Defects in *SPT16* or *POB3* (yFACT) in *Saccharomyces cerevisiae* Cause Dependence on the Hir/Hpc Pathway: Polymerase Passage May Degrade Chromatin Structure

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ABSTRACT

Spt16/Cdc68, Pob3, and Nhp6 collaborate *in vitro* and *in vivo* as the yeast factor SPN, which is homologous to human FACT. SPN/FACT complexes mediate passage of polymerases through nucleosomes and are important for both transcription and replication. An *spt16* mutation was found to be intolerable when combined with a mutation in any member of the set of functionally related genes *HIR1*, *HIR2/SPT1*, *HIR3/HPC1*, or *HPC2*. Mutations in *POB3*, but not in *NHP6A/B*, also display strong synthetic defects with *hir/hpc* mutations. A screen for other mutations that cause dependence on *HIR/HPC* genes revealed genes encoding members of the Paf1 complex, which also promotes transcriptional elongation. The Hir/Hpc proteins affect the expression of histone genes and also promote normal deposition of nucleosomes; either role could explain an interaction with elongation factors. We show that both *spt16* and *pob3* mutants respond to changes in histone gene numbers, but in opposite ways, suggesting that Spt16 and Pob3 each interact with histones but perhaps with different subsets of these proteins. Supporting this, *spt16* and *pob3* mutants also display different sensitivities to mutations in the N-terminal tails of histones H3 and H4 and to mutations in enzymes that modulate acetylation of these tails. Our results support a model in which SPN/FACT has two functions: it disrupts nucleosomes to allow polymerases to access DNA, and it reassembles the nucleosomes afterward. Mutations that impair the reassembly activity cause chromatin to accumulate in an abnormally disrupted state, imposing a requirement for a nucleosome reassembly function that we propose is provided by Hir/Hpc proteins.

THE *Saccharomyces cerevisiae* proteins Spt16/Cdc68, Pob3, and Nhp6 function together as a complex that has been called CP or SPN (Brewster *et al.* 2001; Formosa *et al.* 2001) and that we now call yFACT. Nhp6 is an HMG1-motif DNA-binding protein that binds either to DNA or to nucleosomes (Yen *et al.* 1998; Formosa *et al.* 2001). Spt16 and Pob3 form a stable heterodimer that does not bind to DNA, to nucleosomes, or to Nhp6:DNA complexes, but does bind to Nhp6:nucleosome complexes (Formosa *et al.* 2001). These "SPN:nucleosome" complexes display increased accessibility of the DNA within the nucleosome to DNase I (Formosa *et al.* 2001). SPN/yFACT therefore binds to and alters the structure of the fundamental subunit of chromatin.

Spt16 and Pob3 are both essential for viability, but Nhp6 is nonessential (Costigan *et al.* 1994). Genetic evidence indicates that Nhp6 supports the function of Spt16-Pob3 *in vivo* and it supports the binding of Spt16-Pob3 to nucleosomes *in vitro* (Brewster *et al.* 2001; Formosa *et al.* 2001). However, Spt16-Pob3 must be able to execute its essential function without Nhp6. Human and frog cells contain a factor similar to SPN called FACT or DUF, respectively, although the primary sequence elements found in the yeast Pob3 and Nhp6 proteins are fused to form the single polypeptides SSRP1 and DUF87 in these metazoan complexes (Okuhara *et al.* 1999; Orphanides *et al.* 1999). An HMG1 motif is therefore covalently associated with FACT and DUF, but yeast Nhp6 interacts with Spt16-Pob3 only weakly outside of the context of SPN:nucleosome complexes (Brewster *et al.* 2001; Formosa *et al.* 2001). SPN, FACT, and DUF therefore represent members of a broadly conserved family of factors, but SPN has a somewhat different subunit architecture.

Nucleosomes normally block the progression of RNA polymerase II, but FACT allows Pol II to elongate through these structures *in vitro* (Orphanides *et al.* 1998). Frog oocyte extracts depleted of DUF fail to support DNA replication (Okuhara *et al.* 1999). These results suggest that FACT/DUF promotes progression of both RNA and DNA polymerases along their natural chromatin templates. Genetic analysis in yeast supports this conclusion *in vivo*, because mutations that alter yFACT/SPN components cause defects in both the regulation of

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transcription and the performance of DNA replication (Malone *et al.* 1991; Rowley *et al.* 1991; John *et al.* 2000; Schlesinger and Formosa 2000; Yu *et al.* 2000; Brewster *et al.* 2001; Formosa *et al.* 2001). Although SPN and FACT/DUF are organized somewhat differently, the overall similarities in sequence and activity suggest that these factors all use a common mechanism to facilitate both transcription and replication. We therefore refer here to SPN as yFACT with the adjusted acronym of "facilitates chromatin transactions." This emphasizes the similarities among these factors and incorporates the role they play in both transcription and replication.

The current data suggest that the FACT family functions by making chromatin less inhibitory to the progression of polymerases, perhaps by destabilizing or even disassembling nucleosomes (Orphanides *et al.* 1999). Nucleosomes are composed of ~146 bp of DNA wrapped around a histone octamer composed of a core $(H3\text{-}H4)_2$ tetramer and two H2A-H2B dimers (van Holde 1988). Human FACT binds to free H2A-H2B dimers, suggesting that it might stabilize disrupted nucleosomes (Orphanides *et al.* 1999). One or both H2A-H2B dimers can be removed from a nucleosome (van Holde 1988), resulting in a structure that is less inhibitory to the passage of RNA polymerase II (Kireeva *et al.* 2002). One specific proposal for the mechanism of FACT therefore is that it promotes the disassembly of nucleosomes into incomplete, less restrictive forms (Orphanides *et al.* 1999; Kireeva *et al.* 2002).

Nucleosomes are deposited onto DNA chiefly during DNA replication, and the synthesis of histone proteins is tightly regulated to coincide with this period of increased demand. Histone gene transcription is therefore repressed when cells are not in S phase and is also blocked by elevated levels of histone proteins (Osley 1991). Both the cell cycle regulation and feedback controls fail if any of the *hi*stone *r*egulatory or *hi*stone *p*romoter *c*ontrol (*HIR1*, *HIR2/SPT1*, *HIR3/HPC1*, or *HPC2*) genes are defective (Osley 1991; Xu *et al.* 1992). Mutations in these "*HIR/HPC*" genes therefore lead to increased levels of histone gene transcripts during periods when few nucleosomes are being assembled.

Hir1 and Hir2 proteins are similar to one another and to a family of HIRA proteins that have been identified in several eukaryotes (see references cited in Lorain *et al.* 1998). These proteins bind to histones (Lorain *et al.* 1998; Sutton *et al.* 2001), and the frog HIRA has been shown to act in a replication-independent nucleosome deposition pathway (Ray-Gallet *et al.* 2002). Consistent with a role in chromatin assembly in yeast, the Hir/Hpc proteins display functional overlap and some sequence similarity with the chromatin assembly factor known as CAF-I (Kaufman *et al.* 1998). CAF-I mediates the replication-dependent deposition of nucleosomes *in vitro* and is important for maintaining normal chromatin structure *in vivo* (Smith and Stillman 1989; Kaufman *et al.* 1997). Mild phenotypes result from deletion of individual genes that encode either Hir/Hpc proteins or CAF-I subunits, but combining deletions of genes from both sets causes a strong growth defect, a severe decrease in heterochromatin-mediated gene silencing, and chromosomal instability due to defective kinetochore formation (Kaufman *et al.* 1998; Sharp *et al.* 2002). These data suggest that Hir/Hpc and CAF-I proteins each promote chromatin assembly through independent but partially redundant pathways.

To gain further insight into the function of yFACT, we conducted an unbiased screen for mutations that cause increased reliance on the function of Spt16. Here we report that mutations in any of the four *HIR/HPC* genes cause poor growth or lethality when combined with mutations in *SPT16* or *POB3*. Synthetic phenotypes such as these have proven valuable in discovering common functions for genes (Guarente 1993) and can reflect any of several relationships between the gene products involved. The evidence presented here supports an interpretation that extends our understanding of both FACT and the Hir/Hpc pathway. We propose that FACT not only disrupts nucleosomes but also is responsible for their reassembly after a polymerase has passed. We further propose that the Hir/Hpc proteins constitute a general nucleosome reassembly-repair pathway that becomes essential when yFACT fails to perform its own reassembly function efficiently. Consistent with this model, a screen for mutations that are intolerable in the absence of *HIR1* revealed mutations in the Paf1 complex, another factor required for Pol II elongation. It therefore appears that the processes that allow polymerases to access DNA and are therefore necessary for elongation also disrupt chromatin structure and create a need for a reassembly pathway provided by the Hir/Hpc proteins.

MATERIALS AND METHODS

Strains used are described in Table 1. Media were prepared essentially as described in Hartwell (1967) and Rose *et al.* (1990).

Screen for mutants: Mutations that are lethal when combined with *spt16-11* (T828I P859S) were identified essentially as described in Bender and Pringle (1991) and in Formosa and Nittis (1999). Strain 7915-2-4 (*spt16-11 ade2 ade3 ura3*) carrying the YEp352 derivative pTF149 (*SPT16 ADE3 URA3*) was mutagenized with ultraviolet radiation to ~50% survival on rich medium. About 105,000 colonies were screened for the inability to survive loss of the plasmid and therefore contained no white sectors and were sensitive to 5-fluoroorotic acid (5-FOA; Boeke *et al.* 1987) at 22°. *SPT16* is essential for viability (Malone *et al.* 1991), so candidates were tested for null mutations in *SPT16* by forming diploids with an *spt16-Δ* strain. Isolates unable to form white sectors or to grow on medium containing 5-FOA at a permissive temperature for *spt16-11* mutants (26°) were considered to represent null mutants in *SPT16*, dominant synthetic mutants, or plasmid integrants and were discarded. Isolates able to form white sectors and grow on medium containing 5-FOA at 26° but not at a restrictive temperature for *spt16-11* mutants (37°) were retained. Most combinations of *spt16-11* with defective versions

TABLE 1
Strains

Strain	Genotype
4053-5-2	*MAT***a** *trp1 leu2 ura3 his7*
7373-4-4	*MAT***a** *trp1 leu2 ura3 his3*
7697	*MAT***a** *trp1 leu2 ura3 his7 pob3-Δ(TRP1)*
7737-3-2	*MAT***a** *trp1 leu2 ura3 his3 spt16-Δ(TRP1)*
7740-1-4	*MAT***a** *trp1 leu2 ura3 his3 sas3-Δ(URA3)*
7740-2-1	*MAT***a** *trp1 leu2 ura3 his3 sas3-Δ(URA3) spt16-G132D(TRP1)*
7742-5-3	*MAT***a** *trp1 leu2 ura3 his3 spt16-G132D(URA3)*
7782-x	*MAT***a** *trp1 leu2 ura3 his7 spt16-x*
7809-7	*MAT***a** *trp1 leu2 ura3 his7 pob3-7*
7810-1-3	*MAT***a** *trp1 leu2 ura3 his3 pob3-L78R*
7864-2-1	*MAT***a** *trp1 leu2 ura3 his3 spt16-G132D*
7864-11-1	*MAT***a** *trp1 leu2 ura3 his3 spt16-11*
7915-2-4	*MAT***a** *trp1 leu2 ura3 his7 ade2 ade3 spt16-11 YEp-URA3-SPT16-ADE3*
7973-4-4	*MAT***a** *trp1 leu2 ura3 his3 pob3-7*
7982-3-4 T	*MAT***a** *trp1-Δ2 leu2-Δ0 ura3-Δ0 his7 hta2-htb2-Δ(TRP1)*
7982-5-1 K	*MAT***a** *trp1-Δ2 leu2-Δ0 ura3-Δ0 his3 hta2-htb2-Δ(KanMX)*
7985-3-1	*MAT***a** *trp1-Δ2 leu2-Δ0 ura3-Δ0 his3 hir1-Δ(LEU2)*
7986 K	*MAT***a** *trp1 leu2 ura3 his3 hpc2-Δ(KanMX)*
7988-7-3	*MAT***a** *trp1 leu2 ura3 his3 hpc2-Δ(KanMX) spt16-11 YCp-HPC2*
7989-12-3	*MAT***a** *trp1 leu2 ura3 his3 pob3-L78R hpc2-Δ(KanMX)*
7997-1-4	*MAT***a** *trp1-Δ2 leu2-Δ0 ura3-Δ0 his3 hta2-htb2-Δ(KanMX) spt16-11*
7997-4-4	*MAT***a** *trp1 leu2 ura3 his3 hht1-hhf1-Δ(TRP1) spt16-11*
7998-9-2	*MAT***a** *trp1 leu2 ura3 his7 pob3-7 hta2-htb2-Δ(KanMX)*
8000-1-3	*MAT***a** *trp1 leu2 ura3 his3 spt16-Δ2(TRP1) hpc2-Δ(HIS3) YEp-URA3-SPT16*
8006-2-4	*MAT***a** *trp1 leu2 ura3 his7 hpc2-Δ(TRP1) spt16-G132D*
8008-9-3	*MAT***a** *trp1 leu2 ura3 his3 hir1-Δ(LEU2) pob3-7*
8009-4-4	*MAT***a** *trp1 leu2 ura3 his3 hir1-Δ(LEU2) spt16-11*
8012-2-3	*MAT***a** *trp1-Δ2 leu2-Δ0 ura3-Δ0 his7 hht1-hhf1-Δ(TRP1)*
8012-9-2	*MAT***a** *trp1-Δ2 leu2-Δ0 ura3-Δ0 his3 hht1-hhf1-Δ(TRP1)*
8017-1-4	*MAT***a** *trp1 leu2 ura3 his7 pob3-7*
8019-5-1	*MAT***a** *trp1 leu2 ura3 his7 hht1-hhf1-Δ(TRP1) pob3-7*
8023-2-2	*MAT***a** *trp1 leu2 ura3 his3 hht1-hhf1-Δ(TRP1) spt16-G132D*
8024-3-2	*MAT***a** *trp1 leu2 ura3 his3 hht1-hhf1-Δ(TRP1) spt16-11*
DY150	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*
DY4548	*MAT*α *rpd3-Δ(LEU2) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY7142	*MAT*α *nhp6a-Δ(KanMX) nhp6b-Δ(ADE2) hht1-hhf1-Δ(LEU2) hht2-hhf2-Δ(HIS3) ade2 can1 his3 leu2 lys2 trp1 ura3 YCp50-HHT2-HHF2*
DY7230	*MAT***a** *spt16-11 ade2 can1 his3 leu2 lys2 met15 ura3*
DY7803	*MAT***a** *hht1-hhf1-Δ(LEU2) hht2-hhf2-Δ(KanMX3) ade2 can1 his3 leu2 lys2 trp1 ura3 YCp50-HHT2-HHF2*
DY7805	*MAT***a** *hht1-hhf1-Δ(LEU2) ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY7807	*MAT***a** *hht2-hhf2-Δ(KanMX3) ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY7809	*MAT***a** *spt16-11 hht1-hhf1-Δ(LEU2) hht2-hhf2-Δ(KanMX3) ade2 can1 his3 leu2 lys2 trp1 ura3 YCp50-HHT2-HHF2*
DY7811	*MAT***a** *spt16-11 hht1-hhf1-Δ(LEU2) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY7813	*MAT***a** *spt16-11 hht2-hhf2-Δ(KanMX3) ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY7815	*MAT***a** *spt16-11 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY7818	*MAT***a** *pob3-L78R hht1-hhf1-Δ(LEU2) hht2-hhf2-Δ(KanMX3) ade2 can1 his3 leu2 lys2 met15 trp1 ura3 YCp50-HHT2-HHF2*
DY8106	*MAT***a** *spt16-11 ade2 can1 his3 leu2 met15 trp1 ura3*
DY8145	*MAT***a** *spt16-11 esa1-L254P ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8149	*MAT***a** *esa1-L254P ade2 can1 his3 leu2 lys2 met15 ura3*
DY8152	*MAT***a** *spt16-11 elp3-Δ(LEU2) ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8154	*MAT***a** *spt16-11 gcn5-Δ(HIS3) ade2 can1 his3 leu2 trp1 ura3*
DY8156	*MAT*α *elp3-Δ(LEU2) ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8157	*MAT***a** *gcn5-Δ(HIS3) ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8179	*MAT***a** *sas3-Δ(URA3) ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8181	*MAT***a** *sas3-Δ(URA3) spt16-11 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8259	*MAT*α *rpd3-Δ(LEU2) spt16-11 ade2 can1 his3 leu2 trp1 ura3*
PKY810	*MAT*α *leu2 ura3 his3 trp1 ade2 can1 hpc2-Δ(KanMX) URA3-VIIL*
PKY817	*MAT***a** *leu2 ura3 his3 trp1 ade2 ade3-Δ can1 hir1-Δ(HIS3)*
PKY895	*MAT*α *leu2 ura3 his3 trp1 ade2 can1 asf1-Δ(HIS3)*
PKY938	*MAT*α *leu2 ura3 his3 trp1 ade2 can1 asf1-Δ(TRP1)*
HKY1063-1	*MAT***a** *leu2-k::ADE2-URA3::leu2-k ura3 his3 trp1 ade2 can1 paf1-Δ(HIS3)*
HKY1064-1	*MAT***a** *leu2-k::ADE2-URA3::leu2-k ura3 his3 trp1 ade2 can1 cdc73-Δ(TRP1)*
HKY998-2A	*MAT***a** *leu2-k::ADE2-URA3::leu2-k ura3 his3 trp1 ade2 can1 hpr1-Δ(HIS3)*

Strains with names beginning with letters are isogenic with W303; all others are isogenic with A364a. 7782-x indicates a set of isogenic strains with the number indicating the allele of *spt16* integrated at the normal genomic locus.

of *POB3* were previously shown to cause lethality (Schlesinger and Formosa 2000). Candidates were therefore also tested for complementation of the nonsectoring phenotype and 5-FOA sensitivity by plasmids containing *POB3*. A total of 29 strains were identified that appeared to harbor mutations that were synthetically lethal in combination with *spt16-11* after these tests. A genomic library in the low-copy vector p366 (a generous gift from P. Hieter and F. Spencer) was introduced into some of these strains to identify plasmids that complement the synthetic lethality and therefore produce white colonies resistant to 5-FOA. Plasmids without the *SPT16* gene were sequenced to identify candidate genes. Individual genes were then amplified by PCR (primer sequences available upon request) and tested for complementation in low-copy vectors. In the case of *HPC2* and *HIR1*, the synthetic lethality was further confirmed by deleting these genes and then reconstructing double mutants with *spt16-11* in standard genetic crosses in the presence of a plasmid containing the *HPC2* or *HIR1* genes. These were tested for the ability to survive loss of the *HIR1* or *HPC2* plasmid by testing for growth on medium containing 5-FOA.

Mutations that are lethal when combined with a *hir1*-Δ gene deletion were identified essentially as described above using strain PKY817 (*hir1*-Δ *ade2 ade3 ura3*) carrying the YEp24-derivative pPK171 (*HIR1 ADE3 URA3*). Approximately 200,000 colonies were screened for the inability to survive loss of pPK171. Two isolates were identified that regained the ability to form white sectors and to grow on medium containing 5-FOA in the presence of the pRS425-derivative pPK174 (*HIR1 LEU2*). Previous results showed that *hir1* mutations are lethal when combined with defects in *spt4* (A. Bortvin and F. Winston, personal communication), and tests revealed that one of the mutants from this screen was complemented by a plasmid containing *SPT4*. The other mutant was complemented by plasmids containing only *PAF1*. Genetic crosses with marked gene deletions were then performed to confirm and extend these results (Table 2).

Maximal permissive temperature determination: Aliquots of saturated cultures containing similar numbers of cells were distributed onto solid media and incubated at increments of 1°. The highest temperature supporting at least 10% viability was designated the maximal permissive temperature (MPT).

S1 assays: RNA levels were quantitated by S1 nuclease protection using *HTB1* and *CMD1* probes followed by phosphorimager analysis as described in Bhoite and Stillman (1998) and Yu *et al.* (2000).

RESULTS

A screen for synthetic defects reveals a genetic interaction between *SPT16* and *HIR/HPC* genes: The *spt16-11* (T828I P859S) allele supports robust growth at moderate temperatures, but it causes sensitivity to elevated temperatures, to the dNTP synthesis inhibitor hydroxyurea (HU), and to the transcription elongation inhibitor 6-azauracil (6-AU; Formosa *et al.* 2001). HU blocks progression of replication by limiting the availability of dNTPs and 6-AU inhibits elongation of transcription by causing imbalances in the pools of rNTPs (Exinger and Lacroute 1992; Shaw and Reines 2000). Other alleles of *SPT16* cause more severe defects than *spt16-11* for each of these phenotypes, but no other allele tested affected all of the phenotypes, suggesting a defect in a core function of Spt16 required both for DNA replication and for transcription elongation. Mutations that enhance the *spt16-11* mutation could therefore reveal the function of yFACT in either pathway.

TABLE 2

Results of genetic screens and tests

Screen with	Synthetic lethal mutation in	No. of isolates
spt16-11	*HIR1*	7
	HIR2/SPT1	5
	HIR3/HPC1	7
	HPC2	7
hir1-Δ	*PAF1*	1
Tests with	**Phenotype of double mutant**	
hpc2-Δ *paf1*-Δ	Lethal	
hpc2-Δ *cdc73*-Δ	Slow growth	
hpc2-Δ *hpr1*-Δ	No effect	
asf1-Δ *paf1*-Δ	Lethal	
asf1-Δ *cdc73*-Δ	Slow growth	
asf1-Δ *spt16-11*	MPT decreased 5°	
asf1-Δ *pob3-7*	MPT decreased >2°	

We screened for mutations that cause lethality when combined with *spt16-11* (see materials and methods). A total of 29 strains carrying mutations that make *spt16-11* intolerable were isolated by screening an *spt16-11 ade2 ade3* strain for colonies unable to survive loss of a plasmid carrying *SPT16*, *URA3*, and *ADE3* and therefore uniformly red in color and unable to grow on media containing 5-FOA. A low-copy genomic library was introduced into a set of these mutants, and plasmids that complemented the synthetic defect were identified by their ability to permit growth on media containing 5-FOA and to allow the formation of white sectors. Plasmids with inserts including *HIR1*, *HIR3*, and *HPC2* were identified in this way. Complementation by these genes alone was confirmed using plasmids containing single open reading frames (ORFs). Since mutations in *HIR2/SPT1* cause many of the same phenotypes as mutations in the genes initially identified, a plasmid containing only this gene was constructed and the remaining mutants were tested with all four *HIR/HPC* genes. Twenty-six of the mutations were complemented by one of these genes (Table 2). The unbiased screen therefore indicates very clearly that *spt16-11* mutations cause strong dependence on the function of the *HIR/HPC* genes.

Reconstructions confirm the interaction with *SPT16*: Each mutant was complemented by only one of the *HIR/HPC* genes, suggesting that each mutant had a defect in one of these genes (instead of an alternative in which all synthetic defects with *spt16-11* are suppressed by increasing the amount of any *HIR/HPC* gene). To confirm that individual *hir/hpc* mutations enhance the defects caused by *spt16-11* we constructed deletions of *HIR1* and *HPC2* and attempted to obtain

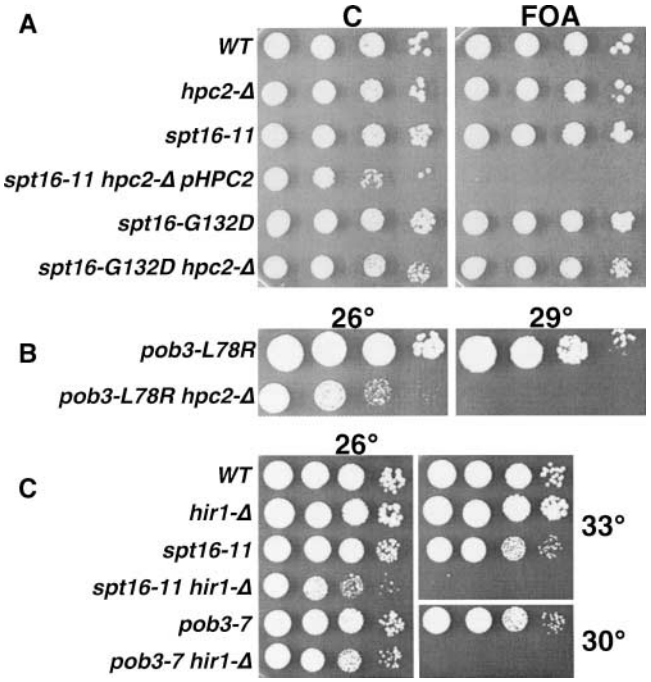

FIGURE 1.—Mutations in *SPT16* and *POB3* cause dependence on *HIR/HPC* genes. (A) Cultures with the relevant genotype indicated were grown to saturation in rich medium and washed in water, and then aliquots of 10-fold dilutions were placed on complete synthetic medium (C) or medium containing 5-FOA and incubated at 26°. Strains (see Table 1) are 7373-4-4, 7986 K, 7864-11-1, 7988-7-3 pSR14, 7782-2, and 8006-2-4. (B and C) Cultures were grown and diluted as in A and then placed on rich medium and incubated at the temperature indicated. Strains are 7810-1-3, 7989-12-3, 7373-4-4, 7985-3-1, 7864-11-1, 8009-4-4, 7973-4-4, and 8008-9-3.

double mutants with *spt16-11* using genetic crosses. Diploids heterozygous for *spt16-11* and either *hpc2*-Δ or *hir1*-Δ and containing *URA3*-marked plasmids with a normal copy of either *HPC2* or *HIR1* were constructed and sporulated. Double mutants were identified among the haploid segregants, and these were tested for the ability to survive loss of the *URA3*-marked plasmid by selection on media containing 5-FOA. As shown in Figure 1A, the combination of *spt16-11* and *hpc2*-Δ is lethal because cells with this genotype do not produce colonies at any temperature on media containing 5-FOA and therefore do not survive the loss of the plasmid with the *HPC2* gene. In contrast, an *spt16-11 hir1*-Δ combination was found to be viable, but impaired for growth relative to single mutants even at 26° (Figure 1C). The growth defect was more pronounced at elevated temperatures, leading to inviability at 33°, a temperature that is permissive for each single mutant. Combining the *spt16-11* and *hir1*-Δ mutations therefore causes a strong synthetic defect even under permissive conditions, a defect severe enough to allow detection in the nonsectoring assay employed in our screen. We conclude from these reconstructions and the results of the genetic screen that mutation of any of the four *HIR/HPC* genes causes a strong defect or lethality when combined with the *spt16-11* mutation.

The interaction between *SPT16* and *HIR/HPC* genes is allele specific: Different alleles of *SPT16* cause significantly different phenotypes (FORMOSA *et al.* 2001 and Table 3), suggesting that different mutations might affect different functional domains of the Spt16 protein that are involved in distinct pathways. To determine whether the interaction with defects in *HPC/HIR* genes is also allele specific, we tested a set of *spt16* mutants for the ability to tolerate loss of *HPC2*. For this purpose a strain with deletions of both *HPC2* and *SPT16* was constructed and the essential *SPT16* gene was provided on a *URA3*-marked plasmid. Low-copy *LEU2*-marked plasmids bearing mutant *spt16* alleles (FORMOSA *et al.* 2001) were then introduced into this strain, and transformants were tested for the ability to survive with only the mutant *spt16* allele after loss of the plasmid with the wild-type gene. The *spt16* alleles varied in their ability to support growth in a strain lacking *HPC2*, indicating that the interaction is allele specific (Table 3). Notably, the commonly used *spt16-G132D* mutation (EVANS *et al.* 1998) supported growth when combined with *hpc2*-Δ. Surprisingly, while some alleles displayed tight lethality in combination with *hpc2*-Δ in this test, *spt16-11* itself supported very weak growth. This suggests that the plasmid-based *SPT16* gene may provide a less stringent test of the synthetic defect with *hpc2*-Δ than does the endogenous genomic context used in the screen and in Figure 1. This is consistent with previous reports that the temperature sensitivity caused by *spt16* mutations can be suppressed by increasing the copy number of the mutated gene (XU *et al.* 1993; EVANS *et al.* 1998). We therefore also tested the effect of combining several genomic *spt16* mutations with *hpc2* using standard genetic crosses (Table 3). The *spt16-G132D hpc2*-Δ double mutant was recovered from a cross at the expected frequency and displayed only slightly reduced growth at 26° (Figure 1A). However, loss of *HPC2* did enhance the temperature sensitivity of the *spt16-G132D* strain somewhat, since the double mutant displayed a maximal permissive temperature 1°–2° lower than that of the single mutant (Table 3). Combining the *spt16-8* or *spt16-22* alleles with an *hpc2* deletion also produced viable cells with enhanced temperature sensitivity, but the synthetic defect was more severe in these cases than with *spt16-G132D*, with the maximal permissive temperature decreasing at least 5° compared to the single mutants (Table 3). Combining *hpc2*-Δ with *spt16* mutations was therefore deleterious in all cases tested, but the severity of the defect was strongly allele specific, suggesting that certain defects in Spt16 cause greater reliance on Hpc/Hir proteins.

***POB3*, but not *NHP6*, also interacts with *HIR/HPC* genes:** Pob3 and Nhp6 function with Spt16 both *in vitro* and *in vivo* (BREWSTER *et al.* 1998, 2001; WITTMEYER *et al.* 1999; SCHLESINGER and FORMOSA 2000; FORMOSA *et*

TABLE 3

Allele specificity of the *hpc2* interaction with *spt16* mutations

Allele	Decrease in MPT with HU	Growth at 30° with HU	Effect with *hpc2*-Δ	
			spt16-x plasmid	*spt16-x* integrated
SPT16	None	+++	Viable	None
spt16-G132D	2°	+++	Viable	Weak defect
spt16-4	2°	+++	Viable	ND
spt16-6	ND	+++	Strong defect	ND
spt16-7	3°	+++	Viable	ND
spt16-8	≥5°	+	Strong defect	Strong defect
spt16-9	ND	−	Lethal	ND
spt16-11	≥6°	+	Defect	Lethal
spt16-12	3°	+++	Weak defect	ND
spt16-16	ND	+/−	Lethal	ND
spt16-22 (A417V)	≥5°	+/−	Lethal	Strong defect
spt16-24	≥4°	+	Weak defect	ND

Isogenic strains (7782-x) with the *spt16* alleles indicated were placed on rich medium with or without 100 mM HU and the difference in maximal permissive temperature was determined as described in MATERIALS AND METHODS. The effect of HU was not determined for alleles that we were unable to integrate, but the qualitative effect of HU on growth at 30° was assessed using strain 7737-3-2 carrying pTF128 (*YCp LEU2 SPT16*) with the allele of *SPT16* indicated. Strain 8000-1-3 (*spt16*-Δ *hpc2*-Δ *YCp-SPT16-URA3*) was transformed with the same pTF128 derivatives and tested for growth on media containing 5-FOA at 26° to assess the effect of losing the *SPT16* plasmid ("*spt16-x* plasmid"). The strength of the defect was scored on the basis of the growth rates observed at 26° and 30°. Selected alleles were also tested by reconstruction of the double mutant in standard genetic crosses ("*spt16-x* integrated"). The maximal permissive temperature of viable combinations was determined; "strong defect" indicates a drop of 5° or more, and "weak defect" indicates a drop of 1°–2°. ND, not determined.

al. 2001). Spt16 and Pob3 are found in cell extracts only in a complex with one another, while Nhp6 associates only weakly with Spt16-Pob3 (BREWSTER *et al.* 2001; FORMOSA *et al.* 2001). We therefore tested the effect of combining mutations in the *POB3* or *NHP6A/B* genes with an *hpc2* deletion to see whether defects in all yFACT components cause dependence on *HIR/HPC* genes. The *pob3-L78R hpc2*-Δ mutants are viable but grow very slowly at 26° and display increased temperature sensitivity compared to single mutants (Figure 1B). Similar results were obtained with a *pob3-7 hir1*-Δ combination (Figure 1C). However, an *nhp6a*-Δ *nhp6b*-Δ *hpc2*-Δ strain was no more compromised for growth than was an *nhp6a*-Δ *nhp6b*-Δ strain, even at temperatures near the restrictive temperature for *nhp6a*-Δ *nhp6b*-Δ strains (data not shown). Therefore, loss of *HIR/HPC* genes was also severely detrimental to cells lacking normal *POB3*, but no strong genetic interaction was detected with Nhp6. The components of yFACT therefore can function both together and independently, with Spt16-Pob3 and Nhp6 defects causing very different responses to the loss of Hir/Hpc proteins.

Mutations in *HIR/HPC* genes are also lethal when combined with other factors that promote elongation: *hir1* mutations were previously found to be lethal when combined with some alleles of *SPT4*, *SPT5*, or *SPT6* (A. BORTVIN and F. WINSTON, personal communication; also see MATERIALS AND METHODS). These three genes are functionally related to one another and promote transcriptional elongation (BORTVIN and WINSTON 1996; HARTZOG *et al.* 1998; WADA *et al.* 1998). To extend these observations, a screen for additional mutations that cannot be tolerated in the absence of *HIR1* was performed, and it revealed that a *paf1* mutation has this property (Table 2). Paf1 is a member of a third complex that promotes transcription elongation and has been linked physically and genetically both to Spt4/5/6 and to yFACT (COSTA and ARNDT 2000; SQUAZZO *et al.* 2002). Reconstructions of double mutants with another member of the Paf1 complex, Cdc73, confirm that loss of function of the Paf1 complex causes reliance on the Hir/Hpc proteins (Table 2).

The histone chaperone Asf1 is involved in some Hir/Hpc functions, such as the ability to promote silent heterochromatin formation at telomeres (SHARP *et al.* 2001; SUTTON *et al.* 2001), but is not required for other functions, such as the role in maintaining kinetochores (SHARP *et al.* 2002). Asf1 is required in cells lacking members of the Paf1 complex since the *asf1*-Δ *paf1*-Δ combination is lethal (Table 2). Mutations in another gene linked to transcription elongation, *HPR1* (CHAVEZ and AGUILERA 1997), did not cause a defect when combined with *hpc2*-Δ, demonstrating that not all elongation factors display this effect. Reconstructions also show that combining *asf1*-Δ with either *spt16-11* or *pob3-7* results in cells that grow normally under conditions used in our genetic screens (data not shown), but cause a severe decrease in the maximal permissive temperature (Table

2). Asf1 is therefore required for full activity of the Hir/Hpc proteins in maintaining the viability of *spt16* and *pob3* mutants. Together, these results indicate that defects in three systems that promote transcriptional elongation (Spt4/5/6, the Paf1 complex, and yFACT) all make the Hir/Hpc-Asf1 pathway more important or essential.

***SPT16* and *POB3* are activators of histone gene transcription:** The *spt16* alleles that cause the strongest synthetic defects with an *hpc2*-Δ deletion overlap the set that caused the greatest sensitivity to HU, although the correlation is imperfect (Formosa *et al.* 2001 and Table 3). This suggests that the same activity of Spt16 that is responsible for allowing growth in the presence of HU is also needed when Hir/Hpc proteins are defective. HU blocks DNA replication by inhibiting the synthesis of dNTP precursors. HU arrest normally represses histone expression, but this response fails in cells lacking normal *HIR/HPC* genes (Osley and Lycan 1987; Xu *et al.* 1992). We therefore considered the possibility that Spt16 and Hir/Hpc proteins each contribute to the repression of histone gene transcription, and the synthetic defects observed reflect the inability of a cell to tolerate very high levels of histones. In this model, some *spt16* mutations cause sensitivity to HU because they fail to repress histone gene expression during S-phase arrest.

It has previously been reported that the *spt16-G132D* mutation causes decreased levels of histone gene transcription and that the repression of this transcription is normal during an HU arrest (Xu *et al.* 1993), but *spt16-G132D* mutants are relatively resistant both to HU and to loss of Hpc2 and thus might not represent other *spt16* alleles. However, as shown in Figure 2, S1 analysis of *HTB1* transcripts in several *spt16* and *pob3* mutants shows that histone gene expression is not increased in any of the mutants tested, but rather is always either unaltered or decreased. The effect is small in some cases, as for *spt16-G132D*, but over twofold in others, as for *spt16-16* and *pob3-1*. The decrease in *HTB1* transcription did not correlate well with sensitivity either to HU or to the loss of Hir/Hpc proteins. For example, *spt16-11* causes relatively strong sensitivity to HU and is lethal when combined with *hir/hpc* mutations but has little or no effect on *HTB1* transcript levels. Thus, none of the *spt16* or *pob3* mutations tested support a role for Spt16-Pob3 as a repressor of histone gene transcription. Further, *hir1*-Δ and *hpc2*-Δ mutations in either W303 or A364a genetic backgrounds grew as well as wild-type strains on 100 mM HU (data not shown), indicating that the elevated level of histone gene expression observed under these conditions is not toxic by itself. Finally, the synthetic defect caused by combining *spt16-11* with *hir/hpc* mutations does not seem to be caused by the Hir− phenotype itself (loss of histone gene repression during exposure to HU), since two other mutations that cause this phenotype, *spt10*-Δ and *spt21*-Δ (Sherwood and Osley 1991), did not cause severe decreases in viability or rate of growth when combined with *spt16-11* (data not shown). We conclude that the interaction between *SPT16-POB3* and *HIR/HPC* genes is not due to overlapping roles as independent repressors of histone gene expression.

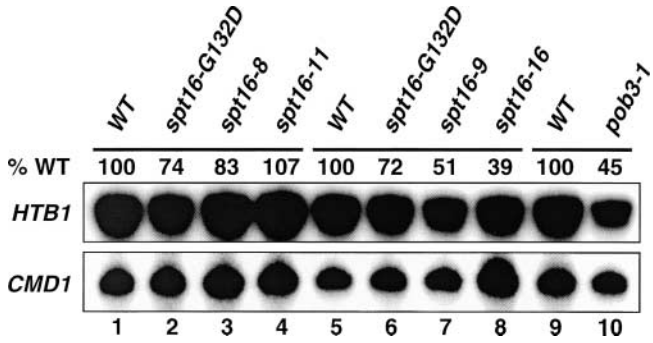

Figure 2.—Spt16 and Pob3 are activators of histone gene expression. Strains with the relevant genotypes indicated were grown to log phase in rich medium, RNA was extracted, and then the *HTB1* and *CMD1* messages were quantitated by S1 nuclease analysis (see materials and methods). The *HTB1* message level normalized to *CMD1* and expressed as a percentage relative to wild type is shown above each lane. Strains in lanes 1–10 are 4053-5-2, 7782-2, 7782-8, 7782-11, 7737-3-2 pTF128, 7737-3-2 pTF128-2, 7737-3-2 pTF128-9, 7737-3-2 pTF128-16, 7697 pTF139, and 7697 pTF139-1. pTF128 is a low-copy vector with normal *SPT16*; a number appearing after the plasmid name designates an *spt16* allele as described previously (Formosa *et al.* 2001). pTF139 is a low-copy vector with normal *POB3*, and pTF139-1 is the same plasmid with the *pob3-1* allele (Schlesinger and Formosa 2000).

***SPT16* and *POB3* mutants are affected by alterations in the number of copies of histone genes:** The *S. cerevisiae* genome includes two copies of each of the genes that encode the four histone proteins. The four genes that encode H2A and H2B are found as two paired sets (*HTA1-HTB1* and *HTA2-HTB2*), as are the genes that encode H3 and H4 (*HHT1-HHF1* and *HHT2-HHF2*). Deleting some histone gene pairs causes the global misregulation of transcription initiation site selection called the Spt− phenotype, as well as other defects in transcription (Clark-Adams *et al.* 1988; Osley 1991). Unbalanced increases in either H2A-H2B or H3-H4 gene sets also cause the Spt− phenotype, as well as chromosome instability (Meeks-Wagner and Hartwell 1986; Clark-Adams *et al.* 1988). The appropriate availability of a balanced set of histone proteins is therefore important for maintaining appropriate regulation of transcription and genomic stability. The *hir/hpc* mutants deregulate expression of both pairs of genes that encode H3-H4, but only one of the H2A-H2B sets (*HTA1-HTB1*; Osley and Lycan 1987; Xu *et al.* 1992), leading to unbalanced overexpression of the histone gene sets. We therefore considered the possibility that *hir/hpc* mutations affect the quality of chromatin by altering the ratio of histones

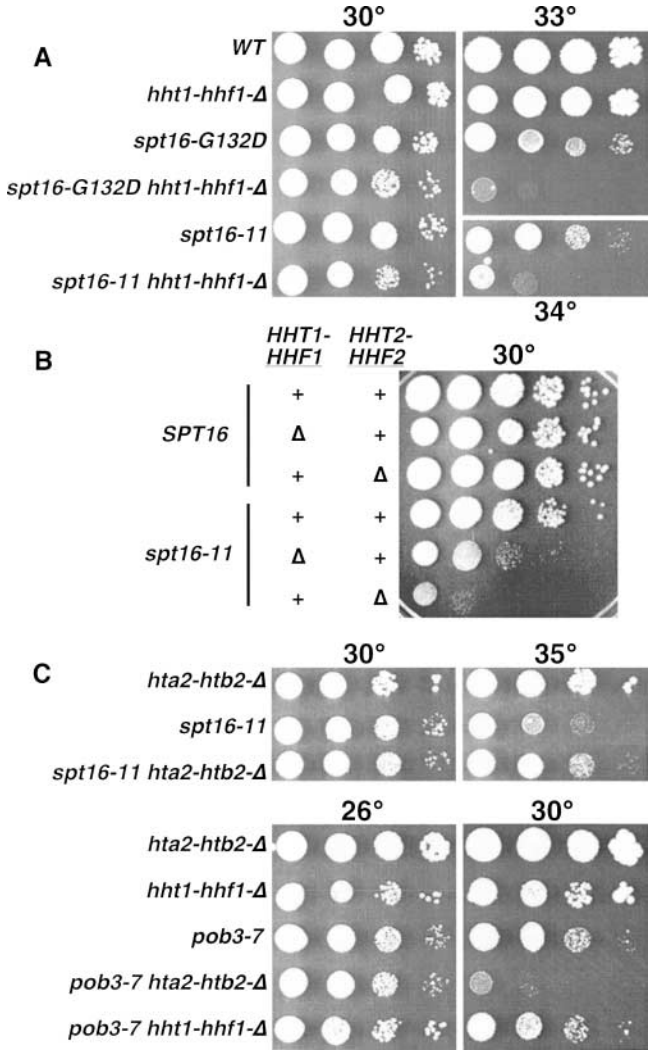

FIGURE 3.—Effects of deletions of histone genes on *spt16* and *pob3* mutants. Strains with the relevant genotypes indicated were grown and diluted as in Figure 1 and then placed on rich media and incubated at the temperatures indicated. Strains are: (A) 7373-4-4, 8012-9-2, 7864-2-1, 8023-2-2, 7864-11-1, and 8024-3-2; (B) DY150, DY7805, DY7807, DY7815, DY7811, and DY7813; and (C) 7982-5-1 K, 7997-4-4, 7997-1-4, 7982-3-4 T, 8012-2-3, 8017-1-4, 7998-9-2, and 8019-5-1.

available and that this alteration in chromatin quality impairs yFACT activity such that mutations in *SPT16* and *POB3* cannot be tolerated. To test this, we determined whether direct perturbations of histone gene copy numbers affect the growth of *spt16* or *pob3* mutants.

As shown in Figure 3, decreasing the histone gene copy number affected the growth of both *spt16* and *pob3* mutants. Surprisingly, these mutants responded in opposite ways to these changes. The growth of an *spt16-11* strain was strongly impaired and the maximal permissive temperature was significantly reduced by deletion of either *HHT1-HHF1* or *HHT2-HHF2*, indicating that a strain with this defect in Spt16 is sensitive to decreased expression of H3-H4 proteins (Figure 3 and Table 4). A similar effect was observed with an *spt16-G132D* strain (Figure 3), so this genetic interaction with diminished H3-H4 copy number is not as strongly allele specific as the interaction with *hpc2*. However, when the same H3-H4 gene deletions were combined with a *pob3-7* mutation, instead of causing an enhanced defect the double mutant grew slightly more rapidly than a *pob3-7* strain and displayed the same maximal permissive temperature (Figure 3 and Table 4). In contrast, deletion of *HTA2-HTB2* was strongly detrimental to the *pob3-7* strain, but caused a slight increase in the growth of an *spt16-11* strain (Figure 3 and Table 4). We have been unable to perform a similar test with a deletion of *HTA1-HTB1* because, unlike results obtained in other labs (*e.g.*, RYKOWSKI *et al.* 1981; NORRIS and OSLEY 1987; CLARK-ADAMS *et al.* 1988), this mutation alone caused inviability in three distinct strain backgrounds in our hands. Defective *POB3* therefore causes sensitivity to decreased expression of H2A-H2B, but not to decreased levels of H3-H4, and defective *SPT16* causes the opposite response (Table 4).

Increasing the levels of histone genes also affected the growth of *spt16* and *pob3* mutants, and the effects were consistent with the results obtained with histone gene deletions. As shown in Figure 4, increasing the expression of H3-H4 by introducing the *HHT2-HHF2* gene pair on a low-copy vector had little effect on a *pob3-7* strain, but partially suppressed the temperature sensitivity of an *spt16-11* strain. [High-copy plasmids carrying these gene pairs impaired the growth of both wild-type and mutant strains significantly (data not shown).] Conversely, increasing the amount of H2A-H2B partially suppressed the growth defect of a *pob3-7* strain, but was deleterious to the growth of an *spt16-11* strain (Figure 4).

Other studies have suggested that the ratio of H2A-H2B to H3-H4 expression is important for normal chromatin function (MEEKS-WAGNER and HARTWELL 1986; KAUFMAN *et al.* 1998). As summarized in Table 4, if we interpret the changes in histone gene dosage described above in terms of H2A-H2B:H3-H4 ratios, then high ratios are deleterious to *spt16* mutants, and low ratios enhance growth of these strains, while *pob3* mutants prefer high ratios and are impaired by low ratios. However, both *spt16* and *pob3* mutations displayed synthetic defects with *hir/hpc* and *asf1* mutations. Therefore, although yFACT function is affected by the levels of histone gene expression, our data suggest that the functional interaction between Spt16-Pob3 and the Hir/Hpc proteins cannot be accounted for by changes in histone gene expression alone.

Spt16-Pob3 does not interact genetically with CAF-I: Hir1 and Hir2 were initially identified as repressors of histone gene expression (OSLEY and LYCAN 1987). However, similar proteins from mammalian and amphibian sources participate in nucleosome assembly in a pathway independent of DNA replication and repair (RAY-GALLET *et al.* 2002). The Hir/Hpc proteins in

TABLE 4

Summary of effects of changes in histone gene copy number on *spt16* and *pob3* mutants

Change in copy number		Gene ratio of	Effect on growth of strain with	
H2A-H2B	H3-H4	H2A-H2B/H3-H4	*spt16-11*	*pob3-7*
Increase		High	Worse	Better
	Decrease	High	Worse	Better
Decrease		Low	(Better)	Worse
	Increase	Low	Better	(Worse)

Changes in growth upon deletion or addition of copies of histone genes are indicated as a function of the change in the ratio of H2A-H2B gene sets to H3-H4 gene sets. Representative data are shown in Figures 3 and 4, but similar results have been obtained in other strains from the A364a, W303, and S288c backgrounds (data not shown). More subtle effects are indicated by parentheses.

yeast also overlap functionally with the DNA replication-dependent nucleosome assembly factor CAF-I (Kaufman *et al.* 1998; Sharp *et al.* 2002). We therefore determined whether loss of CAF-I is detrimental to *spt16* or *pob3* mutants. Neither the growth rate nor the maximal permissive temperature of *pob3-L78R*, *pob3-7*, *spt16-G132D*, or *spt16-11* strains was affected by deletion of *CAC1*, the gene encoding the largest subunit of CAF-I (data not shown). Similar results were obtained with a deletion of *CAC2* combined with an *spt16-11* mutation (data not shown). Therefore, although CAF-I and the Hir/Hpc pathways are redundant for some functions, CAF-I does not appear to have the activity of the Hir/Hpc proteins that becomes essential when yFACT is defective.

***spt16* mutations display interactions with histone acetyltransferase genes:** Spt16 interacts with the histone acetyltransferase (HAT) complex NuA3 (John *et al.* 2000). Evidence supporting this interaction includes partial copurification, a two-hybrid interaction between Spt16 and the catalytic subunit of NuA3 (Sas3), and a synthetic sensitivity to 6-AU when a deletion of *SAS3* is combined with high-level expression of a truncated version of *SPT16* (John *et al.* 2000). Consistent with these data, we found that combining *spt16-G132D* with a *sas3* deletion caused a synthetic growth defect (Figure 5). The double mutant was unable to grow at a temperature permissive for the single mutants and was more sensitive to 6-AU than were the single mutants. The synthetic defect was relatively mild and was not observed with *spt16-11* or *pob3-7* in strains from the A364a background (data not shown). In the W303 background *sas3*-Δ somewhat enhanced the Ts− phenotype caused by *spt16-11* but did not affect sensitivity to 6-AU (Figure

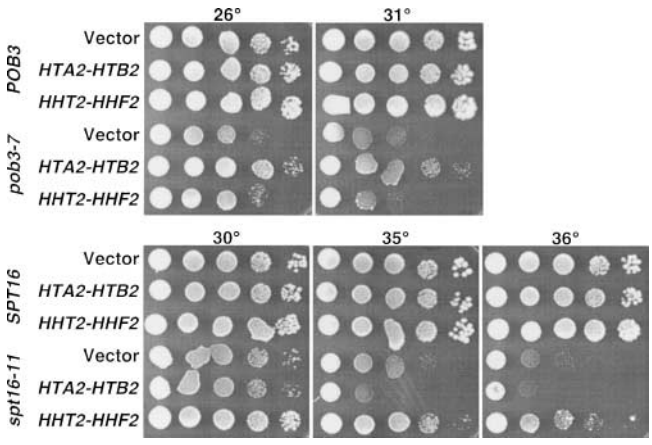

Figure 4.—Effects of extra copies of histone genes on *spt16* and *pob3* mutants. Strains 4053-5-2 (*POB3* and *SPT16*), 7809-7 (*pob3-7*), and 7782-11 (*spt16-11*) were transformed with the low-copy plasmid YCp50 (vector) or with this vector containing the histone gene pairs indicated, grown in media lacking uracil, diluted and plated as in Figure 1 on media lacking uracil, and incubated at the temperatures indicated.

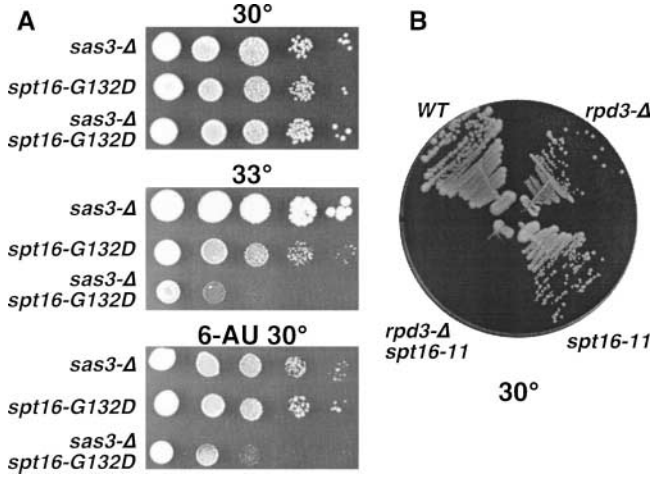

Figure 5.—Genetic interactions between *SPT16* and *SAS3*. (A) Strains with the relevant genotypes indicated (7740-1-4, 7742-5-3, and 7740-2-1; see Table 1) were grown and diluted as in Figure 1 and then placed on rich media and incubated at the temperatures indicated. (B) Approximately equal numbers of cells of strains with the relevant genotypes indicated (DY150, DY4548, DY8106, and DY8259) were struck to rich medium and incubated at 30°.

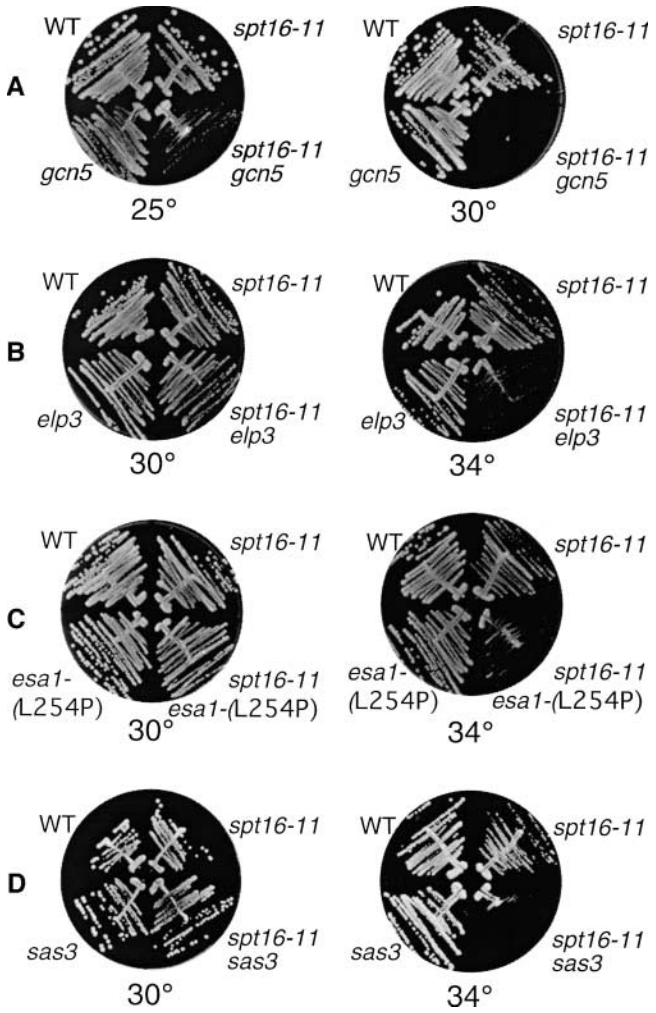

FIGURE 6.—Genetic interactions between *SPT16* and several HAT genes. Equivalent aliquots of strains with the relevant genotypes listed were struck on rich media and incubated at the temperatures indicated. (A–D) WT (wild type) is DY150 and *spt16-11* is DY7230. Other strains are: (A) DY8154, DY8157; (B) DY8152, DY8156; (C) DY8145, DY8149; and (D) DY8179, DY8181.

6 and data not shown). Because of the evidence for a physical interaction between Spt16 and Sas3, this genetic interaction could indicate that the loss of NuA3 enhances some *spt16* mutations by destabilizing functional complexes containing Spt16 protein. Alternatively, Spt16-Pob3 could be more generally sensitive to the modification state of histones and nucleosomes. To further characterize the dependence of yFACT on histone acetylation, we determined whether *spt16-11* interacts genetically with other HATs.

Strains with combinations of *spt16-11* and mutations in various genes encoding HATs were constructed and tested for growth at elevated temperatures and on media containing 6-AU. Deletion of *HAT1* had no effect on the growth of an *spt16-11* strain (data not shown), but *gcn5*-Δ, *elp3*-Δ, and *esa1-L254P* (CLARKE *et al.* 1999) were all detrimental when combined with *spt16-11* (Figure 6). Spt16 function is therefore sensitive to mutations in many HATs. The defect was particularly severe with *gcn5*-Δ, with the double mutant displaying at least a 5° decrease in the maximal permissive temperature relative to single mutants and slow growth even under permissive conditions. The *gcn5*-Δ mutation is synthetically lethal with a *sas3*-Δ mutation (HOWE *et al.* 2001), suggesting that these two enzymes overlap for some critical function. Our results are consistent with participation of Spt16 and Sas3 in a common function, which, when inactivated, causes greater reliance on Gcn5. Overall, the genetic interactions between *SPT16* and multiple HAT genes suggest that yFACT function is affected by the acetylation pattern of histones.

Consistent with a role for histone acetylation in yFACT function, we previously showed that deletion of the histone deacetylase encoded by *RPD3* partially suppresses the temperature sensitivity caused by a *pob3* mutation (FORMOSA *et al.* 2001). Although an *rpd3* deletion did not have a strong effect when combined with an *spt16-G132D* mutation, the *rpd3 spt16-11* double mutant has a significant growth defect at 30° compared to that of the single mutants, representing a decrease of ~5° in the maximal permissive temperature (Figure 5). Therefore, *spt16-11* mutants are also affected by the loss of a deacetylase, but as observed with changes in the copy number of histone genes, *spt16* and *pob3* mutations respond in opposite ways to the loss of *RPD3*.

Defects in *SPT16* cause reliance on normal histone tails: To test whether the effects noted when *spt16* and *pob3* mutations were combined with mutated acetyltransferases and deacetylases result directly from alterations in histones, we combined yFACT mutations with mutations in the genes that encode the histone proteins H3 and H4. For this purpose, strains were constructed that had deletions of both sets of the genes that encode histones H3 and H4, a low-copy plasmid carrying *URA3* and the normal *HHT2-HHF2* locus, and *spt16*, *pob3*, or *nhp6a/b*-Δ mutations. Plasmids with mutated alleles of *HHT2-HHF2* were then introduced into these strains and tested for the ability to replace the wild-type plasmid by selecting on medium containing 5-FOA.

An otherwise normal strain can tolerate the loss of the N-terminal tail of either H3 or H4, but an *spt16-11* strain displayed increased temperature sensitivity when either tail was deleted (Table 5). Point mutations that affect residues known to be acetylated under different circumstances were also tested. Mutations in H3 did not produce strong effects, but changes in the H4 tail caused defects. Changing the lysine residues at positions 5 and 12 in H4 to arginines caused a growth defect in the *spt16-11* strain, whereas changing the same residues to glutamines was tolerated. Arginine residues are chemically similar to lysines, but cannot be acetylated, while glutamine is chemically similar to the acetylated form of lysine. These results therefore suggest that the ability to acetylate these residues of H4 contributes to the exe-

TABLE 5

Effect of histone H3 and H4 N-terminal tail mutations on yFACT mutants

Histone tail					
H3	H4	WT	*spt16-11*	*pob3-L78R*	*nhp6a/b-*Δ
WT	WT	+++	+++	+	+++
Δ3-29	WT	+++	−	−	−
WT	Δ4-19	+++	−	+/−	−
K9Q	WT	+++	+++	+	+++
K9R	WT	+++	+++	+	+++
K14R	WT	+++	+++	+	+++
WT	K5,12R	+++	−	+	+++
WT	K5,12Q	+++	+++	+	+++
WT	K8, 16R	+++	+	−	−
WT	K8,16Q	+++	+++	+	+++
K14Q	K8,16Q	+++	+++	+	+++

Strains DY7803 (WT), DY7809 (*spt16-11*), DY7818 (*pob3-L78R*), and DY7142 (*nhp6a/b-*Δ) carrying YCp50-*HHT2-HHF2* were transformed with plasmids with the mutations indicated (Zhang *et al.* 1998) and then tested on medium containing 5-FOA for ability to grow at 34° (*spt16-11*), 25° (*pob3-L78R*), or 30° (*nhp6a/b-*Δ). The sizes of individual colonies were assessed after several days and compared to WT (defined to be +++). WT, wild type.

cution of the Spt16 function. Newly synthesized H4 is usually acetylated on lysines 5 and 12 (Sobel *et al.* 1995), suggesting that FACT function may be related to the nucleosome assembly process. In contrast, acetylation of lysines 8 and 16 of H4 correlates with transcriptionally active chromatin, and these sites also appear to be important for Spt16 activity. Mutation of residues 8 and 16 to arginines (but not to glutamines) caused a growth defect in an *spt16-11* strain, although one less severe than that of the K5,12R mutations (Table 5). Consistent with the defects observed with multiple HAT mutations, Spt16 activity appears to be affected by the ability of histone tails to be modified at multiple sites. It is notable that the N terminus of Spt 16 shares significant homology with a family of aminopeptidases, but lacks the active site residues found in members of the family with hydrolase activity (Aravind and Koonin 1998; C. Kaplan, personal communication). This suggests that Spt16 could bind directly to the N-terminal tails of histones, and this binding could be affected by modifications of these tails.

As noted for other perturbations of histone expression, *pob3* mutants were also affected by histone tail mutations, but in ways distinct from and sometimes opposite to those noted for the *spt16-11* strain. As with *spt16-11* cells, loss of either the H3 or the H4 tail was detrimental to a *pob3-L78R* strain (Table 5). In contrast, whereas the H4 K5,12R mutations were more detrimental than the K8,16R mutations in an *spt16-11* strain, in a *pob3-L78R* strain the K5,12R mutations had no effect but the K8,16R mutations were intolerable. These experiments again show that the partners Spt16 and Pob3 are affected very differently by changes in the pattern of histone tail modifications.

The function of Nhp6 is also affected by mutations in acetylases and deacetylases (Yu *et al.* 2000), and, as with Spt16-Pob3, the effects appear to directly involve histone tails. As shown in Table 5, combining deletions of both genes that encode Nhp6 with a deletion of the N-terminal tail of either H3 or H4 is lethal at 30°, representing a drop in the maximal permissive temperature of at least 7°. This defect is considerably more severe than that of phenotypes caused by combinations with *spt16-11*. The pattern of effects caused by *nhp6a/b-*Δ is also different from those observed in either the *spt16-11* cells or the *pob3-L78R* cells. For example, the *nhp6a/b-*Δ strain did not respond to the H4 K5,12R mutations that produced the strongest effect with *spt16-11*. Therefore, although Nhp6 and Spt16-Pob3 collaborate *in vitro* and *in vivo*, deficiencies in each protein cause different defects with respect to functional modifications of the N-terminal tail of histone H4. Overall, Nhp6 and Spt16-Pob3 appear to have both common and distinct functional roles.

DISCUSSION

SPN/FACT is a broadly conserved eukaryotic protein complex that binds to and alters the properties of nucleosomes *in vitro* (Orphanides *et al.* 1999; Formosa *et al.* 2001). This factor appears to moderate the inhibitory effects of chromatin, facilitating the progression of polymerases along nucleosomal templates (Orphanides *et al.* 1998). Previous genetic analysis indicated that this activity is important *in vivo* for both transcription and replication (Malone *et al.* 1991; Rowley *et al.* 1991; John *et al.* 2000; Schlesinger and Formosa 2000; Yu *et al.* 2000; Brewster *et al.* 2001; Formosa *et al.*

2001). To further define the role of the yFACT component Spt16, we used an unbiased genetic strategy to identify factors that support its activity or that rely upon it to complete its function efficiently. Of the 29 mutants identified, 26 affected *HIR1*, *HIR2*, *HIR3*, or *HPC2*. Both *spt16* and *pob3* mutations, but not loss of Nhp6, cause this reliance on the *HIR/HPC* pathway, and the effect with *spt16* mutations is strongly allele specific. The Hir/Hpc pathway is therefore revealed as essential for survival when yFACT activity is compromised in specific ways.

Synthetic defects can signal any of several relationships between gene products (Guarente 1993). Spt16-Pob3 and the Hir/Hpc proteins could display mutual dependence because they act within a common complex, but no data have been reported that support physical interactions among these proteins. Each pathway could act independently to repress histone gene expression, but data presented here and elsewhere (Xu *et al.* 1993) indicate that instead Spt16-Pob3 is an activator of transcription at these loci. *hir/hpc* mutations cause misregulation of histone gene expression, which could lead to formation of aberrant nucleosomes. FACT acts on nucleosomes, so *hir/hpc* mutations could impose a requirement for optimal Spt16-Pob3 activity by decreasing the quality of the FACT substrate. However, *spt10* and *spt21* mutations also cause misregulation of histone gene expression (Sherwood and Osley 1991), but these defects are tolerated in combination with the *spt16-11* mutation (although *spt10 hir1* combinations are lethal; D. Hess and F. Winston, personal communication). Further, we find that directly perturbing histone gene expression by altering the number and ratio of histone genes does not recapitulate the lethal effects observed with *hir/hpc* mutations. Instead, to our surprise, *spt16* and *pob3* mutants responded in opposing ways to altered histone gene ratios and to changes in the structure and modifications of histones. In general, high ratios of H2A-H2B to H3-H4 were detrimental to *spt16* mutants, but suppressed the growth defects of *pob3* mutants, and low ratios had the opposite effects (Figures 3 and 4, Table 4). Altering either the potential for H3-H4 tails to be acetylated or the enzymes that modify them also caused opposing effects (Figures 5–7 and Table 5). Appropriate histone availability and modification are therefore important determinants of FACT activity, but this does not appear to be sufficient to explain the genetic relationships observed between the Spt16-Pob3 and Hir/Hpc pathways.

The Hir/Hpc proteins were initially identified through their role in regulating histone gene expression, but recent results show that they also function in nucleosome assembly (Kaufman *et al.* 1998; Lorain *et al.* 1998; Sharp *et al.* 2001; Sutton *et al.* 2001; Ray-Gallet *et al.* 2002). This activity suggests a model for interpreting the results presented here (Figure 7). In this model, we propose that SPN/FACT has two functions. First, as suggested previously (Orphanides *et al.* 1999; Kireeva *et al.* 2002), it mediates the destabilization or disassembly of nucleosomes to facilitate passage of polymerases. Second, it promotes reassembly of chromatin after the polymerase has passed. This could involve maintaining contact with the nucleosomal components so that the same histone proteins that were separated could be reunited, or it could mean recruiting replacement components from a free pool. In this model, some mutations in *SPT16* and *POB3* cause increased levels of aberrant chromatin structures to accumulate, perhaps because the disassembly function is unregulated or because the reassembly activity is deficient. We further propose that the Hir/Hpc proteins act as a general "nucleosome repair" pathway that restores nucleosomes with an aberrant composition to a normal one. Muta-

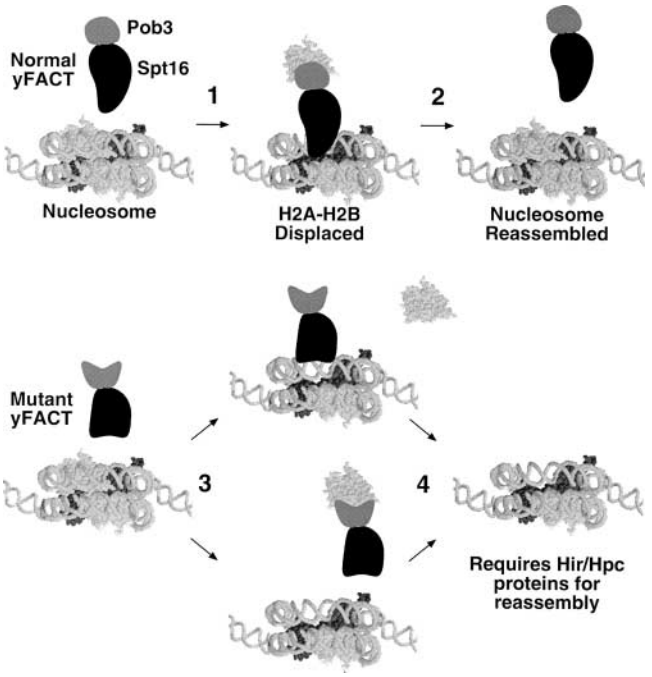

Figure 7.—A model for the interactions among yFACT, polymerase progression, and chromatin structure. (Top) Normal yFACT engages each nucleosome (White *et al.* 2001) to destabilize or disassemble it (step 1), forming a nucleosome that is more permissive for polymerases. This could involve removal of H2A-H2B dimers to form hexasomes (Orphanides *et al.* 1999; Kireeva *et al.* 2002) or other disturbances of the nucleosome structure. yFACT tethers the nucleosomal fragments so that after the polymerase passes it can promote reassembly (step 2), leaving an intact nucleosome. (Bottom) Mutated yFACT is still able to promote the disruption of the nucleosome, but not reassembly. yFACT could fail to retain the displaced H2A-H2B dimer (step 3, top), it could fail to maintain contact with the remainder of the nucleosome (step 3, bottom), or it could fail to promote reassembly (step 4). Polymerase is still capable of progression, but the nucleosome remains in an aberrant form afterward. This altered composition is not tolerable on a broad scale, necessitating action by the Hir/Hpc-Asf1 pathway.

tions in FACT that enhance the formation of aberrant nucleosomes therefore cannot be tolerated when there are defects in the Hir/Hpc pathway because this abrogates the ability to repair the damage.

This model is consistent with the known properties of FACT and also can explain several of the observations presented here. Removing one or both H2A-H2B dimers makes nucleosomes less inhibitory to RNA polymerase II (Kireeva *et al.* 2002). FACT binds both to nucleosomes and to H2A-H2B dimers and promotes RNA Pol II elongation on nucleosomal templates (Orphanides *et al.* 1998, 1999; Formosa *et al.* 2001). Binding to H2A-H2B dimers was initially viewed as a mechanism for disrupting the nucleosome to create a less inhibitory form. In our proposal, the binding of FACT to nucleosomes and to fragments of nucleosomes reflects distinct activities required at different points in a cycle that is completed when the nucleosome is restored to a normal form. Some contacts promote dissociation of H2A-H2B dimers, while others tether the fragments to enhance efficient reassembly after the polymerase has passed. Spt16 and Pob3 are therefore each needed to complete the cycle effectively, and defects in either could lead to the formation of aberrant nucleosomes. Therefore, either *spt16* or *pob3* mutations could cause dependence on the Hir/Hpc pathway, and this would depend on which part of the cycle was defective in each mutant. While both proteins work together to promote this cycle, they contact different parts of the nucleosome and might have very different responsibilities regarding the disassembly and reassembly phases of the cycle. Changes in the availability, structure, and modification status of the histones could therefore affect *spt16* and *pob3* mutants very differently, or even in opposite ways, as we have observed.

Nhp6 promotes binding of Spt16-Pob3 to nucleosomes *in vitro* (Formosa *et al.* 2001) and supports the function of Spt16-Pob3 *in vivo* (Brewster *et al.* 2001; Formosa *et al.* 2001). However, *nhp6* mutants do not cause reliance on the Hir/Hpc system. We propose that Nhp6 acts primarily to enhance loading of Spt16-Pob3 to nucleosomes at promoters or replication origins, but is not involved in the subsequent elongation steps and therefore does not influence the nucleosome reassembly reaction.

Defects in other elongation factors also cause reliance on the Hir/Hpc pathway. This includes the functionally related Spt4, Spt5, and Spt6 proteins (DeSilva *et al.* 1998; F. Winston, D. Hess and A. Bortvin, personal communication), as well as members of the Paf1 complex (Table 2). This could indicate that these factors also promote reassembly of nucleosomes directly (as previously suggested for Spt4-Spt5; Hartzog *et al.* 1998), that they influence the ability of FACT to promote this activity, or that delayed elongation promotes dissociation of nucleosomal components by prolonging the time spent in a tenuous disassembled state. Supporting the notion that Spt4/5/6 and the Paf1 complex affect yFACT function, proteins in these three complexes have been linked physically and genetically to one another (Costa and Arndt 2000; Squazzo *et al.* 2002). Further, the Hir/Hpc proteins have been linked physically with TAFII subunits (Sanders *et al.* 2002), and Asf1 has been linked to the TFIID-associated protein Bdf1 (Chimura *et al.* 2002). The functional association revealed by genetic screens between the Hir/Hpc-Asf1 proteins and factors that promote polymerase passage may therefore be supported by the physical association of Hir/Hpc-Asf1 proteins with general transcription factors.

While many features of this model remain to be demonstrated, it is reasonable that the factor responsible for the disassembly of nucleosomes would have an important role in their reassembly and that some additional pathway would exist to ensure completion of this important function. These results therefore provide insight into the functions of both FACT and the Hir/Hpc system.

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