growth. The strains used were BLY47 (*sth1-1ts*), BLRY181 (*sth1-2ts*), and BLY49 (*sth1-3ts*), and the paired isogenic wild-type strains were BLY46, BLRY139, and BLY76, respectively.

cose-derepressing or galactose-inducing conditions, respectively, was tested by quantitating β-galactosidase activities in *sth1-ts* strains carrying either a *snf/swi*-dependent *SUC2-LEU2-lacZ* reporter gene (Sarokin and Carlson 1985), or a *GAL10-lacZ* 2-μm reporter plasmid (West *et al.* 1984) grown at the permissive and nonpermissive temperatures. The *SUC2* reporter gene was capable of derepressed levels of *lacZ* expression in the *sth1-1ts* and *sth1-3ts* strains grown at 37° that were within twofold of those in the mutants grown at 30°, and levels of induced *lacZ* expression from the *GAL10-lacZ* reporter plasmid in the *sth1-1ts* and *sth1-3ts* mutants grown at 37° were reduced two- to threefold compared to 30° (data not shown). Taken together, these results indicate that *sth1-ts* mutants shifted to the nonpermissive temperature were only slightly defective in transcription of these promoters. The modest effects at *SUC2* and *GAL10* alone cannot therefore account for the conditional lethality caused by the *sth1-ts* alleles.

The *sth1-3ts* allele causes synthetic lethality and synthetic sickness with mutations in histone and nonhistone chromatin-associated proteins: Mutations in genes encoding histones and nonhistone chromatin-assembly proteins were shown previously to suppress the transcriptional and/or growth defects caused by *snf/swi* mutations. Because aspects of the chromatin-remodeling activities of Snf/Swi and RSC are similar (Cairns *et al.* 1996), we examined whether RSC interacted genetically with any of several previously identified mutations that suppress *snf/swi* mutations. Specifically, we tested whether the growth defects caused by the *sth1-3* temperature-sensitive mutation were suppressed by *spt6/ssn20-1*, Δ*hta1-htb1*, or point mutations in histone H3, *hht2-2* (E105K) and *hht2-3* (T118I), or histone H4, *hhf2-8* (V43I), although suppression of Ts^- growth is distinct from suppression of transcriptional defects. An *spt6* mutation was not capable of suppressing the lethality associated with a complete deletion of *STH1* (Laurent *et al.* 1992).

Surprisingly, we found that the *sth1-3ts spt6/ssn20-1* double mutants exhibited more severe growth defects than the *sth1-3ts* or *spt6/ssn20-1* mutant (Figure 7A, compare row 4 with rows 2 and 3), suggesting that the *spt6/ssn20-1* mutation functions synergistically with the *sth1-3ts* mutation. In contrast, deletion of one of the copies of histones H2A and H2B neither suppressed nor enhanced the *sth1-3ts* phenotype (Figure 7A, compare row 6 with rows 2 and 5).

The partially dominant H3 mutation, *hht2-2* (E105K), also caused a synthetic sickness with the *sth1-3ts* mutation (Figure 7B, compare row 4 with rows 2 and 3), although a second partially dominant H3 mutation, *hht2-3* (T118I), did not (Figure 7B, compare row 5 with rows 3 and 4). The *hht2-2* and *hht2-3* mutations in an *STH1* background grew as well as wild-type strain BLY76 at all temperatures assayed (Figure 7A, row 1; data not shown). We next tested the interactions between *sth1-3ts* and the H3 point mutations in strains in which the mutant variants were the sole source of *HHT2* by transforming an *hht2*Δ *sth1-3ts* double mutant with low-copy plasmids expressing the *hht2-2* or *hht2-3* alleles. Importantly, the *sth1-3ts hht2*Δ double mutant recovered from the diploid BLY49-2D × BLY157 displayed growth defects that were more severe than those of either *sth1-3ts* or *hht2*Δ (Figure 7B, compare row 7 with rows 2 and 6), suggesting that like the partially dominant *hht2-2* point mutation and *ssn20-1*, deletion of *HHT2* caused synthetic sickness with *sth1-3ts*. Significantly, we were unable to recover viable *sth1-3ts hht2*Δ cells transformed with the *hht2-2* plasmid, suggesting that the *sth1-3ts hht2-2* double mutant was synthetically lethal. Colonies of 100–200 cells were detected microscopically after 4-day growth on transformation plates at 30°, but these cells were inviable (data not shown). *sth1-3ts hht2*Δ cells trans-

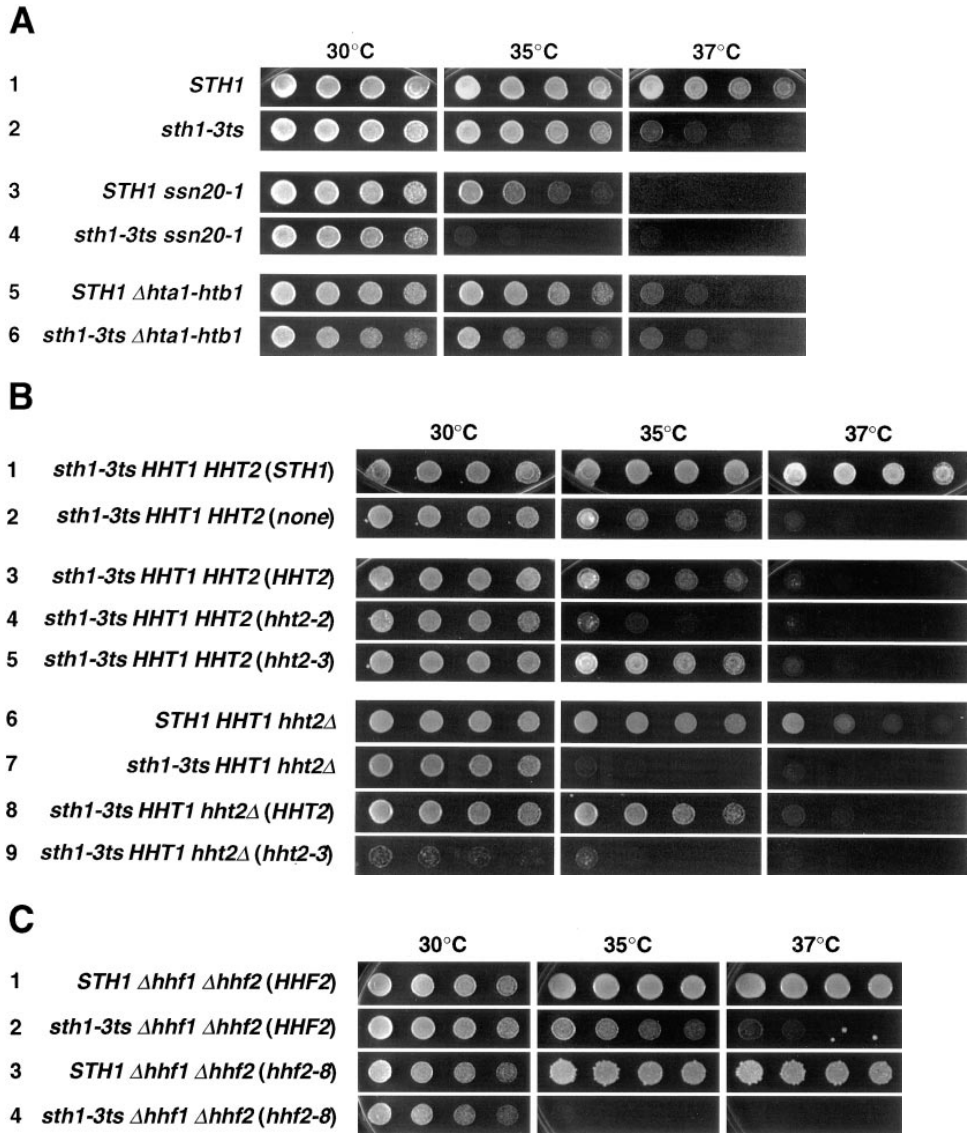

Figure 7.—Genetic interactions between the *sth1-3ts* mutation and mutations in histone and nonhistone chromatin-associated proteins. Threefold serial dilutions of logarithmically grown cells were spotted onto YPD plates grown at the permissive (30°), semi-permissive (35°), and nonpermissive (37°) temperatures for *sth1-3ts*. The plates were photographed after 1 to 2 days of growth. (A) *STH1* is BLY76; the parents for the cross from which the *sth1-3ts ssn20-1* double mutant (BLY150) was isolated are BLY 49 × BLY88. *spt6/ssn20-1* is also temperature sensitive for growth at 37°. BLY150 was a representative double mutant selected from one of the four parental ditype asci (in which Ts^- segregated 2:2) following dissection of 15 complete tetrads. The synthetic enhancement phenotypes segregated with the expected frequencies in a cross of BLY150 to an isogenic wild-type strain. Δ*hta1-htb1* (BLY85), like *sth1-3ts* and *spt6/ssn20-1*, is temperature sensitive for growth at 37°. BLY151 was a typical *sth1-3ts* Δ*hta1-htb1* double mutant identified unambiguously as a Ts^- $Ura3^+$ segregant from a parental ditype ascus following dissection of 3 complete tetrads. The *sth1-3ts* and Δ*hta1-htb1* Ts^- phenotypes from a cross of BLY151 to a wild-type strain segregated as expected. (B) Rows 1–5 are *sth1-3ts* strain (BLY49) carrying low-copy plasmids with the genotypes indicated in parentheses; (none) denotes pRS316 alone. Row 6 is *hht2*Δ strain BLY157; row 7 is *sth1-3ts hht2*Δ strain BLY184; and rows 8 and 9 are *sth1-3ts hht2*Δ strain (BLY275) carrying low-copy plasmids expressing *HHT2* or *hht2-3*. Representative *sth1-3ts hht2*Δ double mutants were identified by following Ts^- $Ura3^+$ segregants in 15 complete tetrads. (C) Row 1 is *hhf1*Δ *hhf2*Δ strain (BLY271) carrying *HHF2* on a low-copy plasmid; row 2 is *sth1-3ts hhf1*Δ *hhf2*Δ strain (BLY272) carrying *HHF2* on a low-copy plasmid; row 3 is *hhf1*Δ *hhf2*Δ strain (BLY273) carrying *hhf2-8* on a low-copy plasmid; and row 4 is *sth1-3ts hhf1*Δ *hhf2*Δ strain (BLY274) carrying *hhf2-8* on a low-copy plasmid. The *sth1-3ts* Δ*hhf1* Δ*hhf2* triple mutants were identified unambiguously by following the relevant phenotype and genetic markers (Ts^- $His3^+$ $Leu2^+$) in segregants from 8 or 13 complete tetrads from crosses of *sth1-3ts* to Δ*hhf1* Δ*hhf2* double mutants carrying either *HHF2* or *hhf2-8* on low-copy plasmids, respectively.

formed with the *hht2-3* plasmid were viable but displayed a severe synthetic sickness even at the fully permissive temperature (Figure 7B, compare row 9 with rows 7 and 8). The synthetic phenotypes with histone H3 point and deletion mutations argue that these interactions are between three loss-of-function mutations, and are not allele specific.

Similarly, we examined *sth1-3ts hhf2-8* interactions in a strain in which both copies of histone H4 were deleted and the only copy of H4 was provided by the point mutation. As for the *hht2-3* mutation, we found that *hhf 2-8* caused a synthetic sickness with the *sth1-3ts* mutation (Figure 7C, compare row 4 with rows 1–3).

In summary, the synthetic sickness and synthetic lethality caused by the *sth1-3ts* mutation with the *ssn20-1*, *hht2-2*, *hht2-3*, *hht2*Δ, and *hhf2-8* mutations (as partially dominant and/or recessive mutations) suggest that Sth1p functions in a parallel genetic pathway with Spt6p and histones H3 and H4.

DISCUSSION

Yeast Snf/Swi and RSC are distinct nucleosome remodeling complexes: Despite similarities, at least five lines of evidence indicate that Snf/Swi and RSC have distinct functions in the cell. First, RSC is approximately

10-fold more abundant than Snf/Swi in yeast cells, suggesting that it plays a broader role. Second, the *RSC* and homologous *SNF/SWI* genes do not function in redundant pathways. Third, unlike the case in *snf/swi* mutants, *SUC2* derepression and *GAL10* induction are only slightly defective in *rsc* mutants, suggesting that the *sth1-ts* growth defects on sucrose, raffinose, and galactose are not due to primary defects in transcription at these promoters. Fourth, RSC is essential for mitotic growth and Snf/Swi is not. Finally, mutations in histone and nonhistone chromatin assembly genes that partially suppress the transcriptional or growth defects of *snf/swi* enhance the temperature-sensitive defects of the *sth1-3ts* mutation, suggesting differences in the way in which Snf/Swi and RSC interact with chromatin.

Point substitutions within conserved helicase motifs: Genetic and biochemical analyses of the lethal and conditional lethal mutations localized to the conserved ATPase/helicase-related domain of Sth1p reveal that this region is essential for several Sth1p functions, including DNA-stimulated ATP hydrolysis, progression through the mitotic cell division cycle, and one or more general cellular processes required for growth on several nonglucose carbon sources. On the basis of a comparison to the recently solved X-ray crystal structure of a DNA helicase (Subramanya *et al.* 1996), the amino acids altered by the *sth1* mutations are predicted to have roles in the enzymatic activity of Sth1p.

None of the point substitution mutations recovered from the mutagenesis of *STH1* affects the ability of the encoded Sth1 protein to hydrolyze ATP *in vitro*. However, the unconditional lethality of the K501R mutation could explain the failure to recover conditional ATPase-sensitive alleles; alternatively, it is possible that the *sth1-ts* mutations affect the ATPase activity of RSC *in vivo*. Importantly, the *sth1-3ts* allele confers G_2/M arrest, suggesting that cell cycle progression and *in vitro* ATP hydrolysis functions are separable. Assembly of RSC may also occur independently of ATP hydrolysis, as Sth1p expressing the ATPase-sensitive K501R allele binds as well as the wild-type to Sfh1p, a yeast Snf5p homolog, *in vivo* in the two-hybrid assay (data not shown). This result supports a model in which ATPase activity is downstream of RSC assembly, and is consistent with the finding that mutation of the corresponding amino acid of Snf2p permitted assembly of Snf/Swi (Peterson *et al.* 1994; Richmond and Peterson 1996).

The *sth1-ts* mutants exhibit cell cycle and non-cell cycle phenotypes. The similarity of arrest phenotypes caused by the *sth1-3ts* allele and the *sth1*Δ deletion allele in *STH1* depletion experiments strongly suggests that *sth1-3ts* affects *STH1* function. The differences in phenotypes of the three *sth1-ts* mutants suggests a model that Sth1p carries out important functions not only at G_2/M but also throughout the cell cycle, although we cannot rule out the possibility that *sth1-1ts* and *sth1-2ts* are leaky alleles. The importance of the ATPase/helicase-related domain for *STH1* function is further underscored by the finding that six out of seven of the disease-causing mutations in *XH2*, a human *SNF2*-related gene, are missense alleles located throughout the corresponding ATPase/helicase-related domain (Gibbons *et al.* 1995). Ultimately, it will be important to characterize the link between the ATP hydrolysis, chromatin modeling, and cell division cycle functions of RSC *in vivo*.

Functional importance of the Sth1p bromodomain: A function for the highly conserved bromodomain, a protein sequence motif found in one to five copies in several different proteins, is currently unknown. Of the proteins in which its functional importance has been tested, only Gcn5p requires the bromodomain for full complementation, although it is dispensable for Gcn5p histone acetyltransferase (HAT) activity (Candau *et al.* 1997). Interestingly, the bromodomain of the human GCN5 (hGCN5) HAT was shown recently to interact with the Ku70 subunit of the DNA-PK holoenzyme that phosphorylates hGCN5 thereby repressing HAT activity, suggesting a role for the bromodomain in the negative modulation of protein activity (Barlev *et al.* 1998). The bromodomain in Snf2p, the most closely related protein to Sth1p in the *S. cerevisiae* genome, is dispensable (Laurent *et al.* 1993). In contrast, deletion mutant analysis demonstrates the functional importance of the Sth1p bromodomain and further suggests that this region carries out a discrete function. Although all *STH1* C-terminal truncation mutants are temperature sensitive for growth, *sth1*Δ*1338* and *sth1*Δ*1349*, which lack all or a portion of the bromodomain α3 helix/helix C (Jeanmougin *et al.* 1997), show the strongest phenotype and arrest in G_2/M of the cell cycle. These cells arrest with abnormally elongated buds, a phenotype not detected in two additional *sth1* mutants that arrest at the same point in the cell cycle: *sth1-3ts* cells and *sth1*Δ null cells in which the expression of *GAL1,10* promoter-driven *STH1* is repressed. Several of these cells were multiply budded and bud growth was often hyperpolarized, terminal phenotypes that are reminiscent of the G_2/M *cak1* (Kaldis *et al.* 1996) or the G_1/S sextuple *clb1-6* and the *cdc34* (Schwob *et al.* 1994) mutants, suggesting defects in mitotic cyclins. This result suggests that the Sth1p bromodomain is engaged in a novel cellular function. The morphological differences of cells expressing *sth1*Δ*1338* and *sth1*Δ*1349* compared to those expressing *sth1*Δ*1274-1335* and *sth1*Δ*1274* further suggest a functional communication between α3 and the two amino-terminal α-helices of the bromodomain. We speculate that amino acids on the surface of the α3 helix regulate intramolecular or intermolecular protein-protein interactions to modulate Sth1p activity.

Roles of the Sth1 protein in the mitotic cell cycle: Several mutations in *STH1* lead to a mitotic cell division cycle arrest at the G_2/M transition. At least four models could explain this result. The first is that Sth1p is a Cdc (cell division cycle) protein with a direct role in regulating cell cycle progression. Our data suggest a

role for Sth1p at, or just preceding, the G_2/M transition, although the mitotic indices and microcolony assays of *sth1-3ts* and *sth1Δ1338* cells indicate that these are not first-cycle arrest mutants.

The second model is that RSC is required indirectly for the mitotic cell division cycle. According to this model, mutation of one or more RSC components leads to loss of RSC function or decreased levels of RSC, either of which causes a cell cycle arrest at the G_2/M stage. The finding that a conditional mutation in another RSC component, *SFH1*, also arrests cells at the same position in the cell cycle (Cao *et al.* 1997) argues in favor of this model. In addition, two of the other proteins in the Snf2p subfamily regulate cell cycle progression (Dunaief *et al.* 1994; Strober *et al.* 1996). At present, we can rule out a requirement for transcription of two genes whose function is necessary for cell cycle progression through mitosis, *CLB2*, a B-type cyclin and *CSE4*, which is required for chromosome condensation, as transcription of neither was dependent on *STH1*.

A third model is that the *sth1* mutants activate a G_2/M checkpoint control pathway. Significantly, mutations in the Drosophila *lodestar* gene, a *SNF2* homolog, result in chromatid tangling and fragmentation at anaphase (Girdham and Glover 1991), defects that trigger mitotic checkpoints in yeast. At least three independent checkpoint pathways exist in *S. cerevisiae* to ensure the accurate transmission of genetic material during mitosis (Weinert and Hartwell 1988; Hoyt *et al.* 1991; Li and Murray 1991; Lew and Reed 1995). So far, we have shown that neither the *RAD9* nor the *MEC2* genes needed for DNA damage and replication checkpoints (Murray 1995) are required for the *sth1-3ts* arrest. However, it is still possible that loss-of-function mutations in any of the other genes involved in the G_2/M checkpoint (Paulovich *et al.* 1997) could alleviate the cell cycle arrest of the *sth1-3ts* allele. Alternatively, the *sth1-3ts* allele could activate a novel checkpoint pathway that surveys the chromatin structure of DNA.

The fourth model is that RSC is required for proper assembly or organization of chromatin. Histones are synthesized in late G_1-early S phase of the cell cycle and are required for nucleosome assembly during S phase (Osley and Lycan 1987; Norris *et al.* 1988; Ling *et al.* 1996). Moreover, other experiments indicate the need for histones in progression through G_2 (Norris and Osley 1987; Kayne *et al.* 1988; Morgan *et al.* 1991; Megee *et al.* 1995). A role for RSC in mitotic chromosome condensation or sister chromatid segregation, like the highly conserved yeast Smc or Cse4 (related by sequence to histone H3) proteins, respectively (Stoler *et al.* 1995; Strunnikov *et al.* 1995), is also consistent with the terminal morphology produced by *sth1-3ts* cells. Importantly, the Drosophila ACF and CHRAC multiprotein complexes that each contain the Snf2p-related protein ISWI, have recently been shown to function in the ATP-dependent assembly of chromatin (Ito *et al.* 1997; Varga-Weisz *et al.* 1997). In addition, human homologs of Snf/Swi have been shown to associate with nuclear matrix, suggesting a role in organizing chromatin (Reyes *et al.* 1997). By analogy, RSC could also direct assembly of chromatin in mitotic yeast cells, in addition to its established role in nucleosome reconfiguration *in vitro*. The synthetic sickness and lethality observed between the *sth1-3ts* mutation and *spt6*/*ssn20-1* and histone H3 and H4 mutations support this hypothesis.

We reasoned that if RSC plays a general role in organizing chromatin, the structure of bulk chromatin would be altered with inactivation of *STH1* function. To test this, nuclei prepared from *sth1-3ts* cells and isogenic wild-type cells grown at various times following a shift to 37° were digested with micrococcal nuclease. No major differences in micrococcal nuclease sensitivity were detected in the $STH1^+$ and $sth1^-$ nuclei (data not shown), suggesting that Sth1p does not function in establishing or maintaining bulk chromatin. Further genetic and biochemical experiments will help us to distinguish among the models above, although the role of RSC in the cell cycle could be explained by a combination of these models.

Sth1p and chromatin modeling: Purified RSC can remodel nucleosomes in an ATP-dependent manner *in vitro* (Cairns *et al.* 1996) although it remains to be shown that RSC has such activity *in vivo*. Significantly, several of the mutations in histone and chromatin assembly genes that suppress *snf*/*swi* failed to suppress the growth defects caused by temperature-sensitive mutations in *STH1*. Instead, we found that the *sth1-3ts* allele is synthetically sick or synthetically lethal with mutations in the histone H3, H4, and *SPT6* genes, implying that these genes function synergistically in parallel genetic pathways.

The specific histone H3 and H4 mutations examined here affect histone-DNA interactions to varying degrees (Kurumizaka and Wolffe 1997; Luger *et al.* 1997; Wechser *et al.* 1997) and all alter amino acids within the histone fold regions of H3 and H4, either within α-helices or in loops linking α-helices (Luger *et al.* 1997). Inexplicably, the E105K mutation, which is synthetically lethal with *sth1-3ts*, has relatively minor effects on nucleosome structure (Kurumizaka and Wolffe 1997) and is predicted to have little direct effect at the mononucleosome level (Luger *et al.* 1997).

Several models could explain the synthetic enhancement between mutations in *sth1-3ts* and mutations in histones H3, H4, or *SPT6*. One possibility is that Sth1p is required for expression of the histone or *SPT6* genes. At present, we favor a model that the synthetic enhancement phenotypes between mutations in *sth1-ts* and mutations in *SPT6* and histones reflect functional interactions. Our genetic analysis showed that the strongest synthetic phenotypes were between *sth1-3ts* and *hht2*, *hhf2*, and *spt6*. Interestingly, Bortvin and Winston (1996) have shown that Spt6p interacts directly with

histones H3 and H4 (and to a lesser extent with H2A-H2B) and probably plays a role in nucleosome assembly. Our results suggest that RSC and Snf/Swi interact oppositely with histones and chromatin-associated proteins and that RSC plays an important role in the organization or assembly of chromatin.

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Note added in proof: An *sth1/nps1* temperature-sensitive allele has been shown recently to activate the *MAD1*-dependent checkpoint and cause alterations in centromeric chromatin structure (Tsuchiya, E., T. Hosotani and T. Miyakawa, 1998. Nucleic Acids Res. **26:** 3286–3292).

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