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28. We thank N. Kley, H. Lu, J. Lin, and A. Levine for plasmids, A. Levine for critical reading of the manuscript, J. Cong for able technical assistance, and T.D. thanks H. Wolf for support in Regensberg. This work was supported by grants from NIH (T.S.) and the Infektionsforschung AIDS-Stipendienprogramm DKFZ (T.D.). S.R. received scholarship support from the University of Regensberg, and T.S. is an American Cancer Society Professor and an Investigator of the Howard Hughes Medical Institute.

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Evidence That Spt6p Controls Chromatin Structure by a Direct Interaction with Histones

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Genetic analysis has implicated *SPT6*, an essential gene of *Saccharomyces cerevisiae*, in the control of chromatin structure. Mutations in *SPT6* and particular mutations in histone genes are able to overcome transcriptional defects in strains lacking the Snf/Swi protein complex. Here it is shown that an *spt6* mutation causes changes in chromatin structure in vivo. In addition, both in vivo and in vitro experiments provide evidence that Spt6p interacts directly with histones and primarily with histone H3. Consistent with these findings, Spt6p is capable of nucleosome assembly in vitro.

Chromatin structure plays an important role in the regulation of eukaryotic transcription (*1*). One subject of intensive study has been the Snf/Swi protein complex (*2*). Genetic, molecular (*3*), and biochemical analyses of the Snf/Swi complex from both yeast (*4*) and humans (*5*) have provided strong evidence that this complex helps transcription factors to bind to nucleosomal DNA. Genetic studies showed that particular histone mutations, such as a deletion of one of the two *H2A-H2B* gene pairs in the yeast genome (*hta1-htb1*)Δ, suppress *snf/swi* null mutations (*6*, *7*). The suppression of *snf2* and *snf5* mutations by this histone mutation occurs at the level of chromatin structure, as has been shown at *SUC2*, a Snf/Swi–dependent gene (*6*). In addition to mutations in histones, mutations in the nonhistone genes *SPT4*, *SPT5*, and *SPT6* also suppress *snf/swi* mutations (*3*). This and other common phenotypes suggest that the Spt4p, Spt5p, and Spt6p proteins are required for control of chromatin structure in yeast (*8*).

We analyzed the function of Spt6p, a large acidic nuclear protein that is essential for growth in *S. cerevisiae* (*9*). Mutations in *SPT6* restore the high levels of *SUC2* mRNA that are reduced by *snf/swi* mutations, thereby allowing growth on raffinose (*8*). The levels of suppression by *spt6* mutations are comparable to those achieved by the (*hta1-htb1*)Δ mutation (Fig. 1A) (*6*, *8*). However, mutations in *SPT6* have no significant effect on histone mRNA or protein levels (*10*).

Given the common phenotypes caused by (*hta1-htb1*)Δ and *spt6* mutations, we tested whether an *spt6* mutation also caused similar changes in chromatin structure. We therefore examined *SUC2* chromatin structure in *snf5* single mutants and *snf5 spt6* double mutants by indirect end-labeling of micrococcal nuclease (MNase)–digested chromatin (*11*). These results (Fig. 1B) demonstrate that in a *snf5* background, an *spt6* mutation causes the same changes in chromatin structure as does the (*hta1-htb1*)Δ histone mutation. In both *snf5 spt6* and *snf5* (*hta1-htb1*)Δ double mutants, cleavage by MNase around the TATA box and near the upstream activator sequence (UAS) was increased relative to the cleavage in a *snf5* single mutant (compare sites B, C, E, and F). These alterations in *SUC2* chromatin structure suggest that, in the *snf5* mutant, the presence of the nucleosomes over the promoter (*6*) is dependent on Spt6p. As shown previously for the suppression of *snf5* by (*hta1-htb1*)Δ, these *SPT6*-dependent changes in chromatin structure at the *SUC2* promoter are independent of the level of transcription because they occur at an enfeebled *SUC2* promoter that contains no TATA element (*10*). These results suggest that the primary effect of Spt6p in controlling transcription occurs by way of changes in chromatin structure.

Because *spt6* mutations cause changes in chromatin structure, it seemed possible that Spt6p might interact with histones. We reasoned that if the defect in *spt6* mutants is the result of a weakened interaction between Spt6p and histones, then an increased level of histones might restore the interaction. To overexpress histone genes,

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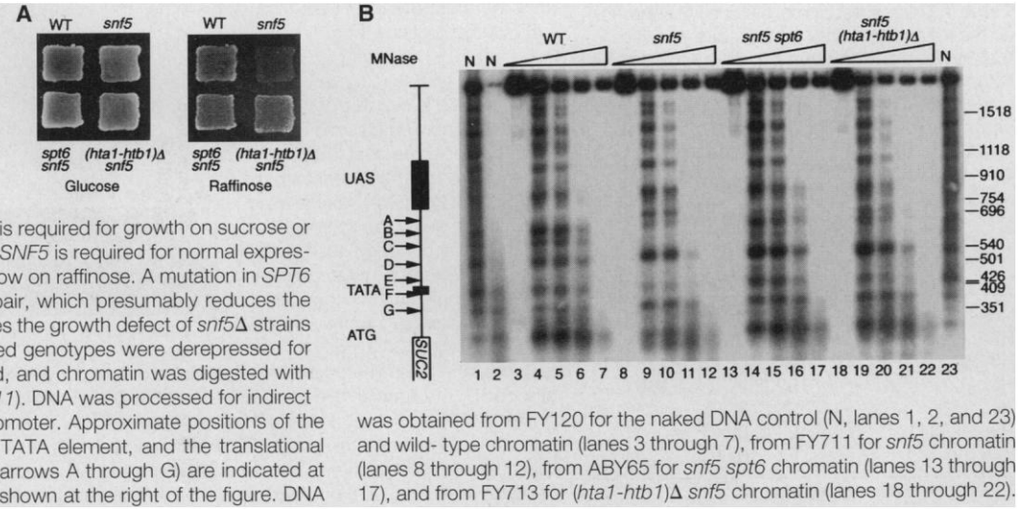

Fig. 1. An *spt6* mutation suppresses an *snf5* defect at the level of chromatin structure. (**A**) Patches of wild-type (WT) (FY120), *snf5*Δ (FY711), *spt6-140 snf5*Δ (ABY65), and (*hta1-htb1*)Δ *snf5*Δ (FY713) strains were grown on rich media and then replica-plated onto media containing either glucose or raffinose (*14*). The *SUC2* gene encodes invertase, which is required for growth on sucrose or raffinose as carbon sources. Because *SNF5* is required for normal expression of *SUC2*, *snf5*Δ mutants fail to grow on raffinose. A mutation in *SPT6* or deletion of the *HTA1-HTB1* gene pair, which presumably reduces the level of histones H2A and H2B, restores the growth defect of *snf5*Δ strains on raffinose. (**B**) Strains of the indicated genotypes were derepressed for *SUC2* expression, nuclei were purified, and chromatin was digested with increasing concentrations of MNase (*11*). DNA was processed for indirect end-labeling analysis of the *SUC2* promoter. Approximate positions of the *SUC2* promoter elements (UAS, the TATA element, and the translational start site) and MNase cleavage sites (arrows A through G) are indicated at left; positions of the size markers are shown at the right of the figure. DNA was obtained from FY120 for the naked DNA control (N, lanes 1, 2, and 23) and wild- type chromatin (lanes 3 through 7), from FY711 for *snf5* chromatin (lanes 8 through 12), from ABY65 for *snf5 spt6* chromatin (lanes 13 through 17), and from FY713 for (*hta1-htb1*)Δ *snf5* chromatin (lanes 18 through 22).

we used a derivative of a divergent promoter for one of the two *H2A-H2B* gene pairs (*12*). Genes under the control of this mutant promoter are expressed throughout the cell cycle rather than solely during the late G_1/early S phases (*13*).

In a first set of experiments, strains overexpressing the individual histone pairs H2A-H2B or H3-H4 were examined (*14*, *15*). Overexpression of the H3-H4 pair strongly suppressed *spt6* lethality (Table 1), whereas overexpression of H2A-H2B had no significant effect. To determine whether both H3 and H4 were required for the suppression, we overexpressed each of these histones individually. Overexpression of H3 alone suppressed *spt6*, whereas overexpression of H4 did not (Table 1), which suggests that Spt6p and histone H3 interact.

To determine which part of H3 is important for the suppression of *spt6* lethality, we overexpressed two different H3 mutants. The first mutant is missing amino acids 4 to 30, which comprise most of the flexible NH_2-terminal tail. Although this part of H3 is not essential for nucleosome assembly, it is important for the normal expression of many genes (*1*, *16*). The NH_2-terminal tail of H3 interacts directly with Sir3p and Sir4p (*17*), which are proteins required for silencing of the mating type information at *HML* and *HMR*, and with Tup1p (*18*), a global repressor. Overexpression of this mutant H3 suppresses *spt6* (Table 1), suggesting that, in contrast to Sir3p, Sir4p, and Tup1p, Spt6p does not require the H3 NH_2-terminal tail for interaction with this histone. The second H3 mutant has a single amino acid change (Thr^{119Ile}) in the globular domain (*7*, *19*). This domain is required for assembly of the histone octamer and for interaction with DNA (*20*). Overexpression of this mutant H3 fails to suppress *spt6* (Table 1), which suggests that Spt6p may interact with the globular domain of H3. This possibility is supported by the fact that this H3 mutant has phenotypes very similar to those of *spt6* mutants (*19*). These genetic results suggest that Spt6p interacts with histone H3 and that this interaction with H3 or with an H3-H4 tetramer, the form in which these histones likely exist in vivo, is a critical aspect of Spt6p function.

To determine if the in vivo interaction between Spt6p and histones occurs by a direct interaction, we tested for an Spt6p-histone interaction in vitro. First, we purified a functional glutathione S-transferase (GST)–Spt6p fusion protein from yeast by binding it to glutathione Sepharose beads (*21*). Then, native human histones [present as H2A-H2B dimers and H3-H4 tetramers under the conditions of the experiment (*22*)] were passed over a column of GST-Spt6p–saturated beads, and the bound histones were eluted by washes in the presence

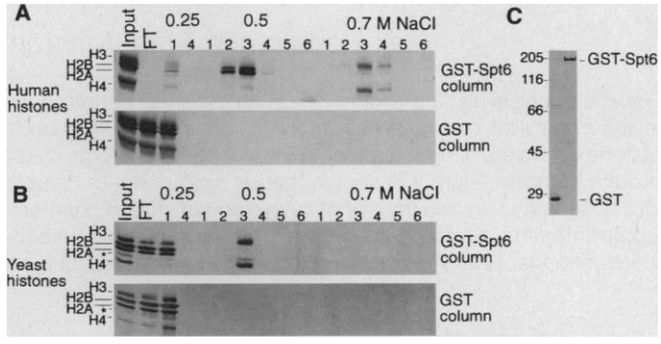

Fig. 2. Human and yeast tetramers of H3-H4 show strong binding to Spt6p. (**A**) Native human histones (35 μg) were loaded onto GST-Spt6p and GST columns. The bound proteins were eluted with the following sequence of washes: four column volumes (CVs) of A250, 3 CVs of A500, and 3 CVs of A700. A500 and A700 were A250 with NaCl concentrations at 0.5 M and 0.7 M, respectively (*21*). No additional protein was eluted by washes with higher salt, and no significant amount of the histone proteins remained bound on beads. 0.5 CV fractions (250 μl) were collected for each wash, and 12 μl of particular fractions for each wash were separated in 15% SDS-polyacrylamide gels. Input lanes contained 0.8 μg of total protein. FT, flow-through. Proteins were detected by silver staining (*34*). (**B**) Acid-extracted yeast histones (35 μg) were analyzed as described for (A). The protein marked with the asterisk is a proteolytic fragment of histone H3 beginning at amino acid 23, as determined by protein microsequencing (*23*). (**C**) A Coomassie-stained 10% SDS-polyacrylamide gel showing purified GST and GST-Spt6 proteins used in the study. Numbers on the left indicate the positions of size markers (Sigma).

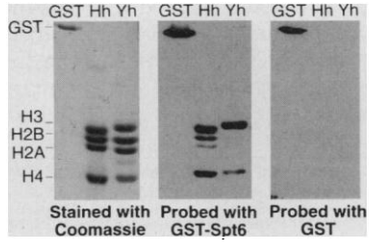

Fig. 3. Spt6p interacts with specific histones in far-western analysis. Samples of GST (1 μg), human histones (Hh, 3.5 μg), and yeast histones (Yh, 3.5 μg) were separated on 15% SDS-polyacrylamide gels and stained with Coomassie or were transferred to Immubilon membrane (Millipore) (*25*). The positions of the transferred proteins were marked after staining with Ponceau S. The membrane was then blocked and probed with purified GST-Spt6 or GST proteins. Protein complexes were then detected with antisera to GST [GST (Z5), Santa Cruz Biotechnology], goat secondary antisera (Bio-Rad), and the ECL detection kit (Amersham). The detection of both yeast and human histone H3 proteins with GST-Spt6p was the most efficient. Detection of the other histones was most reproducible with freshly purified GST-Spt6p protein (*10*).

Table 1. Overexpression of histone H3 suppresses the inviability of *spt6* temperature-sensitive lethal mutants at the nonpermissive temperature. Strains were incubated at 37°C for 8 hours before measurement of cell viability (*14*, *15*). The viability of a wild-type strain, FY120, was not affected by either incubation at 37°C or histone overexpression. ND, not done.

Histones overexpressed	Viable cells (%)*	
	spt6-14	*spt6-1*
None	13 (0.9)	16 (1.5)
H2A, H2B	23 (2.0)	14 (2.5)
H3, H4	94 (2.0)	68 (4.0)
H3	92 (2.4)	66 (2.4)
H4	20 (1.7)	15 (1.6)
H3Δ(4-30), H4	87 (0.8)	ND
H3 (Thr^{119Ile}), H4	22 (2.0)	ND

*Standard errors are in parentheses.

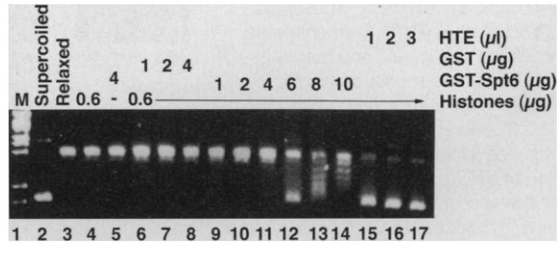

Fig. 4. Spt6p is capable of nucleosome assembly on plasmid DNA in vitro. Nucleosome assembly reactions were performed essentially as in (*26*). pUC19 DNA was relaxed with wheat germ topoisomerase I (Promega) and added to the nucleosome assembly mixes containing the manufacturer-recommended topoisomerase I buffer, bovine serum albumin (400 μg/ml), human histones, and the indicated proteins. Reactions (total volume of 20 μl) were incubated at 37°C for 1 hour, stopped by the addition of 6 μl of DSB buffer [3% SDS, 0.1 M EDTA, 50 mM tris-HCl (pH 7.5), and 25% glycerol] (*26*) and treated with 10 μg of Proteinase K. Plasmid topoisomers were resolved on 1% agarose gels run in 2× tris-borate-EDTA buffer and visualized by staining with ethidium bromide. A time course demonstrated that maximum supercoiling occurred by 30 min. Heat-treated extract (HTE) from *Xenopus* was used as a positive control. M indicates molecular weight markers.

of 0.25 M, 0.5 M, and 0.7 M NaCl. The H3-H4 tetramers showed the highest affinity for GST-Spt6p, remaining bound until the 0.7 M NaCl wash (Fig. 2A). The H2A-H2B dimers also bound, eluting at 0.5 M NaCl. When yeast histones (*23*) were fractionated in a similar experiment, the yeast H3-H4 tetramers showed strong binding to GST-Spt6p, eluting at 0.5 M NaCl, whereas the H2A-H2B dimers flowed through the column in the loading buffer containing 0.25 M NaCl (Fig. 2B). A proteolytic fragment of yeast histone H3, beginning at amino acid 23, bound to the GST-Spt6p column as well as full-length H3. This H3 proteolytic fragment has a charge of +15 and full-length H3 has a charge of +22, suggesting that positive charge cannot account entirely for the specificity of the Spt6p-H3 interaction. This result fits with the ability of an H3 mutant without an NH_2-terminal tail to suppress an *spt6* mutation.

Thus, the strong interaction between Spt6p and histones H3 and H4 in vitro supports the in vivo results. The difference in the NaCl concentration required to elute the human histones versus the yeast histones is not understood, especially since they have virtually the same net charges. This difference is not likely to be the result of the histone preparation method, because either acid-extracted histones (Fig. 2B) or a relatively crude preparation of native yeast histones showed the same elution profile (*24*). Thus, the different elution profiles between human and yeast histones suggest a distinction between the two that is recognized by Spt6p. Nevertheless, Spt6p clearly binds preferentially to H3-H4 tetramers as compared with H2A-H2B dimers from either species.

Because the chromatography experiments described above examined the interaction of Spt6p with H2A-H2B dimers and H3-H4 tetramers, we used a second in vitro approach to determine which individual histones interact with Spt6p directly. We performed far-western analysis, examining the interaction of purified GST-Spt6p protein with both human and yeast histones separated on a polyacrylamide gel (*25*). Among the yeast histones, H3 was strongly recognized by Spt6p (Fig. 3). Yeast H4 was also bound by Spt6p, whereas interaction with histones H2A and H2B was not detectable. In contrast, Spt6p recognized all four human histones to varying degrees. As was consistent with the yeast histone results, Spt6p interacted most strongly with human H3 and H4.

The strong interaction between Spt6p and yeast H3 shown by far-western analysis is consistent with the in vivo suppression of *spt6* mutations by H3 overexpression. The weaker interaction between Spt6p and yeast H4 hints at a weak in vivo interaction between these two proteins as well. The differences in the pattern of Spt6p recognition for yeast and human histones shown by far-western analysis is consistent with the differences in the profiles of histone binding in the chromatography experiments. The relatively greater affinity of Spt6p for human histones H4, H2A, and H2B, as compared with their yeast counterparts, may be responsible for the tighter binding of human histones overall to the GST-Spt6p column.

Our results suggest several possible roles for the in vivo interaction between Spt6p and H3-H4 tetramers in yeast. To address one of these possibilities, we have tested for the ability of Spt6p to assemble nucleosomes with a plasmid supercoiling assay (*26*). Purified GST-Spt6p protein converted up to 50% of the relaxed plasmid DNA into a supercoiled form in the presence of topoisomerase I and human histones (Fig. 4). This reaction required both GST-Spt6p and histones. The nucleosome assembly activity of GST-Spt6p in this assay was unaffected by the addition of adenosine triphosphate (ATP) (*10*) and was less than that of a heat-treated extract from *Xenopus*, which contains the nucleosome assembly factors nucleoplasmin and N1/N2 (Fig. 4) (*26*). Other ATP-independent nucleosome assembly activities have been identified (*27*). Finally, treatment of the GST-Spt6p assembled chromatin with MNase showed a protected region of approximately 150 base pairs of DNA, which is characteristic of a nucleosome core particle (*10*, *20*). Thus, purified GST-Spt6p is capable of transferring histones onto DNA to form nucleosomes. The moderate level of activity of GST-Spt6p may indicate that additional factors are required for efficient assembly, as is the case for similar activities from higher eukaryotes. For example, chromatin assembly factor 1 from *Drosophila* requires both dCAF1 and dCAF4 for efficient nucleosome assembly (*28*). Because previous analysis suggests that Spt6p interacts with at least two other proteins in vivo, Spt4p and Spt5p (*8*), these factors may be required for a more robust nucleosome assembly by Spt6p in vitro.

Our results suggest that Spt6p controls chromatin structure by direct interactions with histones H3 and H4. Spt6p may function in nucleosome assembly or could serve as a donor or acceptor of histones in genomic regions undergoing extensive chromatin reorganization, such as those that occur at highly regulated genes. Further biochemical and genetic analysis, aided by the recent identification of *SPT6* homologues in *Caenorhabditis elegans* and humans (*29*), and a functional *SPT4* homolog in humans (*30*) will tell us more about Spt6p function.

Table 2. *Saccharomyces cerevisiae* strains used in this study.

Strain	Genotype
FY120	*MATa his4-912δ lys2-128δ ura3-52 leu2Δ1*
FY711	*MATα trp1Δ63 snf5-5::URA3 his4-912δ lys2-128δ ura3-52 leu2Δ1*
FY713	*MATα trp1Δ63 snf5-5::URA3 (hta1-htb1)Δ::LEU2 his4-912δ lys2-128δ ura3-52 leu2Δ1*
FY889	*MATa his4-912δ lys2-128δ suc2ΔUAS(-1900/-390) ura3-52 trp1Δ63 bur5-1*
FY957	*MATa spt6-14 his4-912δ lys2-128δ ura3-52 leu2Δ1*
FY1202	*MATa trp1Δ63 spt6-140 snf5-5::URA3 his-4-912δ lys2-128δ ura3-52 leu2Δ1*
FY1204	*MATa spt6-14 (HTA1-HTB1)Δ16′ his4-912δ lys2-128δ ura3-52 leu2Δ1*
FY1205	*MATa spt6-14 (HHT1-HHF1)Δ16′::LEU2 his4-912δ lys2-128δ ura3-52 leu2Δ1*
FY1206	*MATa spt6-14 (HHT1-hhf1Δ)Δ16′::LEU2 his4-912δ lys2-128δ ura3-52 leu2Δ1*
FY1207	*MATa spt6-14 (hht1Δ-HHF1)Δ16′::LEU2 his4-912δ lys2-128δ ura3-52 leu2Δ1*
FY1208	*MATa spt6-14 (hht1{Δ4-30}-HHF1)Δ16′::LEU2 his4-912δ lys2-128δ ura3-52 leu2Δ1*
FY1209	*MATa spt6-14 (hht1{T119I}-HHF1)Δ16′::LEU2 his4-912δ lys2-128δ ura3-52 leu2Δ1*
ABY396	*MATa leu2 trp1 ura3-52 prb1-1122 pep4-3 prc1-407 GAL2⁺*
ABY418	*MATa spt6-1 leu2Δ1 his4-539 ura3-52 (HHT1-HHF1)Δ16′::LEU2*
ABY419	*MATa spt6-1 leu2Δ1 his4-539 ura3-52 (hht1Δ-HHF1)Δ16′::LEU2*
ABY420	*MATa spt6-1 leu2Δ1 his4-539 ura3-52 (HHT1-hhf1Δ)Δ16′::LEU2*
ABY437	*MATa spt6-1 (HTA1-HTB1)Δ16′ his4-912δ lys2-128δ ura3-52 leu2Δ1*
MCY939	*MATa his4-539 spt6-1 ura3-52*

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11. The isolation and analysis of yeast chromatin were done as previously described (*6*).
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14. *S. cerevisiae* strains used are listed in Table 2. Strain MCY939 was provided by M. Carlson; all other strains are from our lab. All strains are congenic to S288C. FY strains are *GAL2*$^+$ derivatives of S288C [F. Winston, *et al.*, *Yeast* **11**, 53 (1995)]. Strain ABY396 is a *GAL2*$^+$ derivative of BJ2168 [E. Jones, in *Methods Enzymol.* **194**, 428 (1991)]. Standard yeast genetic methods were used [F. Sherman, G. R. Fink, C. W. Lawrence, *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1978)].
15. To overexpress histones H2A and H2B, the Δ16' promoter was recombined into the *HTA1-HTB1* locus, replacing the wild-type promoter, after a two-step integration protocol with the use of plasmid pUC13$^+$Δ16' (a gift from M. A. Osley) (*31*). To overexpress histones H3 and H4, the *HHT1* (H3) and *HHF1* (H4) genes were amplified by polymerase chain reaction (PCR) and subcloned under control of the Δ16' promoter to create plasmid pAB157. This integrating plasmid, which also contains the *LEU2* gene, was then integrated at *leu2Δ1* in yeast strains. We achieved expression of either *HHT1* or *HHF1* individually by dropping out the *HHT1* or *HHF1* fragments from pAB157. Sequences encoding H3Δ(4-30) were created by PCR amplification of *HHT1*, with the use of an oligonucleotide that spans the junction from codons from 4 to 30 of *HHT1*. The mutation encoding H3 (Thr^{119}Ile) was PCR-amplified from genomic DNA of strain FY889, which contains this *hht1* mutation. All PCR products were verified by sequencing. We determined the effect of histone overexpression on cell viability by shifting liquid cell cultures to the restrictive temperature (37°C) for 8 hours, after which cells were sonicated to disperse any clumps, dilutions were plated in duplicate, and plates were incubated at 30°C. Percent viability represents the fraction of viable cells at time zero that were able to grow after 8 hours of incubation at the restrictive temperature. Similar results were obtained when colony formation was monitored by light microscopy.
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21. Plasmid pGH85, encoding the GST-Spt6p fusion, is full-length *SPT6* in pEMBL-GST (*32*). GST was expressed from pSYB305. The GST and GST-Spt6p fusions were isolated from ABY396 after a 3-hour induction with 2% galactose. Proteins were routinely purified from 10 mg of protein lysate prepared with glass beads and buffer A250 [25 mM tris-HCl (pH 7.5), 15 mM $MgCl_2$, 15 mM EGTA, 10% glycerol, 0.3% Triton X-100, 0.02% NaN_3, 250 mM NaCl, and 1 mM dithiothreitol (DTT)] and the protease inhibitors chymostatin, leupeptin, pepstatin, benzamidine, and phenylmethylsulfonyl fluoride. For the column chromatography experiments, 10 mg of protein lysate was incubated with 0.5 ml of glutathione beads (Pharmacia) for 45 min in a final volume of 5 ml. The beads were then extensively washed with A250 and with A1000 (A250 with NaCl at 1 M).
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23. Acid-extracted yeast histones were prepared from purified nuclei (*6*) as described (*33*), except that no butyrate was used in extraction buffers A and B, and extraction with 0.4 N sulfuric acid was done only once. The acid-soluble protein was precipitated in 20% trichloroacetic acid and the pellet was washed in acidified acetone, air-dried, and suspended in 10 mM tris-HCl (pH 8.0). Protein microsequencing was done at the Biopolymer Facility of the Howard Hughes Institute and the Department of Genetics, Harvard Medical School.
24. A. Bortvin, D. M. O'Rourke, F. Winston, data not shown.
25. GST and GST-Spt6p proteins used in far-western analysis were purified as described above (*21*), except that 1.5 ml of glutathione beads was used for every 10 mg of protein lysate. After the beads were washed with 30 ml of 50 mM tris-HCl (pH 7.5), 50 mM NaCl, and 1 mM DTT, the bound proteins were eluted in 2 ml of the same buffer plus 10 mM glutathione (Sigma) and concentrated on Centricon concentrators (Amicon). Far-western analysis was performed as described in the Fig. 3 legend.
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Inhibition of Myogenic bHLH and MEF2 Transcription Factors by the bHLH Protein Twist

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The myogenic basic helix-loop-helix (bHLH) and MEF2 transcription factors are expressed in the myotome of developing somites and cooperatively activate skeletal muscle gene expression. The bHLH protein Twist is expressed throughout the epithelial somite and is subsequently excluded from the myotome. Ectopically expressed mouse Twist (Mtwist) was shown to inhibit myogenesis by blocking DNA binding by MyoD, by titrating E proteins, and by inhibiting trans-activation by MEF2. For inhibition of MEF2, Mtwist required heterodimerization with E proteins and an intact basic domain and carboxyl-terminus. Thus, Mtwist inhibits both families of myogenic regulators and may regulate myotome formation temporally or spatially.

Members of the bHLH family of transcription factors play important roles in controlling cell type determination and differentiation in both vertebrates and invertebrates (*1*). Vertebrate skeletal muscle development is controlled by four members of this family: MyoD, Myf-5, myogenin, and MRF-4. Gene disruption in mice has established that the myogenic bHLH proteins are necessary for establishment of the myoblast cell lineage and for proper execution of the skeletal muscle differentiation program (*2*). In addition to the MyoD family, members of the MEF2 family of transcription factors also play a role in muscle differentiation. There are four members of the MEF2 family of proteins (MEF2A through MEF2D), and all share a region of homology with the MADS family of transcription factors and an adjacent region known as the MEF2 domain (*3*). MEF2 factors are highly induced during skeletal myogenesis (*4*), and many skeletal muscle–specific genes require both myogenic bHLH and MEF2 binding sites for maximal skeletal muscle expression (*3*). High-level expression of both myogenic bHLH and MEF2 family members in the somite are confined to the myotome (*5–7*).

Originally identified in *Drosophila* as a gene necessary for embryonic gastrulation and formation of mesoderm (*8*), Twist is a bHLH protein that is specifically expressed in the mesoderm (*9*). Twist homologs have been isolated from *Xenopus* (*10*), mouse (*11*), and chick (*12*). Vertebrate Twist is initially expressed throughout the somitic mesoderm. As the somites develop, Twist is excluded from the forming myotome but continues to be expressed in other mesodermal structures such as the dermomyotome, sclerotome, lateral plate mesoderm, and cranial neural crest (*10–13*). The exclusion of Twist from the myotome suggested that it may negatively regulate the myogenic bHLH proteins and that the mutually exclusive expression of these transcription factors may partition the somitic mesoderm into alternative cell fates. We now demonstrate that ectopically expressed mouse Twist (Mtwist) inhibits skeletal myogenesis by blocking the activity of both myogenic

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