

Fermentation of High Concentrations of Maltose by *Saccharomyces cerevisiae* Is Limited by the COMPASS Methylation Complex[†]

Jens Houghton-Larsen and Anders Brandt*

Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby, Denmark

Received 20 July 2006/Accepted 4 September 2006

In *Saccharomyces cerevisiae*, genes encoding maltose permeases and maltases are located in the telomeric regions of different chromosomes. The COMPASS methylation complex, which methylates lysine 4 on histone H3, controls the silencing of telomeric regions. Yeast strains deleted for *SWD1*, *SWD3*, *SDC1*, *SET1*, *BRE2*, or *SPP1*, encoding components of the COMPASS complex, fermented a medium containing 22% maltose with noticeably higher attenuation than did the wild type, resulting in production of up to 29% more ethanol. The least effective strain was *spp1*. Absence of COMPASS components had no effect on the fermentation of media with 20% glucose, 20% sucrose, or 16% maltose. Deletion of *SWD3* resulted in larger amounts of *MAL12* transcript, encoding maltase, at the late stages of fermentation of 22% maltose. A similar effect on maltase activity and maltose uptake capability was seen. The lysine 4 residue of histone H3 was trimethylated in wild-type cells at the late stages, while only small amounts of the dimethylated form were detected. Trimethylation and dimethylation of this residue were not detected in strains deleted for *SWD1*, *SWD3*, *SET1*, *BRE2*, or *SDC1*. Trimethylated lysine 4 was apparent only at the early stages (48 and 96 h) of fermentation in an *spp1* strain. This work indicates that the COMPASS complex represses the expression of maltose utilization genes during the late stages of fermentation of a high concentration of maltose.

The carbohydrate of brewer's wort made from barley malt consists mainly of maltose (60 to 70%), and efficient assimilation of this disaccharide is important for fast and efficient fermentation. Incomplete fermentation of high-gravity worts can be a problem in beer production (9, 18, 36), leading to unwanted sweetness and abnormal flavor. The transcription of genes required for maltose and maltotriose assimilation is repressed in the late stages of brewery fermentations (16). Constitutive expression of a maltose permease gene in a brewer's yeast strain improved maltose utilization and resulted in faster and more complete fermentation of very high gravity wort (19).

Assimilation of maltose by *Saccharomyces cerevisiae* requires a least one of five unlinked *MAL* loci (*MAL1* to *MAL4* and *MAL6*). Each locus, designated *x* in the following list, consists of up to three functionally distinct genes encoding a maltose permease, *MAL(x)1*; a maltase, *MAL(x)2*; and a transcriptional activator, *MAL(x)3* (5, 6, 34). *Saccharomyces cerevisiae* strains can contain maltose permeases encoded by genes from any of these loci. In addition, *MPH2* and *MPH3* encode permeases for α -glucosides, including maltose (8). The maltose permeases transport maltose across the plasma membrane by proton symport, and efficient transport requires an intact plasma membrane proton gradient (32). The transcription of genes encoding maltose permease and maltase is repressed through binding of the Mig1p and/or Mig2p repressor in the presence of glucose (14, 15). Activation of transcription occurs through binding of the *MAL(x)3*-encoded transcription activator when maltose constitutes the carbon source. In the absence of a repressing or an inducing carbon source, *MAL* genes are tran-

scribed at a low basal level (38). Nitrogen starvation or the presence of glucose leads to downregulation of maltose transport by inactivation and proteolytic degradation of the maltose permease in the vacuole (25, 29).

MAL loci and the α -glucoside permease genes *MPH2* and *MPH3* map to telomeric regions on different chromosomes in *Saccharomyces cerevisiae* (28, 8). In certain regions of chromosomes, repression of genes transcribed by RNA polymerase II can occur by transcriptional silencing (30). Silencing of gene expression at telomeres and subtelomeric regions requires posttranslational modifications of histones (17, 33). Deacetylation of lysine residues in the tail of histone H3 or H4 by Sir2p leads to silencing, as does trimethylation of lysine 4 of histone H3 by the COMPASS complex (30, 27, 21, 26), which is responsible for the mono-, di-, and trimethylation of this residue. The COMPASS complex consists of eight subunits. One, Swd2p, is essential for viability (7), and *SET1* encodes the methyl transferase, the catalytic component of the complex. Together, Swd1p, Swd3p, and Set1p constitute a core of the complex, and the absence of any of these subunits abolishes methylation of lysine 4 on histone H3. Subunits encoded by the genes *BRE2* and *SDC1* are essential for trimethylation and important for mono- and dimethylation. Spp1p is important for trimethylation, while the absence of Shg1p results in a minor reduction in the capacity of the COMPASS complex for trimethylation (26). Mono- and/or dimethylation of lysine 4 of histone H3 is important for the proper growth of *S. cerevisiae*, while trimethylation is needed for silencing in the ribosomal DNA (rDNA) and telomere regions (10, 26). Silenced regions are hypomethylated compared to actively transcribed euchromatic regions, and methylated lysine 4 on histone H3 is associated with genes actively transcribed by RNA polymerase II (3). However, chromatin immunoprecipitation analysis has revealed methylation of lysine 4 of

* Corresponding author. Mailing address: Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby, Denmark. Phone: (45) 3327 5236. Fax: (45) 3327 4765. E-mail: anders.brandt@crc.dk.

[†] Published ahead of print on 15 September 2006.

TABLE 1. Primers used to generate PCR fragments^a

Primer	Sequence
BRE2_F	AGGTGGCCATAATTGGACGAAGACAAATAATTCAC
BRE2_R	CTTCCTTAATAGCTTCGTACGCTGCAGGTGCGAC
SET1_F	GAAACACACTTTTCAGTGTGTTTAAATTATCTTCT
SET1_R	TTGAATGCTGGGCCACTAGTGGATCTGATATC
SPP1_F	TTCCTTATTGTTGAATCTTTATAAGAGGTCTCTG
SPP1_R	CGTTTAGAGAGCTTCGTACGCTGCAGGTGCGAC
SDC1_F	CGATATGTAAATCAGGAAGCTCCAAACAAATCA
SDC1_R	ATGTATCATCGGGCCACTAGTGGATCTGATATC
SWD1_F	GAAAAGGCTACTTCGACCTCAATAATTTCTCAGCC
SWD1_R	TATCTTTCTAGCTTCGTACGCTGCAGGTGCGAC
SWD3_F	TATATATATATGTAGAACTGATATTTGATTAGGC
SWD3_R	TCCAACGCCGGGCCACTAGTGGATCTGATATC
SWD3_F	CCTTCATAAGCAGGTTCTCTTAAGAGAATTAGGG
SWD3_R	AAACCTATACAGCTTCGTACGCTGCAGGTGCGAC
SWD3_F	GTAAGGAAAAATAAATACACATGTATATTTATAT
SWD3_R	AATTATGGTTAGGCCACTAGTGGATCTGATATC
SWD3_F	GACCGAGTCTTTTATGTTGTGTATATCAGCTGGT
SWD3_R	TCTTTTCGTTGCTTCGTACGCTGCAGGTGCGAC
SWD3_F	TCAGTGTCTAGTTGCTATTTAACTTTCTCCTTTTG
SWD3_R	AAGAGAAACGCCCACTAGTGGATCTGATATC
SWD3_F	TTGAGCGTAGGCTCATTCATTGCAAGTGCCATT
SWD3_R	ATATGCTAACGCTTCGTACGCTGCAGGTGCGAC
SWD3_F	GTTACACCTTAAGGGAATGTCTGCTGTACATA
SWD3_R	ACGCAGATTCTGGCCACTAGTGGATCTGATATC

^a The fragments were used to delete genes encoding COMPASS components.

histone H3 in the silenced left arm of chromosome VI and the rDNA regions (26).

According to the “histone code” hypothesis, different combinations of histone modifications are read by different proteins, resulting in distinct downstream events. Differences between the histone code at actively transcribed genes and that in silenced regions could explain how methylated lysine 4 on histone H3 could be important for both active transcription and silencing (33).

How maltose uptake and utilization in *S. cerevisiae* are influenced by telomeric silencing mediated by the COMPASS complex has not previously been studied. The present study describes a negative influence of an intact COMPASS complex on the fermentation of media with high concentrations of maltose.

MATERIALS AND METHODS

Yeast strains. All yeast strains used in this study are based on the prototrophic *Saccharomyces cerevisiae* Y55 (24) derivatives JT20149 (*MATa MAL1*), JT20150 (*MATα MAL1*), and JT20151 (*MATa/MATα MAL1/MAL1*), which were obtained from Johan Thevelein, Katholieke Universiteit, Leuven, Belgium. A PCR-based deletion strategy (2, 37) was used to delete single components of the COMPASS complex in JT20149. Chromosomal DNA constituting open reading frames of genes encoding components of the COMPASS complex was replaced by a PCR fragment amplified from the plasmid pUG6 (11), containing the *kanMX* cassette flanked by two *loxP* sites and generated with primers with sequences that allowed integration by homologous recombination. All deletion strains were checked by restriction analysis of PCR fragments amplified by primers flanking the inserted fragment. PCR was carried out with Phusion High-Fidelity DNA polymerase (Finnzymes OY). Primers used to create deletion fragments are listed in Table 1. Removal of the *kanMX* cassette by the *lox-out* procedure with *Cre* recombinase was performed by transformation with the pAUR112 vector (13) containing the *SacI*-*KpnI* fragment from plasmid pSH62 (12). This fragment contains a *GAL* promoter, the *Cre* recombinase gene, and a *CYC1* terminator. As expected, an *swd3::kanMX* deletion strain with the *kanMX* cassette fermented like the *swd3::kanMX* strain. Thus, deletion of the gene and not the presence of the *kanMX* cassette causes the fermentation phenotype of COMPASS deletion strains. Diploid strains were made by mating *a* and *α* strains. Strains constructed for use in this study are listed in Table 2.

TABLE 2. *Saccharomyces cerevisiae* strains constructed and used in this study

Strain	Genotype
M5675	<i>MATa MAL1 swd3::kanMX</i>
M5676	<i>MATα MAL1 swd3::kanMX</i>
M5681	<i>MATa/MATα MAL1/MAL1 swd3::kanMX/SWD3</i>
M5680	<i>MATa/MATα MAL1/MAL1 swd3::kanMX/swd3::kanMX</i>
M5707	<i>MATa/MATα MAL1/MAL1 swd3::lox4/swd3::lox4</i>
M5673	<i>MATa MAL1 swd1::kanMX</i>
M5674	<i>MATa MAL1 sdc1::kanMX</i>
M5704	<i>MATa MAL1 set1::kanMX</i>
M5705	<i>MATa MAL1 bre2::kanMX</i>
M5706	<i>MATa MAL1 spp1::kanMX</i>

Media and fermentations. For spot testing of growth on plates, cells were grown overnight in 5 ml of YPD (1% yeast extract, 2% peptone, 2% glucose). Cells at an optical density at 600 nm (OD₆₀₀) of 1 were pelleted by centrifugation and washed in sterile water. Tenfold serial dilutions were applied to synthetic complete medium (4) with or without 50 nM rapamycin. Plates were photographed after 7 days at room temperature.

Small-scale stirred fermentations were performed as described previously (35) with complex media containing 1% yeast extract, 2% peptone, and one of the sugars maltose, sucrose, or glucose. The concentrated sugar solution was autoclaved separately. This created a slight variation in the final sugar concentration of the medium from batch to batch. Therefore, the densities were determined with a Density Meter (Anton Paar DMA35n). Densities were expressed in degrees Plato (°P) corresponding to the percentage of sucrose by weight. Wort fermentation was done in all-malt 22.5°P wort. The stirred miniaerobic fermentations, containing 200 ml medium, were inoculated to an OD₆₀₀ of 0.2 from a preculture grown for 72 h in YP (1% yeast extract, 2% peptone) containing 2% of the carbon source that was used in the fermentations. Fermentations were incubated at 22°C in 250-ml measuring cylinders, continuously stirred at 130 rpm, and covered with an inverted beaker. The weight of each cylinder was determined at the start and subsequently at all indicated time points. Weight loss by evaporation was corrected for by monitoring the weight of a parallel cylinder without added yeast. The density of the medium in degrees Plato was determined at the start and end of the fermentation. The weight loss at all intermediate points was used to calculate the density of the culture medium at those time points.

Maltose uptake capability. Initial uptake rates were determined as described previously (23), with minor modifications. Samples taken during fermentation were pelleted by centrifugation at 4000 × g, washed with sterile water, and resuspended in 0.1 M tartaric acid (pH 4.2) to an OD₆₀₀ of 30 units/ml. Aliquots of 80 μl were added to 20 μl of 25 mM [¹⁴C]maltose (0.25 μCi/μmol) (Amersham Biosciences, United Kingdom) and incubated at 20°C for 20 s. Maltose uptake was stopped by the addition of 10 ml ice-cold water, and cells were collected on GC-50 glass fiber filters (Advantec; Toyo Roshi Kaisha Ltd., Japan). The filters were washed with water and immersed in vials containing 5 ml liquid scintillation fluid (Ecoscint O; National Diagnostics, Atlanta, Ga.). Radioactivity was counted in a Beckman LS6500 liquid scintillation counter (Beckman Instruments, Fullerton, Calif.).

Maltase activity. Maltase activity was determined as described previously (20). Briefly, cells at an OD₆₀₀ of 40 were harvested and resuspended in 1 ml of 0.1 M potassium phosphate buffer (pH 7.0), 1 mM dithiothreitol, 4 mM MgCl₂, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride at 4°C. Cells were broken with glass beads by repeated whirlimixing for 15 s, with 15 s of resting on ice, for a total of 7 min. Lysates were cleared at 20,000 × g for 5 min. Maltase activity was determined with 6 mM *p*-nitrophenyl- α -D-glucopyranoside as a substrate for 15 min at 37°C. The reaction was stopped by adding Na₂CO₃ to 0.15 M, and the OD₄₀₀ was measured. One unit is defined as the amount of maltase that produces 1 μmol *p*-nitrophenol per minute. A millimolar extinction coefficient of 18.1 for *p*-nitrophenol was used. The protein level was determined with the BCA protein assay reagent kit (Pierce).

Ethanol determinations. Samples were taken at the end of fermentation and cleared by centrifugation at 3,000 × g for 10 min. The ethanol level was determined in 1,000-fold diluted aliquots with the K-ETOH kit (Megazyme International Ireland Ltd.).

Sugar analysis. Samples were taken at the end of a 22.5°P all-malt wort fermentation. Cells were pelleted by centrifugation at 3,000 × g. The concentrations of fermentable carbohydrates in aliquots of the beer and in unfermented

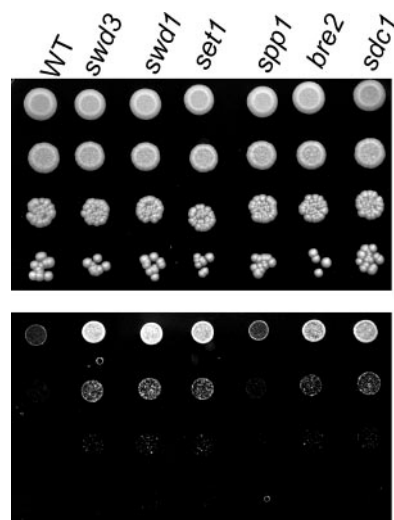


FIG. 1. Lack of COMPASS components causes rapamycin tolerance. Cell suspensions of the wild-type (WT) parental strain JT20149 and strains deleted for genes encoding COMPASS components were serially diluted and spotted onto synthetic complete medium without (upper panel) or with (lower panel) 50 nM rapamycin.

wort were determined by chromatography on a Dionex HPLC system with a Carpac PA1 column and an electrochemical detector.

RNA isolation and Northern blotting. RNA was isolated from cells at an OD_{600} of 50 by phenol extraction, according to the method of Burke et al. (4). Twenty-five micrograms of total RNA was subjected to electrophoresis in a MOPS (morpholinepropanesulfonic acid) buffer system (31) and blotted onto Hybond-plus nitrocellulose filters. As a probe, we amplified 571 bp of the *MAL12* coding region and labeled the DNA with ^{32}P using the Prime-a-Gene labeling system (Promega). Hybridization was carried out in 50% formamide, $5\times$ SSC ($1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and 0.1% Denhardt solution at 42°C. Hybridizing RNAs were detected by a phosphorimager screen and analyzed with a Storm 860 scanner (Molecular Dynamics).

Western blotting. Aliquots of cells at an OD_{600} of 1 were pelleted by centrifugation at $15,000\times g$ for 1 min. Pellets were resuspended in sodium dodecyl sulfate sample buffer (0.2 M Tris, 20% glycerol, 5 mM EDTA, 0.01% bromophenol blue, 0.01 M phenylmethylsulfonyl fluoride, and 0.01 dithiothreitol) and placed at 96°C for 10 min, and soluble proteins were loaded onto NuPAGE 4 to 12% bis-Tris gels (Invitrogen Life Technologies). Equal amounts of protein were loaded. The amount of protein was visualized both with Coomassie brilliant blue R250 staining of separate gels and with Ponceau Red staining of each nitrocellulose filter following electroblotting (22). Filters were incubated with anti-trimethyl-histone H3 (Lys4) rabbit antiserum (Upstate Cell Signaling Solutions) diluted 1:500 or with anti-dimethyl-histone H3 (Lys4) rabbit monoclonal immunoglobulin G (Upstate Cell Signaling Solutions) diluted 1:1000 as the primary antibody. Horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin (DakoCytomation Denmark A/S) diluted 1:1,000 was used as the secondary antibody. Reacting polypeptides were visualized with the ECL Plus Western blotting detection system (Amersham Biosciences) and a Storm 860 scanner (Molecular Dynamics).

RESULTS

Deletion of *SET1*, *BRE2*, *SPP1*, *SWD1*, *SWD3*, or *SDC1* results in two distinct growth phenotypes. The genes *SET1*, *SWD1*, *SWD3*, *BRE2*, *SDC1*, and *SPP1*, encoding components of the COMPASS lysine 4 histone H3 methylation complex, were individually deleted in the haploid *Saccharomyces cerevisiae* laboratory strain JT20149. Deletion of *SDC1*, *SWD1*, or *SWD3* in the *S. cerevisiae* BY4741 strain background has been reported to result in rapamycin tolerance (39). Similarly, all mutants in the JT20149 strain background, except the *spp1*Δ

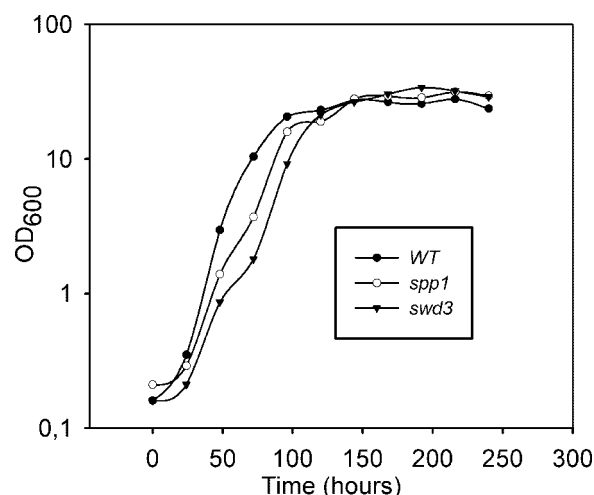


FIG. 2. Lack of COMPASS components causes a decreased growth rate. Growth of the wild-type (WT) strain (JT20149) and two strains each deleted for a gene encoding a COMPASS component was monitored during fermentation of complex medium containing 22% maltose (single determinations).

strain, displayed rapamycin resistance (Fig. 1). Thus, a functional COMPASS complex is required for the rapamycin sensitivity of yeast strain JT20149.

The *spp1* and *swd3* deletions caused decreased growth rates at the beginning of small-scale stirred fermentations of complex medium containing 22% maltose (roughly 22% maltose; strictly, a solution with a density of that of 22% sucrose) (Fig. 2), demonstrating that the presence of a functional COMPASS complex is important for the initiation of yeast growth in medium containing high concentrations of maltose. After approximately 5 days, however, the cell densities of the three cultures had become approximately equal.

Deletion of *SET1*, *BRE2*, *SPP1*, *SWD1*, *SWD3*, or *SDC1* improves the capacity to ferment high concentrations of maltose. Strains lacking any one of the COMPASS subunits Swd1p, Swd3p, Sdc1p, Bre2p, or Set1p displayed an improved final attenuation of a 22% maltose fermentation (Fig. 3A). The *spp1* strain was slightly less efficient. The strains lacking COMPASS components displayed a slightly lower fermentation rate than the wild type at the early stages of fermentation. The lower fermentation rate corresponded to the slower growth of the deletion strains during the early stages of fermentation (Fig. 2). The *spp1* strain had a fermentation rate at the beginning of fermentation comparable to that of the wild type. At the end of a 22% maltose fermentation, the wild type had produced 57 g/liter ethanol; the *swd1* and *swd3* strains had produced 74 g/liter; the *sdc1*, *bre2*, and *set1* strains had produced 70 g/liter; and the *spp1* strain, which fermented 22% maltose less completely, produced 68 g/liter. Thus, in the absence of a functional COMPASS complex, more ethanol is produced during fermentation of a medium containing high concentrations of maltose. Diploid a/α strains with one or both copies of *SWD3* deleted were also constructed. The *swd3/swd3* strain showed improved attenuation, whereas the *swd3/SWD3* strain did not (data not shown). One copy of the *SWD3* gene is apparently sufficient for the proper functioning of the COMPASS com-

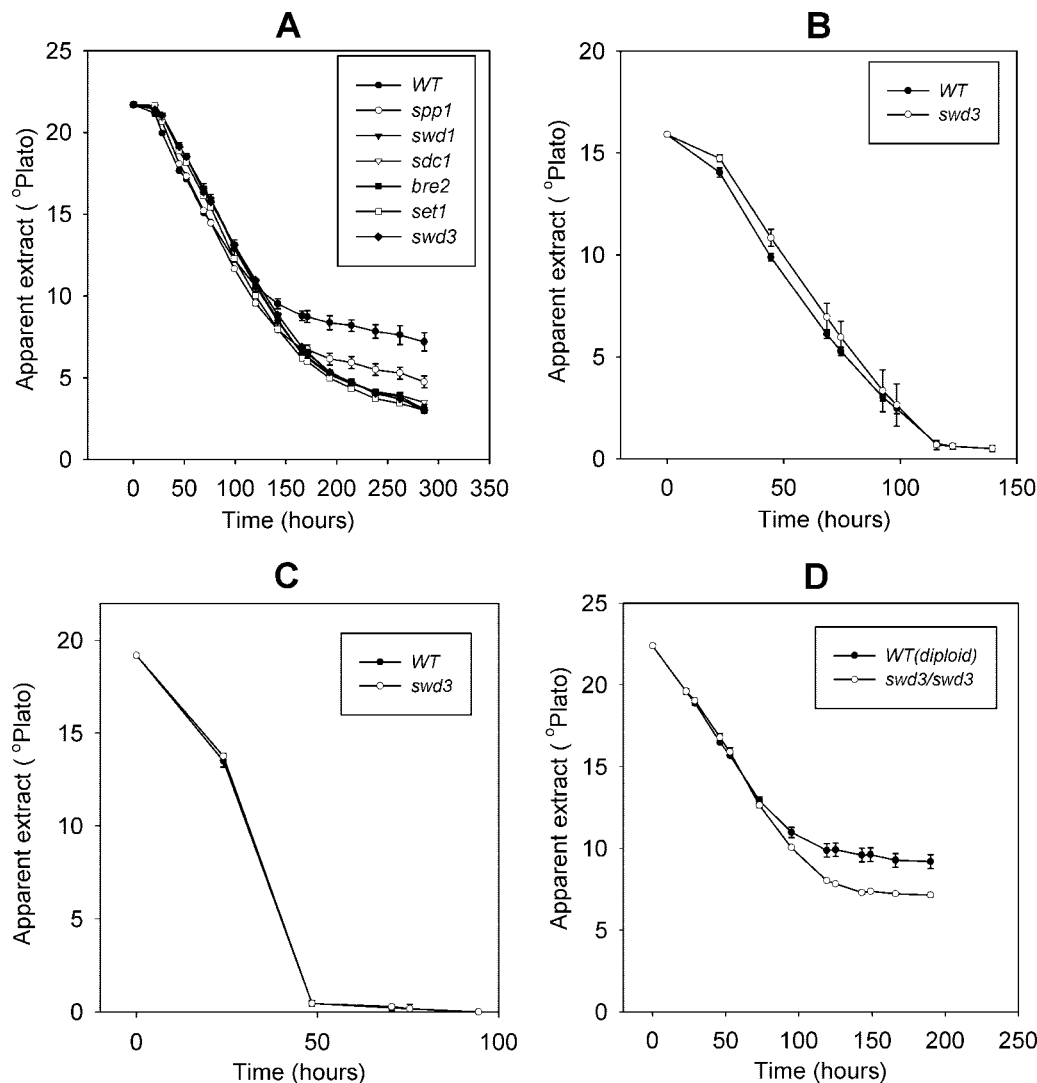


FIG. 3. Apparent extract during fermentations with the wild-type (WT) strain (JT20149) and yeast strains deleted for genes encoding COMPASS components. (A) Fermentation of complex medium with 22°P maltose. (B) Fermentation of complex medium with 16°P maltose. (C) Fermentation of complex medium with 20°P glucose. (D) Fermentation of 22.5°P brewer's wort. Fermentations were done in duplicate, and the error bars indicate the range between duplicate experiments.

plex in diploid yeast. The *swd3* strain and the wild type fermented 16°P maltose, 20°P glucose (Fig. 3B and C), and 22°P sucrose (not shown) equally well, indicating that the improved attenuation in the absence of COMPASS components is restricted to fermentations of media containing high initial maltose concentrations (22°P). The improved fermentation of *swd3* was also apparent in 22.5°P all-malt high-gravity brewer's wort (Fig. 3D). Sugar analysis of the wort and the beer showed that glucose, fructose, sucrose (together comprising approximately 20% of the fermentable sugars in the wort), and maltose (approximately 60%) were all utilized completely by the *swd3* strain, while only 81% of the maltose was utilized by the wild type. Maltotriose, which cannot be utilized by any of the strains investigated here, comprised approximately 16% of the wort sugar.

Increased initial maltose uptake rate and maltase activity in the absence of COMPASS components. During a 22°P maltose fermentation, the initial maltose uptake capability was highest

at the beginning of the fermentation process and then declined (Table 3). The reduction in maltose transport activity was more pronounced in the wild type than in the *swd3*Δ strain (Table 3). During later stages of fermentation, the initial maltose uptake

TABLE 3. Initial uptake rates of [¹⁴C]maltose and maltase activities in cells from a fermentation of complex medium containing 22°P maltose^a

Time (h)	Maltose uptake (nmol/OD ₆₀₀ /min) ± SEM		Maltase activity (U/mg of protein/min) ± SEM	
	JT20149	JT20149 <i>swd3</i>	JT20149	JT20149 <i>swd3</i>
48	1.20 ± 0.34	1.76 ± 0.09	10.84 ± 0.42	6.05 ± 0.77
96	0.71 ± 0.04	0.71 ± 0.09	ND	ND
120	ND	ND	3.57 ± 0.37	5.06 ± 0.31
192	0.46 ± 0.10	0.67 ± 0.06	0.70 ± 0.06	2.26 ± 0.15
216	0.43 ± 0.03	0.69 ± 0.07	1.13 ± 0.02	2.62 ± 0.06

^a Each analysis was done in triplicate. ND, not determined.

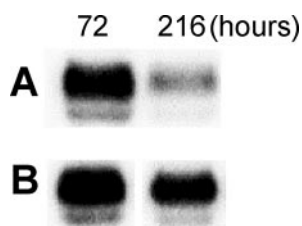


FIG. 4. Northern blot of RNA from yeast taken after 72 and 216 h of fermentation of a complex medium with 22°P maltose. (A) Wild-type strain (JT20149). (B) *swd3* strain. Equal loadings of rRNA were done. The probe was a *MAL12*-specific 571-bp fragment.

capability of the *swd3* strain was approximately 50% higher than that of the wild type. Maltase was approximately 40% lower at 48 h in the *swd3* strain, while in the late stages of fermentation the maltase activity was two- to threefold higher in the *swd3* strain than in the wild type (Table 3). Thus, both maltose permease and maltase activity were increased in the absence of a functional COMPASS complex during the late stages of a 22°P maltose fermentation.

Maltose utilization genes are repressed during the late stages of fermentation (16). The amount of *MAL12* maltase transcript was therefore determined during a 22°P maltose fermentation (Fig. 4). Equal amounts of rRNA were loaded onto the gel. The amount of *MAL12* transcript was large after 72 h in both the wild type and the *swd3* strain. At 216 h, the amount was small in the wild type, while the *swd3* strain still contained significant amounts of *MAL12* transcript. Thus, a functional COMPASS complex is important for repression to occur during the late stages of very high gravity maltose fermentations.

Methylation of lysine 4 on histone H3. We analyzed the methylation status of lysine 4 of histone H3 by Western blotting using antibodies recognizing tri- or dimethylated lysine 4. The strains lacking *SWD1*, *SWD3*, *SET1*, *BRE2*, or *SDC1* had no detectable levels of di- or trimethylation, while the *spp1* strain retained the ability to methylate lysine 4 on histone H3, although trimethylation appeared to be reduced (Fig. 5A).

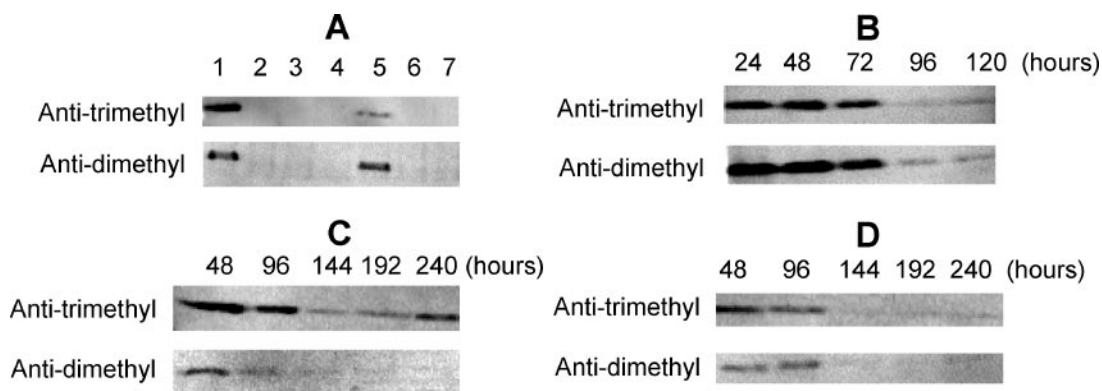


FIG. 5. Methylation of histone H3 by the COMPASS complex during maltose fermentation. Western blots were probed with antibodies recognizing dimethylated or trimethylated lysine 4 in histone H3. (A) The wild-type strain (JT20149, lane 1) or strains deleted for genes encoding COMPASS components were, as a control, grown for 20 h in YPD (complex medium with 2°P glucose). Lanes: 2, *swd3*; 3, *swd1*; 4, *set1*; 5, *spp1*; 6, *bre2*; 7, *sdcl*. (B) Samples of wild-type (JT20149) cells taken during fermentation in complex medium containing 16°P maltose. (C) Samples of wild-type (JT20149) cells taken during fermentation in complex medium containing 22°P maltose. (D) Samples of the *spp1* strain taken during fermentation in complex medium containing 22°P maltose.

The effects on methylation caused by removing individual COMPASS components in the JT20149 strain background were similar to the effects in the MBY1198 strain background reported by Mueller et al. (26). We were, however, unable to detect dimethylation in the JT20149-derived *sdcl* and *bre2* strains (Fig. 5A, lanes 6 and 7).

Methylation of lysine 4 on histone H3 was subject to a series of changes in wild-type cells. In the first half of a 16°P maltose fermentation, when the cells were growing, large amounts of both di- and trimethylated lysine 4 on histone H3 were detected (Fig. 5B, 24, 48 and 72 h). This methylation almost disappeared in the later stages of fermentation (Fig. 5B, 96 and 120 h). Throughout the growth phase of a 22°P maltose fermentation, trimethylated lysine 4 on histone H3 was detected (Fig. 5C, 48 and 96 h). Dimethylated lysine 4 was also present. When cell growth slowed down, the amounts of trimethylated lysine 4 became low, and dimethylation was barely detected (Fig. 5C, 144 h). After cessation of growth, the reduction in methylation of lysine 4 on histone H3 was followed by an increase in trimethylation during the later stages of fermentation (Fig. 5C, 192 and 240 h). Dimethylation was barely detected at these stages. The overall methylation pattern of the *spp1* strain during fermentation of 22°P maltose (Fig. 5D) resembled the methylation pattern of the wild-type strain (Fig. 5C). However, both tri- and dimethylation were hardly detectable after 96 h of fermentation. The patterns of dimethylation of the wild type and the *spp1* strain during a 22°P maltose fermentation were similar, suggesting that the improved attenuation of the *spp1* strain was a result of a reduced trimethylation of lysine 4 of histone H3. As expected, di- or trimethylation of lysine 4 on histone H3 could not be detected in the *swd3* strain during a 22°P maltose fermentation (data not shown).

DISCUSSION

During fermentation of high-gravity worts, yeast is exposed to many environmental stresses (36, 16). While a complex medium containing high concentrations of glucose (20°P) was fermented completely by the wild-type strain JT20149, fermenta-

tation of a medium containing high concentrations of maltose (22°P) was incomplete (Fig. 3A and C). Kodama et al. (19) achieved more complete high-gravity wort fermentations by constitutive overexpression of a maltose permease gene. This shows that incomplete fermentation of media containing high concentrations of maltose can be improved by increased transcription of *MAL* genes. The expression of the maltase gene in the JT20149 strain was repressed in the late stages of fermentation of 22°P maltose (Fig. 4), even though a substantial amount of maltose was present in the medium (5 to 10°P residual maltose). The repression resulted in reduced maltose uptake capability and reduced maltase activity (Table 3). A functional COMPASS complex contributed to the repression of maltose uptake and maltase activity in the late stages of fermentation (Table 3). In view of the subtelomeric location of the *MAL* genes, this is consistent with the role of the COMPASS complex in telomeric silencing (26).

Fingerman et al. (10) showed that trimethylation of lysine 4 of histone H3 was required for silencing of telomeres, while mono- and dimethylation was not. In agreement with this, we found that the *spp1* strain, which has a reduced capacity for trimethylation, fermented high-gravity maltose medium to a higher attenuation than did the wild-type strain. Complete prevention of lysine 4 methylation of histone H3 by deleting *SWD3* resulted in a further improvement of the final attenuation.

During the early stages of fermentation, the *spp1Δ* strain grew slower than the wild type and faster than the *swd3Δ* strain (Fig. 2). This is in agreement with the finding that mono- and dimethylation of lysine 4 of histone H3 are important for proper growth of yeast (10).

Ai et al. (1) showed that in *S. cerevisiae*, Sir3p is phosphorylated by the mitogen-activated protein kinase Slt2p, and the presence of hyperphosphorylated Sir3p is correlated with reduced subtelomeric silencing. They also reported that TOR inhibits Sir3p phosphorylation and that rapamycin activates Slt2p. We found that deletion of *SET1*, *SDC1*, *BRE2*, *SWD1*, or *SWD3* in the JT20149 strain resulted in increased tolerance to rapamycin (Fig. 1). The distinct rapamycin sensitivity of the *spp1* strain, rather closely resembling that of the wild type, showed that a mere reduction in trimethylation of lysine 4 of histone H3 was insufficient for the rapamycin tolerance seen in the other mutants.

Reduced telomere silencing during the late stages of very high gravity maltose fermentations is expected to influence the expression of many genes. Some, such as the *MAL* genes, may contribute to the improved fermentation phenotype, and some, such as the seripauperin (*PAU*) gene family, may contribute to increased stress resistance. The construction and analysis of transcriptional fusions of *MAL* genes to promoters that are active during the late stages of fermentation with high concentrations of maltose may reveal whether increased transcription of maltose utilization genes is sufficient to improve fermentation of media with high concentrations of maltose.

Future experiments may further improve our understanding of the negative effects of telomere silencing on maltose fermentation by, e.g., establishing whether telomere silencing by Sir2p-mediated histone deacetylation also limits the fermentation of high concentrations of maltose.

ACKNOWLEDGMENTS

We thank Jytte Tarnø and Steen Bech Sørensen, of the Carlsberg Research Laboratory, for performing the sugar analysis of brewer's wort. We are grateful to Morten C. Kielland-Brandt for valuable discussions and critical reading of the manuscript.

REFERENCES

1. Ai, W., P. G. Bertram, C. K. Tsang, T. F. Chan, and X. F. Zheng. 2002. Regulation of subtelomeric silencing during stress response. *Mol. Cell* **10**: 1295–1305.
2. Baudin, A., O. Ozier-Kalogeropoulos, A. Denouel, F. Lacroute, and C. Cullin. 1993. A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **21**:3329–3330.
3. Bernstein, B. E., E. L. Humphrey, R. L. Erlich, R. Schneider, P. Bouman, J. S. Liu, T. Kouzarides, and S. L. Schreiber. 2002. Methylation of histone H3 Lys 4 in coding regions of active genes. *Proc. Natl. Acad. Sci. USA* **99**:8695–8700.
4. Burke, D., D. Dawson, and T. Stearns. 2000. Methods in yeast genetics. A Cold Spring Harbor Laboratory course manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
5. Charron, M. J., and C. A. Michels. 1988. The naturally occurring alleles of *MAL1* in *Saccharomyces* species evolved by various mutagenic processes including chromosomal rearrangement. *Genetics* **120**:83–93.
6. Charron, M. J., E. Read, S. R. Haut, and C. A. Michels. 1989. Molecular evolution of the telomere-associated *MAL* loci of *Saccharomyces*. *Genetics* **122**:307–316.
7. Cheng, H., X. He, and C. Moore. 2004. The essential WD repeat protein Swd2 has dual functions in RNA polymerase II transcription termination and lysine 4 methylation of histone H3. *Mol. Cell* **24**:2932–2943.
8. Day, R. E., V. J. Higgins, P. J. Rogers, and I. W. Dawes. 2002. Characterization of the putative maltose transporters encoded by YDL247w and YJR160c. *Yeast* **19**:1015–1027.
9. Dietvorst, J., J. Londeborough, and H. Y. Steensma. 2005. Maltotriose utilization in lager yeast strains: *MTT1* encodes a maltotriose transporter. *Yeast* **22**:775–788.
10. Fingerman, I. M., C. L. Wu, B. D. Wilson, and S. D. Briggs. 2005. Global loss of Set1-mediated H3 Lys4 trimethylation is associated with silencing defects in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **280**:28761–28765.
11. Güldener, U., S. Heck, T. Fiedler, J. Beinhauer, and J. H. Hegemann. 1996. A new efficient gene disruption cassette for repeated use in budding yeast. *Nucleic Acids Res.* **24**:2519–2524.
12. Güldener, U., J. Heinisch, G. J. Köhler, D. Voss, and J. H. Hegemann. 2002. A second set of *loxP* marker cassettes for Cre-mediated multiple gene knock-outs in budding yeast. *Nucleic Acids Res.* **30**:e23.
13. Hashida-Okado, T., A. Ogawa, M. Endo, R. Yasumoto, K. Takesako, and I. Kato. 1996. *AUR1*, a novel gene conferring aureobasidin resistance on *Saccharomyces cerevisiae*: a study of defective morphologies in Aur1p-depleted cells. *Mol. Gen. Genet.* **251**:236–244.
14. Hu, Z., J. O. Nehlin, H. Ronne, and C. A. Michels. 1995. *MIG1*-dependent and *MIG1*-independent glucose regulation of *MAL* gene expression in *Saccharomyces cerevisiae*. *Curr. Genet.* **28**:258–266.
15. Hu, Z., Y. Yue, H. Jiang, B. Zhang, P. W. Sherwood, and C. A. Michels. 2000. Analysis of the mechanism by which glucose inhibits maltose induction of *MAL* gene expression in *Saccharomyces*. *Genetics* **154**:121–132.
16. James, T. C., S. Campbell, D. Donnelly, and U. Bond. 2003. Transcription profile of brewery yeast under fermentation conditions. *J. Appl. Microbiol.* **94**:432–448.
17. Jenuwein, T., and C. D. Allis. 2001. Translating the histone code. *Science* **293**:1074–1080.
18. Jespersen, N., L. B. Cesar, P. G. Meaden, and M. Jakobsen. 1999. Multiple α -glucoside transporter genes in brewer's yeast. *Appl. Environ. Microbiol.* **65**:450–456.
19. Kodama, Y., N. Fukui, T. Ashikari, and Y. Shibano. 1995. Improvement of maltose fermentation efficiency: constitutive expression of *MAL* genes in brewing yeasts. *J. Am. Soc. Brew. Chem.* **53**:24–29.
20. Khan, N. A., and N. R. Eaton. 1967. Purification and characterization of maltase and α -methyl glucosidase from yeast. *Biochim. Biophys. Acta* **146**:173–180.
21. Krogan, N. J., J. Dover, S. Khorrami, J. F. Greenblatt, J. Schneider, M. Johnston, and A. Shilatifard. 2002. COMPASS, a histone H3 (lysine 4) methyltransferase required for telomeric silencing of gene expression. *J. Biol. Chem.* **277**:10753–10755.
22. Kyhse-Andersen, J. 1994. The semi-dry electroblotter. *J. Biophys. Biochem. Methods* **10**:203–209.
23. Lucero, P., E. Penalver, E. Moreno, and R. Lagunas. 1997. Moderate concentrations of ethanol inhibit endocytosis of the yeast maltose transporter. *Appl. Environ. Microbiol.* **63**:3831–3836.
24. McCusker, J. H., and J. E. Haber. 1988. Cycloheximide-resistant temperature-sensitive lethal mutations of *Saccharomyces cerevisiae*. *Genetics* **119**: 303–315.
25. Medintz, L., X. Wang, T. Hradek, and C. A. Michels. 2000. A PEST-like

- sequence in the N-terminal cytoplasmic domain of *Saccharomyces* maltose permease is required for glucose-induced proteolysis and rapid inactivation of transport activity. *Biochemistry* **39**:4518–4526.
26. Mueller, J. E., M. Canze, and M. Bryk. 2006. The requirement for COMPASS and Paf1 in transcriptional silencing and methylation of histone H3 in *Saccharomyces cerevisiae*. *Genetics* **173**:557–567.
 27. Nagy, P. L., J. Griesenbeck, R. D. Kornberg, and M. L. Cleary. 2002. A trithorax-group complex purified from *Saccharomyces cerevisiae* is required for methylation of histone H3. *Proc. Natl. Acad. Sci. USA* **99**:90–94.
 28. Naumov, G. I., E. S. Naumova, and C. A. Michels. 1994. Genetic variation of the repeated *MAL* loci in natural populations of *Saccharomyces cerevisiae* and *Saccharomyces paradoxus*. *Genetics* **136**:803–812.
 29. Penalver, E., P. Lucero, E. Moreno, and R. Lagunas. 1998. Catabolite inactivation of the maltose transporter in nitrogen-starved yeast could be due to the stimulation of general protein turnover. *FEMS Microbiol. Lett.* **166**:317–324.
 30. Rusche, L. N., A. L. Kirchmaier, and J. Rine. 2003. The establishment, inheritance, and function of silenced chromatin in *Saccharomyces cerevisiae*. *Annu. Rev. Biochem.* **72**:481–516.
 31. Sambrook, J., T. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
 32. Serrano, R. 1977. Energy requirements for maltose transport in yeast. *Eur. J. Biochem.* **80**:97–102.
 33. Strahl, B. D., and C. D. Allis. 2000. The language of covalent histone modifications. *Nature* **403**:41–45.
 34. Vanoni, M., P. Sollitti, M. Goldenthal, and J. Marmur. 1989. Structure and regulation of the multigene family controlling maltose fermentation in budding yeast. *Prog. Nucleic Acid Res. Mol. Biol.* **37**:281–322.
 35. van Voorst, F., J. Houghton-Larsen, L. Jonson, M. C. Kielland-Brandt, and A. Brandt. 2006. Genome-wide identification of genes required for growth of *Saccharomyces cerevisiae* under ethanol stress. *Yeast* **23**:351–359.
 36. Vidgren, V., L. Ruohonen, and J. Londesborough. Characterization and functional analysis of the *MAL* and *MPH* loci for maltose utilization in some ale and lager yeast strains. *Appl. Environ. Microbiol.* **71**:7846–7857.
 37. Wach, A., A. Brachat, R. Poehlmann, and P. Philippsen. 1994. New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast* **10**:1793–1808.
 38. Wang, X., and C. A. Michels. 2004. Mutations in *SIN4* and *RGR1* cause constitutive expression of *MAL* structural genes in *Saccharomyces*. *Genetics* **168**:747–757.
 39. Xie, M. W., F. Jin, H. Hwang, S. Hwang, V. Anand, M. C. Duncan, and J. Huang. 2005. Insights into TOR function and rapamycin response: chemical genomic profiling by using a high-density cell array method. *Proc. Natl. Acad. Sci. USA* **102**:7215–7220.