# Fine-Tuning of Histone H3 Lys4 Methylation During Pseudohyphal Differentiation by the CDK Submodule of RNA Polymerase II

**Michael J. Law[1] and Kerri Ciccaglione**
Department of Molecular Biology, Rowan University School of Osteopathic Medicine, Stratford, New Jersey 08084
ORCID ID: 0000-0001-8976-7459 (M.L.)

**ABSTRACT** Transcriptional regulation is dependent upon the interactions between the RNA pol II holoenzyme complex and chromatin. RNA pol II is part of a highly conserved multiprotein complex that includes the core mediator and CDK8 subcomplex. In *Saccharomyces cerevisiae*, the CDK8 subcomplex, composed of Ssn2p, Ssn3p, Ssn8p, and Srb8p, is thought to play important roles in mediating transcriptional control of stress-responsive genes. Also central to transcriptional control are histone post-translational modifications. Lysine methylation, dynamically balanced by lysine methyltransferases and demethylases, has been intensively studied, uncovering significant functions in transcriptional control. A key question remains in understanding how these enzymes are targeted during stress response. To determine the relationship between lysine methylation, the CDK8 complex, and transcriptional control, we performed phenotype analyses of yeast lacking known lysine methyltransferases or demethylases in isolation or in tandem with *SSN8* deletions. We show that the RNA pol II CDK8 submodule components *SSN8*/*SSN3* and the histone demethylase *JHD2* are required to inhibit pseudohyphal growth—a differentiation pathway induced during nutrient limitation—under rich conditions. Yeast lacking both *SSN8* and *JHD2* constitutively express *FLO11*, a major regulator of pseudohyphal growth. Interestingly, deleting known *FLO11* activators including *FLO8*, *MSS11*, *MFG1*, *TEC1*, *SNF1*, *KSS1*, and *GCN4* results in a range of phenotypic suppression. Using chromatin immunoprecipitation, we found that *SSN8* inhibits H3 Lys4 trimethylation independently of *JHD2* at the *FLO11* locus, suggesting that H3 Lys4 hypermethylation is locking *FLO11* into a transcriptionally active state. These studies implicate the CDK8 subcomplex in fine-tuning H3 Lys4 methylation levels during pseudohyphal differentiation.?

**KEYWORDS** lysine methylation; transcription; pseudohyphal growth; cyclin/Cdk

A robust and dynamic transcriptional response requires cells to integrate extracellular signaling cues into an appropriate output. Inappropriate responses to external cues can lead to developmental defects, programmed cell death, and cancer. Both transcriptional induction and repression require the coordinated activity of transcription factors, histone-modifying enzymes, chromatin-remodeling proteins, and histone chaperone proteins (reviewed in Rando and Winston 2012). The budding yeast *Saccharomyces cerevisiae* has proven to be a powerful model in the understanding of how extracellular environmental signals elicit transcriptional responses.


doi: 10.1534/genetics.114.172841
Manuscript received August 21, 2014; accepted for publication November 24, 2014; published Early Online December 1, 2014.
Supporting information is available online at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.172841/-/DC1.
[1]Corresponding author: Rowan University School of Osteopathic Medicine, Department of Molecular Biology, 2 Medical Center Dr. #122, Stratford, NJ 08084.
E-mail: lawmj@rowan.edu

Post-translational histone modifications play a central role in a signaling network that regulates transcriptional activation, attenuation, or repression (Rea *et al.* 2000; Strahl and Allis 2000; Jenuwein and Allis 2001; Berger 2007; Smith and Shilatifard 2010; Rando and Winston 2012). Histone proteins that are responsible for packaging DNA in the nucleus can be post-translationally modified via acetylation, ubiquitination, sumolation, phosphorylation, and methylation (Strahl and Allis 2000). These modifications are dynamic and are controlled by opposing classes of enzymes, termed "writers" and "erasers" (Ruthenburg *et al.* 2007a,b). These enzymes, as well as the protein domains that interpret the modifications termed "readers," are well conserved throughout eukaryotes (reviewed in Rando and Winston 2012). Therefore, the coordinated regulation of histone writing, erasing, and reading is of central importance to transcriptional responses and phenotypic outcomes. Recent studies focused on histone methylation have been important in forwarding our understanding of histone modifications in transcription.

In budding yeast, lysine methylation targets include histone H3 Lys4, Lys36, and Lys79 and histone H4 Lys5, Lys12, and Lys18. Methylation of these residues is catalyzed by the enzymes Set1p, Set2p, Dot1p, and Set5p, respectively (Krogan *et al.* 2002; Strahl *et al.* 2002; van Leeuwen *et al.* 2002; Edwards *et al.* 2011; Green *et al.* 2012). Lysine can be modified by one, two, or three methyl groups, and each level of methylation results in different functional consequences (Fingerman *et al.* 2005). Conversely, lysine demethylases have been identified for H3 Lys4 and H3 Lys36; Jhd2p demethylates H3 Lys4 (Liang *et al.* 2007; Seward *et al.* 2007), while Jhd1p, and the paralogs Rph1p and Gis1p target H3 Lys36 for demethylation (Tu *et al.* 2007). Yeast harboring deletions of these enzymatic regulators of methylation have a myriad of phenotypes including loss of telomeric silencing and ribosomal DNA (rDNA), sensitivity to cellular stressors, and misregulation of apoptosis and meiosis (Singer *et al.* 1998; San-Segundo and Roeder 2000; Deutschbauer *et al.* 2002; Krogan *et al.* 2002; Santos-Rosa *et al.* 2002; Boa *et al.* 2003; Schaft *et al.* 2003; Sollier *et al.* 2004; Carrozza *et al.* 2005; Fingerman *et al.* 2005; Morohashi *et al.* 2005; Trelles-Sticken *et al.* 2005; Merker *et al.* 2008; Walter *et al.* 2014).

The best-characterized histone methyl mark occurs on histone H3 Lys4. While histone H3 Lys4 trimethylation at the promoters of genes has been associated with active transcription, the methyltransferase *SET1* is also required for transcriptional silencing at rDNA and telomeres (Nislow *et al.* 1997; Bernstein *et al.* 2002; Bryk *et al.* 2002; Krogan *et al.* 2002; Nagy and Denison 2002; Santos-Rosa *et al.* 2002; Boa *et al.* 2003). In yeast, both *SET1* and *JHD2* are required for efficient meiotic differentiation. *SET1* yeast mutants have major defects in meiosis due to delayed meiotic S-phase, defects in centromere and telomere structure, and inefficient double-strand break formation (Sollier *et al.* 2004; Trelles-Sticken *et al.* 2005; Borde *et al.* 2009). The *JHD2* demethylase has a critical function in completing meiosis and supporting gamete fitness (Xu *et al.* 2012). These functions are controlled in part by regulating meiotic noncoding RNA (ncRNA), rRNA, and protein-coding gene expression during spore morphogenesis (Xu *et al.* 2012). H3 Lys4 methylation is also important for pseudohyphal differentiation. For example, deleting the *SWD3* component of COMPASS, the Set1p-containing enzymatic complex, results in enhancement of flocculation, one of the hallmarks of psuedohyphal growth (Dietvorst and Brandt 2008). Together, these data highlight the importance of tight control of H3 Lys4 methylation levels during yeast cell fate determination.

Lysine methylation can be regulated by a diverse array of molecular interactions, including those with sequence-specific transcription factors, crosstalk with other histone modifications, and interactions with the RNA pol II holoenzyme. For example, histone H3 Lys4 and Lys79 methylation are dependent upon H2B ubiquitylation, which is regulated in part by the polymerase-associated factor (PAF1) complex subunit Rtf1p (Krogan *et al.* 2003a; Ng *et al.* 2003a; Wood *et al.* 2003a). In addition, phosphorylation of the RNA pol II large subunit C-terminal domain (RNA pol II CTD) stimulates interactions with the H3 Lys36 methyltransferase Set2p during transcriptional elongation (Krogan *et al.* 2003b; Xiao *et al.* 2003). Both H3 Lys36 trimethylation and phosphorylated Rpo21p combine to recruit the histone deacetylase complex Rpd3S into coding regions, leading to deacetylation in coding regions of actively transcribed genes (Carrozza *et al.* 2005; Li *et al.* 2007; Govind *et al.* 2010). These studies highlight the intimate communication between lysine methylation, the PAF complex, and phosphorylated RNA pol II CTD.

Gene-specific transcriptional activation depends upon a modular, multisubunit RNA pol II holoenzyme complex, which is composed of RNA pol II and mediator. The CDK8 submodule associates with mediator, but is genetically and biochemically distinct from the core mediator (Liao *et al.* 1995; Carlson 1997; Cooper and Strich 1999; Borggrefe *et al.* 2002). This submodule, containing Ssn2p (Med13p), Ssn3p (Cdk8p), Ssn8p (Cyclin Cp), and Srb8p (Med12p), is a highly conserved protein complex that can both positively and negatively regulate transcription in a locus-specific fashion (Strich *et al.* 1989; Hirst *et al.* 1994; Myers *et al.* 1998; Chi *et al.* 2001; Vincent *et al.* 2001; van de Peppel *et al.* 2005). CDK8 submodule function requires the Ssn3p/Ssn8p kinase complex and acts in part via the RNA pol II CTD. *In vivo* work showed that it can suppress growth defects due to RNA pol II CTD truncations, while *in vitro* studies suggested that it may function by phosphorylating the CTD (Liao *et al.* 1995; Hengartner *et al.* 1998). Ssn8p also has a nontranscriptional role in stress response as it transits to the mitochondria inducing mitochondrial fission during reactive oxygen stress (Cooper *et al.* 2014). Further support of its central importance in nutritional and stress responses is evident as it is of critical importance to meiosis (Cooper and Strich 2002), apoptosis (Cooper *et al.* 2014), diauxic shift (Holstege *et al.* 1998), and pseudohyphal growth (Nelson *et al.* 2003). Due to the genetic evidence indicating that *SSN8* and *SSN3* impinge upon RNA pol II CTD and that the CTD can influence histone methylation, we hypothesized that *SSN8/SSN3* and histone methylation are interdependent.

In this study, we examined the genetic relationship between the histone lysine methylase and demethylase enzymes and the *SSN8/SSN3* complex. Surprisingly, we observed that *SSN8* and the histone demethylase *JHD2* are required to repress pseudohyphal growth in rich media. Pseudohyphal growth occurs when yeast are deprived of nitrogen, fermentable carbon, or amino acids and is characterized by enhanced cell–cell adhesion, changes in cell polarity, increases in cell length, adherence to plastic surfaces, and invasive growth into substrates (Gimeno and Fink 1992; Gimeno *et al.* 1992; Ljungdahl *et al.* 1992; Cullen and Sprague 2000). Similarly, diploid yeast can enter meiotic differentiation when deprived of both fermentable carbon and nitrogen. Historically, studies focused on pseudohyphal growth have been performed in Σ1278B yeast strains, which execute meiotic differentiation at low efficiencies. More

recently, the meiotic strain SK1 was shown to undergo pseudohyphal growth (Strudwick *et al.* 2010), making it well suited for understanding the transitions from mitosis to either pseudohyphal growth or meiosis.

The morphological changes of pseudohyphal yeast are highly correlated with expression of the mannoprotein-encoding *FLO* gene family (Dranginis *et al.* 2007). Work focused on understanding transcriptional regulation of *FLO* genes has centered around *FLO11*, the only nonsubtelomeric family member and thus not subject to transcriptional silencing (Guo *et al.* 2000; Verstrepen *et al.* 2004; Chen and Thorner 2007). Studies of *FLO11* have indicated that it is necessary for pseudohyphal growth, but other investigations have suggested that *FLO1* and *FLO10* compensate in strains lacking *FLO11* (Guo *et al.* 2000; Smukalla *et al.* 2008; Bester *et al.* 2012). Previous work from many laboratories has shown that *FLO11* is regulated by the integration of multiple cell-signaling pathways, including RAS/PKA, MAPK, SNF, TOR, and mitochondrial retrograde transport (Halme *et al.* 2004; reviewed in Bruckner and Mosch 2012; Cullen and Sprague 2012). These signaling pathways converge to either downregulate transcriptional repressors or stimulate transcriptional activators leading to *FLO11* transcription. Additionally, *FLO11* transcription is subject to multiple types of "epigenetic control" including a ncRNA toggle, histone deacetylation, and chromatin-remodeling proteins (Bumgarner *et al.* 2009, 2012; Barrales *et al.* 2012).

In this study, we identify an unexpected genetic relationship between *SSN8* and *JHD2*. We find that mutant yeast lacking both *SSN8* and *JHD2* constitutively activate *FLO11* transcription and that this activation requires known *FLO11* transcriptional activators at varying degrees. We further characterized a role for *SSN8* in repressing H3 Lys4 trimethylation independently of *JHD2*. This study uncovers an important relationship between the CDK8 subcomplex and locus-specific control of H3 Lys4 methylation, raising the possibility that it can guide cell-fate decisions by regulating the dynamic balance of H3 Lys4 methylation levels.

## Materials and Methods

### *Yeast strains, microbiological techniques, and growth conditions*

Yeast used in this study are listed in Table 1. All strains are in the SK1 genetic background unless otherwise noted. Yeast deletions were generated using homologous recombination, and gene deletions were shuttled from the Research Genetics strain collection (KanMX) or using hygromycin B resistance cassettes (Goldstein and Mccusker 1999). Construction of diploid homozygous double-null yeast strains utilized either homologous recombination or crosses between each single-deletion mutant. Following generation of heterozygous diploids, cells were sporulated and dissected. The resulting haploid double-null yeast strains were then transformed with Ycp50-ho, giving rise to diploid homozygous mutants. Yeast were cultured in rich media (YEPD) or Synthetic Low Ammonia Dextrose (SLAD; 0.17% yeast nitrogen base, 50 μM ammonium sulfate, 2% dextrose) liquid or plates (2% washed agar) (Ryan 1950; Gimeno *et al.* 1992) supplemented with amino acids for auxotrophies. For SLAD time-course experiments, yeast were grown to mid-logarithmic phase in YEPD containing 0.5% peptone, harvested, washed with water, and split into either SLAD or YEPD media. Initial experiments analyzed *FLO11* messenger RNA (mRNA) expression in wild-type yeast 0, 1, 2, and 4 hr postshift. These experiments determined that the optimal time to assay *FLO11* expression was at $t = 0$ and $t = 4$ hr.

### *Microscopy, cytometry, and phenotype characterization*

Bright-field and fluorescence microscopy images were acquired with a Nikon Eclipse 90i microscope equipped with a Retiga Exi CCD camera and NIS software for data analysis.

Flocculation rates were determined for overnight cultures grown in YEPD media using a Klett–Summerson photoelectric colorimeter. Absorbances were measured at the indicated time points, and the reading at $t = 0$ was set equal to 100%.

Invasive growth assays were performed essentially as described (Roberts and Fink 1994). Briefly, individual colonies of yeast were spread onto YEPD containing 2% agar and grown for 3 days at 30°. Yeast were then washed away from the plate using a gentle stream of water with light scrubbing.

Budding patterns were determined using calcofluor staining essentially as previously described (Pringle *et al.* 1989). Briefly, mid-logarithmic YEPD cultures were fixed with 3.7% formaldehyde for 30 min at room temperature. Fixed cells were then washed with $ddH_20$ and resuspended in 100 μg/ml Calcofluor white (Sigma, catalog no. 18909) and incubated in the dark at 4° for <48 hr. After light sonication to remove flocs, bud scars were analyzed using the DAPI filter. Budding patterns were scored as follows: cells containing proximal and distal bud scars were bipolar, those with only distal bud scars were unipolar, and cells with equatorial bud scars were random. At least 200 cells per genotype were assayed.

Length-to-width measurements were performed on mid-logarithmic cultures in rich media (Gimeno *et al.* 1992). Photographs were taken on the Zeiss axioscope and measurements were performed using Axiovision 4.3. At least 100 cells were measured per genotype. Statistically significant differences were determined using the Mann–Whitney *U*-test with a correction for multiple comparisons.

Cell cytometry was performed on an Accuri C6 instrument. Cells containing *flo11*-GFP were grown in the indicated media to mid-logarithmic phase. Following light sonication to disrupt flocs, 30,000 cells were analyzed for their GFP expression using the FL1 channel. Once collected, data were analyzed using Flowjo software (Tree Star, Ashland, OR). All samples were gated equally, and median GFP expression was reported for cells that are in the positive population.

### *RT-qPCR*

Total nucleic acids were prepared from 20-ml mid-logarithmic cultures. Approximately 500 ng of total nucleic acid preparations

**Table 1 Yeast strains used in this study**

| Strain | Genotype[a] | Source |
|---|---|---|
| RSY883 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ* | Strich *et al.* (2004) |
| MLY2 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 jhd2::KanMX* | This study |
| MLY3 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ jhd2::KanMx* | This study |
| MLY4 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1* | This study |
| MLY10 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn3::KanMx::hisG-URA3-hisG jhd2::KanMX* | This study |
| MLY11 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn3::KanMX* | This study |
| MLY25 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 jhd2::KanMX ime1::KanMX* | This study |
| MLY37 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 jhd2::KanMX flo8::KanMX* | This study |
| MLY43 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 jhd2::KanMX mss11::KanMX* | This study |
| MLY66 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 rph1::KanMX* | This study |
| MLY67 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 set2::KanMX* | This study |
| MLY68 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 jhd2::KanMX kss1::KanMX* | This study |
| MLY71 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 jhd1::KanMX* | This study |
| MLY72 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 gis1::KanMX* | This study |
| MLY74 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ jhd1::KanMX* | This study |
| MLY80 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 dot1::KanMX* | This study |
| MLY83 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ dot1::KanMX* | This study |
| MLY84 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 set1::KanMX* | This study |
| MLY85 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 jhd2::KanMX tec1::KanMX* | This study |
| MLY86 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ set1::KanMX* | This study |
| MLY108 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ gcn4::HphMX* | This study |
| MLY109 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ rtg3::HphMX* | This study |
| MLY110 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ snf1::HphMX* | This study |
| MLY121 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 jhd2::KanMX rtg3::HphMX* | This study |
| MLY124 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ set5::KanMX* | This study |
| MLY125 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 set5::KanMX* | This study |
| MLY128 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 jhd2::KanMX gcn4::HphMX* | This study |
| MLY136 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 jhd2::KanMX snf1::HphMX* | This study |
| MLY140 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn3::Kanmx::hisG-URA3-hisG set1::KanMX* | This study |
| MLY141 | *MATa/MATα lys2 TRP1 ura3 LYS2::hoΔ set2::KanMx* | This study |
| MLY142 | *MATa/MATα lys2 TRP1 ura3 LYS2::hoΔ gis1::KanMx* | This study |
| MLY143 | *MATa/MATα lys2 TRP1 ura3 LYS2::hoΔ rph1::KanMx* | This study |
| MLY 147 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ mss11::KanMX* | This study |
| MLY148 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ flo8::KanMX* | This study |
| MLY201 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ mfg1::HphMX* | This study |
| MLY203 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 jhd2::KanMX mfg1::HphMX* | This study |
| MLY209 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ flo11::GFP-KanMX* | This study |
| MLY210 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ssn8::TRP1 jhd2::hisG flo11::GFP-KanMX* | This study |
| MLY218 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ ime1::KanMX* | This study |
| MLY224 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ tec1::KanMX* | This study |
| MLY225 | *MATa/MATα lys2 trp1::hisG ura3 LYS2::hoΔ kss1::KanMX* | This study |

[a] All strains are isogenic to RSY883 except as noted. All genotypes are homozygous except as noted.

were then treated with DNase I (New England Biolabs), followed by reverse transcription using Mu-MLV reverse transcriptase (New England Biolabs) in oligo(dT)-primed reactions to allow reverse transcription of poly(A) mRNA. Subsequent qPCR reactions were prepared using the Power SYBR Master mix (Applied Biosystems) containing primers listed in Table 2. All $C_T$ values were normalized first to *NUP85* and then to wild-type values ($\Delta\Delta C_T$). Values reported are the average of three or more independent biological replicates; error bars represent the standard error of the mean.

### *Chromatin immunoprecipitation*

Chromatin immunoprecipitation (ChIP) was performed essentially as described previously (Meluh and Koshland 1997) with the following modifications. Fifty milliliters of mid-log dextrose cultures were cross-linked with 1% formaldehyde (15 min at room temperature) followed by quenching of cross-linked protein/DNA complexes with 140 mM glycine for 5 min. Cross-linked cells were then spheroplasted, washed extensively, and sonicated using a Bioruptor UCD-200 (Diagenode) to generate fragments ~300–750 nt in length. IPs were performed on 50 μg of chromatin solution using antibodies directed toward tri-methylated H3 Lys4 (Abcam, ab8580) or histone H3 CTD (Abcam, ab1791). Immune complexes were collected, washed sequentially with TSE-150 and -500, LiCl/Det, and TE and then eluted prior to reversing cross-links. DNA was precipitated, treated with proteinase K, and subjected to qPCR. The percentage of input of each IP was calculated using a standard curve for each genomic locus assayed. Each ChIP experiment was performed on three or more independent biological repeats.

**Table 2 qPCR primers used in this study**

| Primer name | Primer sequence (5′ → 3′) |
|---|---|
| *NUP85*-coding forward | TTCGCGAAGGAGCATAATGC |
| *NUP85*-coding reverse | ACACTTCCAATTCATTCAGAATCG |
| −733 *FLO11* promoter forward | CAACAATACGGGCACAACTCA |
| −733 *FLO11* promoter reverse | TCACACCACCGATAGGCAATAG |
| −1325 *FLO11* promoter forward | GAACGCCGGTAGGCAAATT |
| −1325 *FLO11* promoter reverse | TGGGCGACATTCTTGTCAAG |
| *FLO11*-coding forward | GTTCAACCAGTCCAAGCGAAA |
| *FLO11*-coding reverse | GTAGTTACAGGTGGGTAGGTGAAGTG |
| *IME1*-coding forward | TCCCCTAGAAGTTGGCATTTTG |
| *IME1*-coding reverse | CCAAGTTCTGCAGCTGAGATGA |
| −250 *SUC2* promoter forward | GGTACGCCCGATGTTTGC |
| −250 *SUC2* promoter reverse | AGTCGTTTAAGCATTCCTCGAAA |
| *18S* rDNA forward | AATAAGGGTTCGATTCCGGAG |
| *18S* rDNA reverse | TGGATGTGGTAGCCGTTTCTC |

## Results

### *SSN8 and JHD2 are required to inhibit pseudohyphal growth under rich conditions*

We wanted to determine whether *SSN8* and histone methylation are interdependent in regulating transcription. To do this, strains lacking a known lysine methyltransferase or demethylase, with and without SSN8, were generated (Table 3). Since both lysine methylation and *SSN8* play major roles in transcription, one might expect that removal of these genes would cause pleiotropic affects, resulting in reduced doubling times under rich conditions. To test this possibility, growth rates of the mutant yeast strains cultured in rich media were measured, but this did not identify any statistically significant differences (Supporting Information, Table S1). While conducting these measurements, we noted that the strain lacking both *SSN8* and *JHD2* displayed a pseudohyphal budding pattern (Figure 1A). This phenotype was not observed in any of the other *ssn8Δ* yeast mutants analyzed in this study (Figure 1A and Figure S1A). These data indicate that both *SSN8* and *JHD2* are required to inhibit pseudohyphal differentiation under rich conditions.

Pseudohyphal yeast display enhanced cell-to-cell adhesion, a unipolar budding pattern, elongated buds, and invasive growth into solid substrates (reviewed in Cullen and Sprague 2012). To assess the contribution of *SSN8* and histone methylation regulators to pseudohyphal growth, these individual phenotypes were determined in each genetic background (Table 3 and Table S1). First, cell-to-cell adhesion was assayed using a quantitative flocculation assay. Overnight cultures were grown in rich media and assayed for their flocculation rates using measurements from a Klett colorimeter. Initial Klett readings were set equal to 100%, and decreases in absorbance were monitored over a 16-min time-course experiment (Figure 1B and Figure S1B). None of the strains containing *SSN8* displayed significant enhancement of flocculation over the duration of the time course (Figure 1B and Figure S1B). However, deleting *SSN8* resulted in increased flocculation rates, which is consistent with earlier reports documenting this phenotype (Nelson *et al.* 2003; Raithatha *et al.* 2012). Surprisingly, the histone H3 Lys4 methylation regulators *JHD2* and *SET1* influenced flocculation in the *SSN8* mutant. Removal of the *JHD2* demethylase increased the flocculation rate of the *ssn8Δ* yeast strain, while deleting *SET1* completely abrogated flocculation (Figure 1B). The enhanced flocculation observed in the *ssn8Δjhd2Δ* strain is most pronounced at the earliest time points; after only 1 min an ∼20% decrease in absorbance was observed (Figure 1B), indicating that these mutant yeast contain a high percentage of flocs in rich media. *SET1* was the only lysine methylation regulator that was required for *ssn8Δ* flocculation phenotypes (Figure 1B, Figure S1B, and Table 3), suggesting that the dynamics of H3 Lys4 methylation play a central role in cell-to-cell adhesion.

To determine budding patterns, wild-type and mutant yeast were grown to mid-logarithmic phase in rich media and stained with calcofluor white, and the position of bud scars was quantified (for details see *Materials and Methods*). Notably, yeast lacking *SET1*, *JHD2*, *SET2*, *GIS1*, *RPH1*, or *DOT1* all displayed moderate increases in the percentage of cells with unipolar budding patterns. (Table 3 and Table S1). Consistent with these observations, abnormal budding patterns have been noted for *set1Δ* mutants, cellular morphologies are altered in *gis1Δ* mutants, and a chitin deposition phenotype has been observed in *dot1Δ* mutants (Nislow *et al.* 1997; Sopko *et al.* 2006; Frederiks *et al.* 2009). These minor increases in unipolar buds have not been previously reported for *jhd2Δ*, *set2Δ*, or *rph1Δ* mutants. The impact of *SSN8* on bud-site selection was next determined in double-mutant yeast strains. Removal of *SSN8* alone resulted in modest enhancement of unipolar budding. Deleting *GIS1*, *SET1*, or *JHD2* in the *ssn8Δ* mutant background resulted in dramatic increases in unipolar budding (>80% cells unipolar; Table 3 and Table S1). While *ssn8Δgis1Δ* mutants displayed increased unipolar budding, deleting the *GIS1* paralog *RPH1* in the *ssn8Δ* mutant background did not provide a phenocopy. This indicates that, while both enzymes catalyze H3Lys36 demethylation, they regulate nonoverlapping genes or gene products, which is also consistent with earlier reports (see below; Sopko *et al.* 2006). Interestingly, both H3Lys4 methylation regulators *SET1* and *JHD2* displayed genetic interactions with *SSN8* for bud-site selection. Together, these data indicate that an intermediary level of H3Lys4 methylation is important for bud-site determination. Additionally, they highlight the complexity with which bud-site selection is controlled by histone methylation regulators.

Next, elongated budding was measured by calculating length-to-width ratios. Yeast were cultured to mid-logarithmic phase in rich media, and at least 100 cells were measured per genotype. The ratio of cell length to width was measured and is displayed as box-whisker plots (Figure 1C, Table 3, and Table S1). Statistical analyses of these data show that most of the yeast mutants assayed do not have elongated buds relative to wild type. Statistically significant increases in median length-to-width ratio in cells lacking *RPH1*, *GIS1*,

**Table 3 Phenotype analysis of histone methylation and *SSN8* mutant yeast**

| Histone target | Genotype | *SSN8* | Unipolar buds | Elongated buds | Invasive growth | Flocculation |
|---|---|---|---|---|---|---|
| | Wild type | + | − | − | − | − |
| | | Δ | + | − | + | + |
| | | +, *ssn3Δ* | + | + | + | + |
| H3 Lys4 | *set1Δ* | + | + | − | − | − |
| | | Δ | ++ | − | − | − |
| | | +, *ssn3Δ* | − | − | + | − |
| | *jhd2Δ* | + | + | + | + | − |
| | | Δ | ++ | ++ | ++ | ++ |
| | | +, *ssn3Δ* | ++ | ++ | ++ | ++ |
| H3 Lys36 | *set2Δ* | + | + | ++ | − | + |
| | | Δ | − | + | + | + |
| | *jhd1Δ* | + | − | − | + | − |
| | | Δ | − | + | + | + |
| | *gis1Δ* | + | + | + | + | − |
| | | Δ | ++ | − | ++ | + |
| | *rph1Δ* | + | + | + | − | − |
| | | Δ | + | − | + | + |
| H3 Lys79 | *dot1Δ* | + | + | + | − | − |
| | | Δ | + | − | + | + |
| H4 Lys5, -8, -12 | *set5Δ* | + | − | − | − | + |
| | | Δ | + | + | − | ++ |

Phenotypes were scored from yeast harboring the indicated deletions cultured in rich media to mid-logarithmic phase. Yeast were scored as follows: unipolar buds: "−" < 30%; "+" = 30–80%; "++" ≥ 80%; elongated buds, median length-to-width values <1.3 = "−", ≥ 1.3–1.4 = "+", ≥ 1.4 = "++" (Table S1). Invasive growth and flocculation scores are based on data in Figure 1, C and D

and *SET2* were identified. Since all of these genes regulate H3 Lys36 methylation, these results suggest that this methyl mark may play important roles in the G2/M transition. The most pronounced increase in length-to-width ratio was observed in the strain lacking both *SSN8* and *JHD2* (Figure 1C, Table 3, and Table S1). Yeast harboring these mutations displayed a statistically significant increase in cell length relative to wild type, consistent with the observed pseudohyphal growth patterns ($P < 0.0001$; Mann–Whitney *U*-test).

Finally, invasive growth was determined using a plate wash assay. Cells were grown for 3 days on rich plates and washed with dd$H_2O$ while scrubbing the plate to determine which cells were embedded in the agar. Yeast lacking *JHD1* or *DOT1* display an increase in invasive growth relative to wild type, but these increases are not enhanced upon removal of *SSN8* (Figure 1D and Table 3). Yeast lacking *SSN8* and either *JHD2* or *GIS1* all displayed elevated agar invasion. This may be due to upregulation of multiple genes that are involved in this phenotypic switch, as systematic overexpression studies have shown that invasive growth is a complex phenotype, controlled by diverse gene products including transcription factors and chromatin-modifying enzymes (Shively *et al.* 2013). In agreement with our flocculation assays and length-to-width measurements, the enhanced invasion phenotype of the *ssn8Δ* strain was *SET1*-dependent. Interestingly, the invasive growth phenotypes of yeast lacking *GIS1* or *RPH1* in the *ssn8Δ* mutant background were not identical. Invasive growth in these strains mirrored the unipolar budding phenotypes described above, providing further support for the separable functions of these two demethylase genes. Importantly, the only yeast mutant strain assayed that displayed all of the hallmarks of pseudohyphal growth in rich media (herein referred to as "synthetic pseudohyphal growth") was the strain lacking both *SSN8* and *JHD2* (Table 3). Consistent with the idea that H3 Lys4 methylation dynamics play a central role in the pseudohyphal transition, all filamentation-related phenotypes except for abnormal budding patterns of *ssn8Δ* yeast strains are *SET1*-dependent (Table 3).

### *Synthetic pseudohyphal growth relies on the transcriptional function of SSN8*

*SSN8* encodes a cyclin (cyclin C, *CNC1*) whose gene product physically interacts with the cyclin-dependent kinase Ssn3p (Cdk8p) to regulate the expression of stress response genes (Carlson *et al.* 1984; Kuchin *et al.* 1995; Cooper *et al.* 1997; Liu *et al.* 2001; Cohen *et al.* 2008). In addition to its function as a transcriptional regulator, Ssn8p also plays a regulatory role in inducing mitochondrial fission upon $H_2O_2$ stress that is independent of Ssn3p (Cooper *et al.* 2014). Given the requirement of mitochondrial function for pseudohyphae formation (Starovoytova *et al.* 2013), we wanted to address if pseudohyphal growth in rich media observed in yeast lacking *SSN8* and *JHD2* is primarily attributed to the transcriptional or mitochondrial role of *SSN8*. Two independent approaches were used to test this. First, yeast mutants lacking *SSN3/CDK8* in tandem with *JHD2* or *SET1* were constructed and their phenotypes were scored. Similar to our results observed in the *SSN8/CNC1* yeast mutants, these yeast mutants displayed unipolar budding, increased cell-to-cell adhesion, elongated buds, and enhanced invasive growth (Figure 2, A–D, and Table 3). Interestingly, *ssn3Δ* mutant pseudohyphal phenotypes were dependent upon *SET1*, further supporting a genetic relationship between

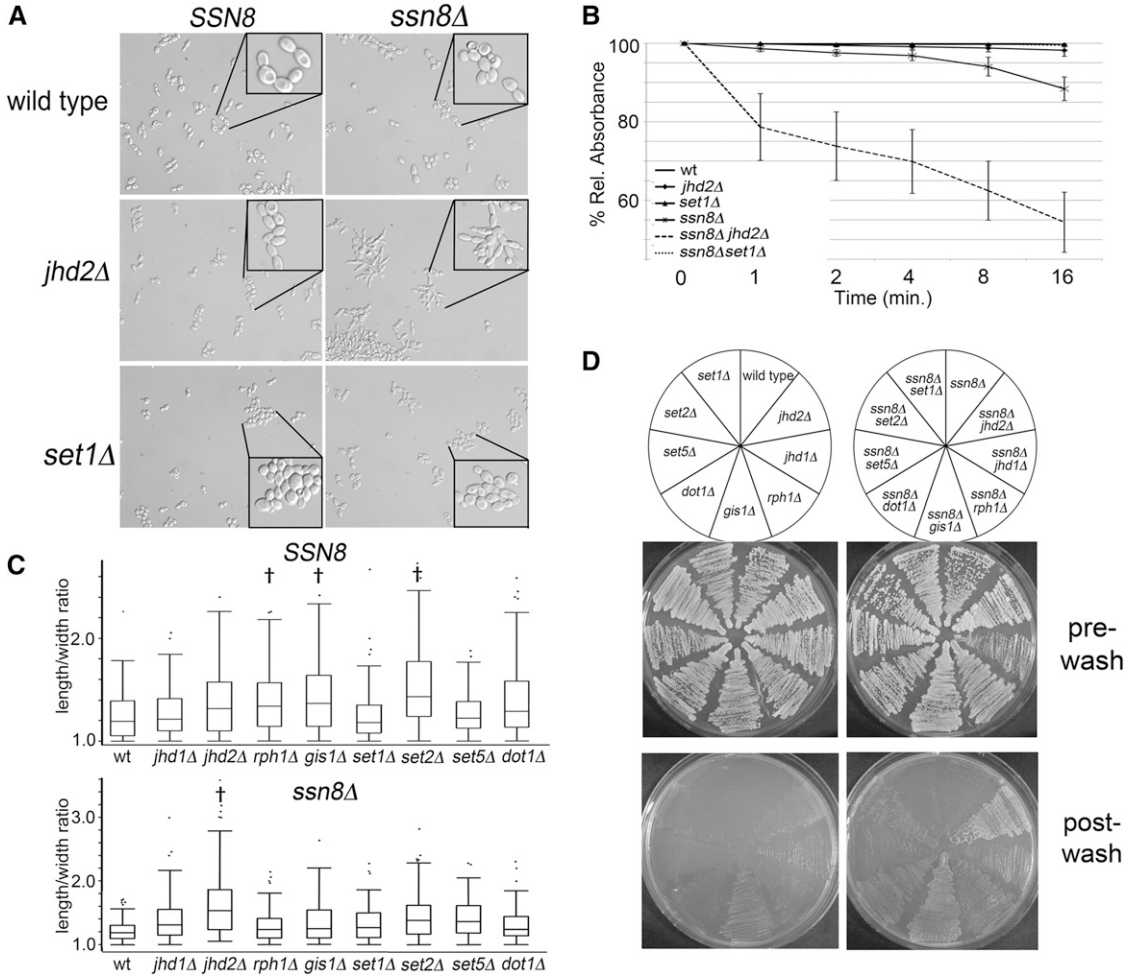


**Figure 1** Phenotype analyses of *SSN8* and histone methylation mutants. (A) Bright-field microscopy images of yeast with the indicated genotypes grown to mid-logarithmic phase in rich media. Note the pseudohyphal budding pattern of yeast lacking both *SSN8* and *JHD2*. Images of remaining genotypes can be seen in Figure S1A. (B) Flocculation time-course experiment. Yeast with the indicated genotypes were grown overnight in rich media, and flocculation rates were measured using a Klett colorimeter. Absorbances were measured at $t = 0$, 1, 2, 4, 8, and 16 min with absorbance at $t = 0$ set equal to 100%. Error bars represent SEM for three independent biological replicates; time courses for the remaining genotypes can be seen in Figure S1B. (C) Box-whisker plots of length-to-width ratios for yeast with the indicated genotypes cultured to mid-logarithmic phase in rich media. At least 100 cells per genotype were measured; the dots above whiskers show the outliers, and the "†" symbol indicates statistically significant differences from wild type (Mann–Whitney *U*-test with correction for multiple comparisons, $P < 10^{-4}$). (D) Invasive growth assays of yeast with the indicated genotypes grown on YEPD plates for 3 days. Invasiveness was determined by washing with water while scrubbing the plate.

the CDK8 complex, H3 Lys4 methylation regulators, and pseudohyphal growth.

To determine if the synthetic pseudohyphal growth observed in *ssn8Δjhd2Δ* yeast mutants was dependent upon mitochondrial retrograde transport, yeast mutants lacking *SSN8*, *JHD2*, and *RTG3* were constructed. *RTG3* is required for expression of filamentation reporter genes and invasive growth in respiration-competent yeast (Chavel *et al.* 2010). Additionally, respiration competency is a prerequisite for filamentous growth via mechanisms that support a major role for retrograde transport genes (Lorenz *et al.* 2000; Kang and Jiang 2005; Jin *et al.* 2008; Chavel *et al.* 2010; Starovoytova *et al.* 2013). Removal of *RTG3* in the *ssn8Δjhd2Δ* mutant background did not suppress any of the hallmarks of filamentous growth (Figure 2, A–D). This indicates that synthetic pseudohyphal growth occurs independently of mitochondrial retrograde transport, supporting a model in which the transcriptional function of *SSN8/SSN3* is primarily responsible for inducing this dimorphic switch.

### *SSN8 and JHD2 are required for FLO11 transcriptional repression in rich media*

The experiments described above suggest that the pseudohyphal phenotype observed in *ssn8Δjhd2Δ* mutant yeast is due primarily to transcriptional defects. Previous work from multiple labs has identified *FLO11* as a central hub that is necessary but not sufficient for the yeast morphological switch to pseudohyphal growth (Pan and Heitman 1999; Conlan and Tzamarias 2001; Reynolds and Fink 2001; Halme *et al.* 2004; Voordeckers *et al.* 2012). To determine if *FLO11* mRNA is elevated in *ssn8Δjhd2Δ* mutant yeast, we performed RT-qPCR analysis in a time-course experiment.

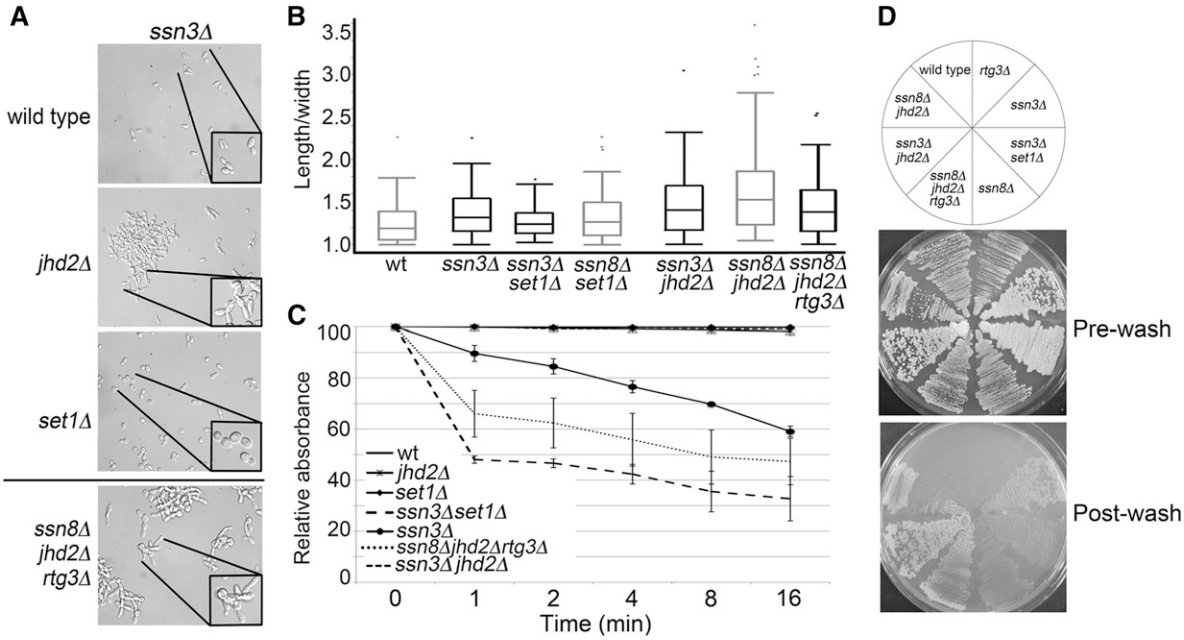


**Figure 2** Phenotype analyses of yeast mutant discriminating between transcriptional and mitochondrial roles in synthetic pseudohyphal growth. (A) Bright-field microscopy. (B) Box-whisker plots showing length-to-width measurements. (C) Flocculation assays. (D) Invasive growth assays of yeast harboring the indicated mutations performed as described in Figure 1.

First, *FLO11* transcriptional induction kinetics were determined in wild-type yeast. Yeast cultures were grown to mid-logarithmic phase in YEPD containing 0.5% peptone ($t = 0$) and then shifted to either rich (YEPD; 2% peptone) or liquid low-ammonia (SLAD) media to induce *FLO11* transcription. Time points were taken at $t = 0.5$, 1, 2, and 4 hr postshift. These initial studies showed that maximal *FLO11* induction in SLAD and repression in YEPD occurred at the 4-hr time point (Figure S2). Using this information, time-course experiments were performed comparing *FLO11* mRNA expression in wild-type, *jhd2Δ*, *ssn8Δ*, and *ssn8Δjhd2Δ* mutant yeast. Wild-type and both single mutants displayed similar levels of *FLO11* transcript at $t = 0$. Shifting these cultures to rich media resulted in *FLO11* repression, while shifting to SLAD resulted in *FLO11* induction (Figure 3A). Interestingly, yeast lacking both *SSN8* and *JHD2* display elevated *FLO11* expression under all three growth conditions assayed (Figure 3A). This indicates that both *SSN8* and *JHD2* are required to repress *FLO11* transcription in rich media.

Since *FLO11* transcription is variegated in a population of pseudohyphal yeast (Halme *et al.* 2004), we wanted to determine if *ssn8Δjhd2Δ* mutant yeast express *FLO11* mRNA in every cell or in a subset of cells. Two independent models could be used to explain the observed increases in *FLO11* mRNA levels. One model is that all yeast in the culture are expressing *FLO11* mRNA at similar elevated levels, while an alternative to this is that *FLO11* mRNA is increased dramatically in some cells in the population while absent in others. To differentiate between these possibilities, the *FLO11* ORF was replaced with GFP in wild-type or *ssn8Δjhd2Δ* mutant yeast (Figure 3, B and C). A SLAD time-course experiment in wild-type strains was performed to examine *flo11*-GFP induction. Cell cytometry showed that 0.6% of wild-type cells express *flo11*-GFP in YEPD media. Shifting wild-type *flo11*-GFP cultures to SLAD media resulted in an ~12-fold increase in the percentage of cells expressing the GFP reporter, consistent with our RT-qPCR data (Figure 3, A and C). While culturing yeast in SLAD liquid media can induce *FLO11* expression, it fails to result in pseudohyphal growth. For this to occur, wild-type yeast must be grown on SLAD plates. To determine the percentage of cells expressing *flo11*-GFP during pseudohyphal induction, wild-type yeast containing the reporter were grown on SLAD plates for 4 days, and cytometry was performed. These assays showed that ~56% of cells were now expressing the reporter gene. Both the percentage of GFP-expressing cells (~99%) and the levels of GFP expressed in each cell were dramatically increased in the *ssn8Δjhd2Δ* mutant (GFP positive median signal = 1300 wild type SLAD liquid, = 5000 wild type SLAD plate, = 26,000 *ssn8Δjhd2Δ* YEPD liquid; Figure 3, B–D). This supports the idea that *FLO11* transcription is locked into the "on" state, even while grown under repressive conditions. Interestingly, replacing the *FLO11* ORF with GFP in the *ssn8Δjhd2Δ* yeast mutant did not inhibit synthetic pseudohyphal growth (Figure 3C). This suggests that other members of the *FLO* gene family may be compensating for the absence of *FLO11*, indicating that *SSN8* and *JHD2* may be responsible for repressing other genes related to pseudohyphal growth. Additionally, these data show that both *SSN8* and *JHD2* are required for the *FLO11* repression in the total population of cells cultured in rich media.

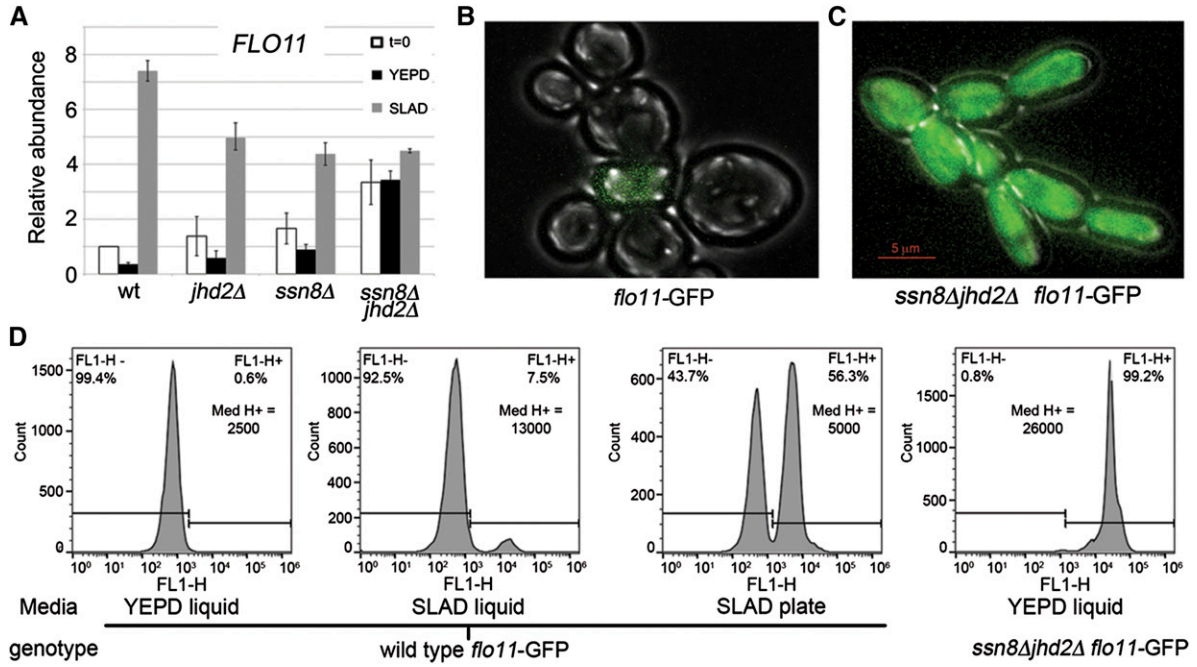


**Figure 3** *FLO11* transcriptional regulation in wild-type and *ssn8Δjhd2Δ* yeast mutants. (A) SLAD time course measuring *FLO11* mRNA levels using RT-qPCR. Wild-type and single or double *SSN8* and *JHD2* mutant yeast strains were grown as described in *Materials and Methods*. Average *FLO11* expression normalized to *NUP85* and wild-type levels at $t = 0$ for three independent biological replicates is reported; error bars represent SEM. (B and C) Representative *flo11*-GFP reporter expression for (B) wild-type yeast cultured in SLAD media for 5 hr or (C) *ssn8Δjhd2Δ* mutants cultured to mid-logarithmic phase in YEPD. (D) Flow cytometry data quantifying *flo11*-GFP reporter expression of wild-type or *ssn8Δjhd2Δ* mutants cultured in the indicated media. The percentages and median levels for GFP-positive signal were determined from 30,000 cells.

### *SSN8 and JDH2 bypass the requirement for IME1 in pseudohyphal growth*

Recent reports using genome-wide approaches to investigate pseudohyphal differentiation have uncovered an important role for meiotic genes (Shively *et al.* 2013). In the SK1 yeast background, *IME1*, the master regulator of meiosis, is required for pseudohyphal growth on nonfermentable carbon sources (Kassir *et al.* 1988; Strudwick *et al.* 2010). Similar to *FLO11* transcription, *IME1* transcription is responsive to mating type, nitrogen, and carbon sources (Kassir *et al.* 1988, 2003). Due to these regulatory overlaps, we hypothesized that *IME1* mRNA is upregulated in the *ssn8Δjhd2Δ* mutant yeast and that this upregulation stimulates *FLO11* transcription. This hypothesis was tested in two ways. First, *IME1* mRNA levels were determined using RT-qPCR in a SLAD time-course experiment. Unlike *FLO11* transcriptional upregulation, *IME1* expression profiles in wild type were similar to those measured in mutant yeast during the time course (Figure 4A). This indicates that *SSN8* and *JHD2* are not required to repress *IME1* mRNA expression in rich media, supporting a model in which both *SSN8* and *JHD2* contribute to locus-specific transcriptional repression.

Next the requirement for *IME1* in synthetic pseudohyphal growth was tested by generating triple-mutant yeast that were deleted for *SSN8*, *JHD2*, and *IME1*. While this strain did exhibit decreased cell length-to-width ratios (Figure 4, B and D), both flocculation and invasive growth were unaffected (Figure 4, C and E). These data indicate that *IME1* is not essential for all of the phenotypes associated with synthetic pseudohyphal induction and support negative control of *FLO* gene transcription by *SSN8* and *JHD2*.

### *Psuedohyphal growth in ssn8Δjhd2Δ mutants requires a subset of the known FLO11 activators*

Our data have shown that *FLO11* transcriptional repression in rich media requires both *SSN8* and *JHD2*. *FLO11* transcriptional induction and pseudohyphal differentiation are dependent upon the integration of multiple signaling pathways including SNF, MAPK, cAMP/PKA, and TOR that converge upon the *FLO11* promoter region to regulate transcription factor activity (reviewed in (Bruckner and Mosch 2012; Cullen and Sprague 2012). Together, these pathways combine to signal when yeast should enter the pseudohyphal differentiation pathway. To address which of these pathways are required for synthetic pseudohyphal cell divisions, we performed phenotypic analysis of yeast lacking components of each pathway in the *ssn8Δjhd2Δ* mutant yeast background.

First, a pathway that responds to low glucose and is required for invasive growth, adhesion to plastic, and pseudohyphal growth was examined by generating triple-null *SNF1* yeast mutants (Cullen and Sprague 2000; Reynolds and Fink 2001). Since *SSN8* was initially characterized as a suppressor of the *SNF1* kinase, it is likely that deleting *SSN8* may result in its hyperactivation (Carlson *et al.* 1984).

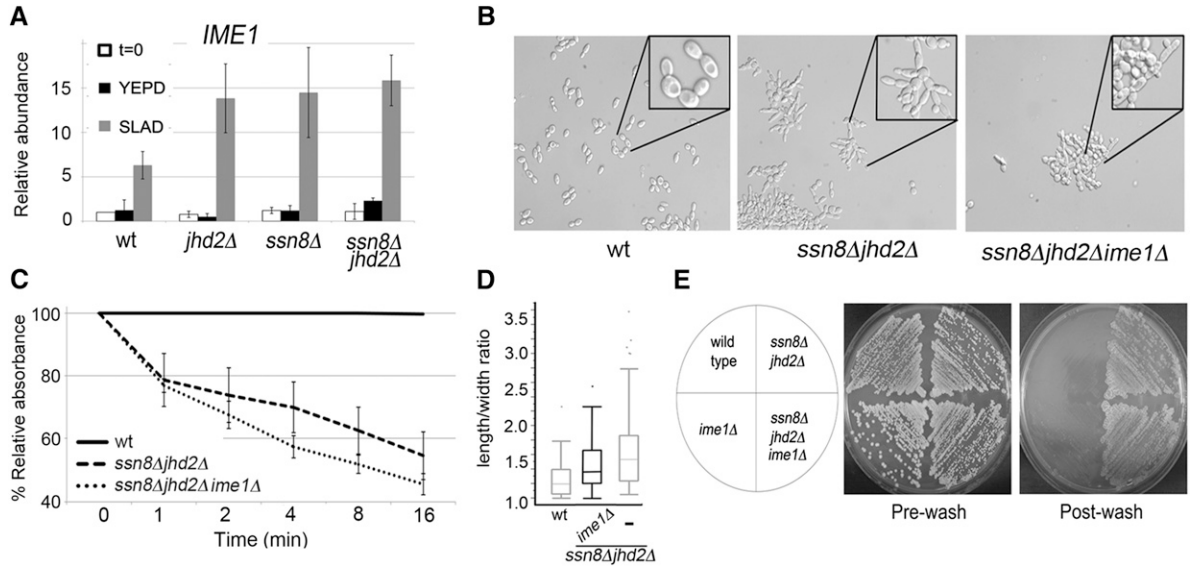


**Figure 4** *IME1* transcriptional regulation and *ssn8Δjhd2Δime1Δ* yeast mutant phenotypes. (A) SLAD time course of wild-type and single or double *SSN8* and *JHD2* yeast mutants measuring *IME1* mRNA levels using RT-qPCR. Average *IME1* expression is normalized to *NUP85* and wild-type levels at $t = 0$ for three independent biological replicates. Error bars represent SEM. (B) Bright-field microscopy. (C) Flocculation time courses. (D) Length-to-width ratios. (E) Plate wash assays for the indicated mutants performed as in Figure 1.

Surprisingly, deleting *SNF1* in the *ssn8Δjhd2Δ* mutant background did not affect flocculation rates, cell length-to-width ratio, unipolar budding, or invasive growth (Figure 5, A–E). It should be noted that the *SNF1* triple-mutant yeast cells appear sick, which is also supported by increased doubling times (data not shown). Together, these data suggest that *SNF1* is not essential for the synthetic pseudohyphal phenotype, indicating that *SSN8* is acting independently of its previously described role as a suppressor of *SNF*.

Next, the relationship between the MAPK-signaling pathway and the *ssn8Δjhd2Δ* mutant phenotypes was investigated. When yeast are cultured in media containing low nitrogen, the MAPK-signaling cascade is initiated, leading to activation of the filamentous growth MAPK, Kss1p (Cullen and Sprague 2012). Kss1p stimulates filamentous growth by phosphorylating and inactivating the inhibitors of *FLO11* expression, Dig1p and Dig2p (Roberts and Fink 1994; Mosch *et al.* 1996; Cook *et al.* 1997). Dig1p and Dig2p inhibit *FLO11* expression by interacting with and repressing the transcriptional activation complex Tec1p/Ste12p; both of these genes are required for pseudohyphal growth (Gavrias *et al.* 1996; Madhani and Fink 1997; Madhani *et al.* 1997; Bardwell *et al.* 1998; Kohler *et al.* 2002; Zeitlinger *et al.* 2003; Borneman *et al.* 2006; Chou *et al.* 2006; Borneman *et al.* 2007a,b). Additionally, previous work has shown that the Ssn8p/Ssn3p complex can inhibit the Tec1p/Ste12p heterodimer by phosphorylating Ste12p and triggering its degradation (Nelson *et al.* 2003). This work suggests that *SSN8* mutant yeast may enhance pseudohyphal growth by acting through the Kss1p MAPK and/or Ste12p/Tec1p heterodimer. To test this possibility, triple-null yeast lacking *SSN8*, *JHD2*, and *KSS1* or *TEC1* were generated. Deleting *KSS1* in *ssn8Δjhd2Δ* mutants resulted in partial suppression of pseudohyphal growth in rich media; yeast harboring this deletion had reduced, but not eliminated, flocs and partial suppression of increased cell length, but still displayed elevated unipolar budding patterns and invasive growth (Figure 5, A–E). These data show that *KSS1* is required for some, but not all, of the *ssn8Δjhd2Δ* mutant pseudohyphal phenotype. This may be due to incomplete inactivation of the Dig1p and Dig2p transcriptional repressors in the *KSS1* mutants.

We next assayed the impact of removing *TEC1* from the synthetic pseudohyphal yeast strain. Strains harboring *TEC1* deletions showed more exaggerated phenotypes than the *KSS1* deletions with an almost complete suppression of elongated buds and flocculation (Figure 5, A–C). However, the *ssn8Δjhd2Δtec1Δ* yeast mutant still displayed increased unipolar budding and agar invasiveness (Figure 5, D and E). The maintenance of unipolar budding in the *ssn8Δjhd2Δtec1Δ* mutants supports previous work indicating that *STE20* and not *STE12* plays a critical role in bipolar budding of diploid cells (Sheu *et al.* 2000; Cullen and Sprague 2002). Importantly, the failure of *TEC1* mutants to completely suppress synthetic pseudohyphal growth indicates that the *ssn8Δjhd2Δ* mutants are not acting solely through the upregulation of Ste12p.

Next the role of the nitrogen limitation response was assayed by examining a component of the TOR pathway, which helps to coordinate cellular response to limited nitrogen. TOR pathway activation signals to the transcription factor Gcn4p to induce filamentation and *FLO11* transcription (Gimeno *et al.* 1992; Crespo and Hall 2002). In addition to its role in general nitrogen response, previous work has shown that Gcn4p can be activated during amino acid limitation even while in the presence of glucose and ammonium (Braus *et al.* 2003; Hinnebusch 2005; Kleinschmidt

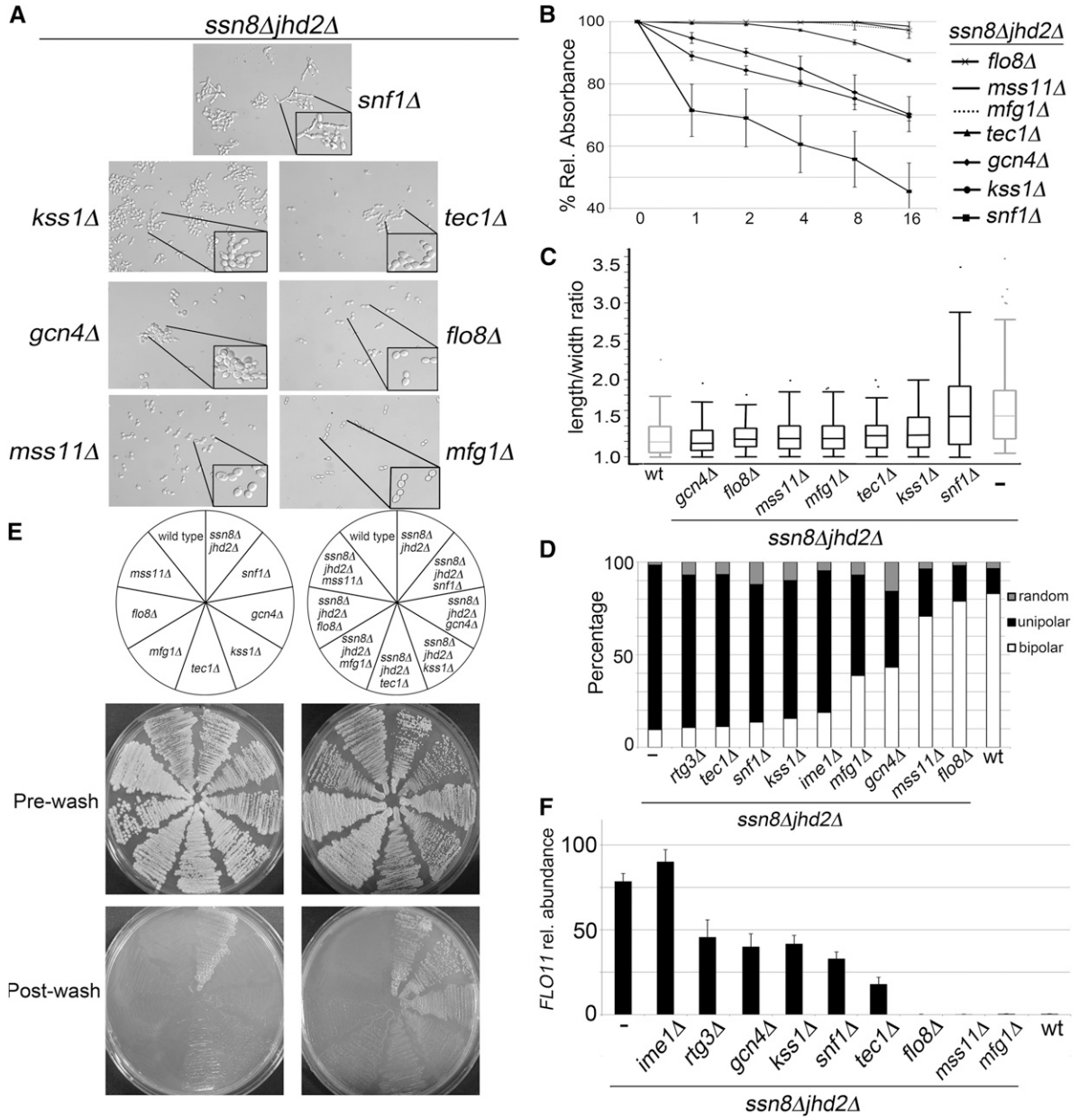


**Figure 5** Phenotype and *FLO11* transcriptional analyses of synthetic pseudohyphal yeast mutants lacking transcriptional activators of pseudohyphal growth. (A–E) Phenotype analyses of synthetic pseudohyphal yeast mutants with each indicated triple deletion were analyzed as described in Figure 1. (A) Bright-field microscopy. (B) Flocculation time courses. (C) Length-to-width ratios. (D) Budding patterns. (E) Invasive growth assays. (F) Steady-state *FLO11* mRNA measured by RT-qPCR of yeast harboring the indicated mutations. Expression levels were normalized to *NUP85* and wild type; data show the average of three or four independent biological replicates; error bars depict SEM.

*et al.* 2005). Therefore, *GCN4* plays important roles in *FLO11* transcriptional induction by sensing both amino acid stress and nitrogen limitation. Analyzing *ssn8Δjhd2Δgcn4Δ* mutants revealed partial suppression of all of the hallmarks of pseudohyphal growth. While *GCN4* was required for elongated, unipolar buds, it was only partially responsible for enhanced flocculation and invasive growth (Figure 5, A–E). This indicates that, unlike *KSS1* and *TEC1*, which are essential for some, but not all, of the hallmarks of pseudohyphal growth, *GCN4* is responsible for the maximal induction of all pseudohyphal phenotypes. Together, these different classes of partial suppressors reflect the complex genetic regulation of the pseudohyphal phenotype.

Finally, the role of the cAMP/PKA pathway in the *ssn8Δjhd2Δ* mutant phenotype was investigated by deleting the transcription factors *FLO8*, *MSS11*, and *MFG1*. The cAMP/PKA pathway is induced by low glucose and may also be regulated by low ammonia levels. The ammonia response requires the ammonium permease Mep2p, which is also required for filamentation (Lorenz and Heitman 1998; Van Nuland *et al.* 2006). Inhibition of the Bcy1p subunit by cAMP leads to activation of the protein kinase Tpk2p. Tpk2p

can induce *FLO11* expression in two ways; it positively regulates the transcriptional activator Flo8p and negatively regulates the transcriptional repressor Sfl1p (Robertson and Fink 1998; Pan and Heitman 2002). The Flo8p, Mss11p, and Mfg1p proteins form a complex that stimulates *FLO11* transcription and pseudohyphal growth (Gagiano *et al.* 1999; Van Dyk *et al.* 2005; Ryan *et al.* 2012; Mayhew and Mitra 2014). The requirement of this complex for synthetic pseudohyphal growth was assayed by deleting each component individually in *ssn8Δjhd2Δ* mutant yeast. Deleting *FLO8* and *MSS11* in *ssn8Δjhd2Δ* mutants resulted in complete suppression of all pseudohyphal phenotypes; deleting *MFG1* suppressed all phenotypes except for unipolar buds, which were reduced, but not eliminated, in *ssn8Δjhd2Δmfg1Δ* mutants (Figure 5, A–E). Given our previous results showing *FLO11* independence for synthetic pseudohyphal growth (Figure 3C), these results also indicate that the other genes required for synthetic pseudohyphal growth are regulated by *FLO8*, *MSS11*, and *MFG1*, perhaps acting as a protein complex.

### *Transcription factor requirements for constitutive FLO11 expression*

Our previous experimentation showed that *ssn8Δjhd2Δ* mutants bypass a subset of the transcriptional activators of *FLO11* for some of the pseudohyphal phenotypes. In addition, we observed constitutive upregulation of *FLO11* in *ssn8Δjhd2Δ* mutants independent of culture conditions. To determine if the suppression phenotypes result from repression of constitutive *FLO11* expression, we assayed *FLO11* mRNA levels in the triple-null yeast mutants discussed above. Yeast harboring triple deletions were cultured to mid-logarithmic phase in YEPD media. *FLO11* expression was monitored in these cultures using RT-qPCR. *IME1* was expendable for constitutive *FLO11* activation, supporting our previous observations indicating that control of synthetic pseudohyphal growth by *IME1* is bypassed (Figure 5F). These experiments showed an approximately twofold reduction in *FLO11* mRNA levels in triple-null yeast mutants lacking *RTG3*, *GCN4*, *KSS1*, and *SNF1*, and an approximately threefold reduction in yeast lacking *TEC1* (Figure 5F). These results are intriguing, given the results of our suppression of the pseudohyphal phenotypes in these yeast mutants. For example, *SNF1* and *RTG3* appear to be partially required for constitutive *FLO11* transcription, but are mostly bypassed for the synthetic pseudohyphal phenotypes. The partial suppressors *GCN4*, *KSS1*, and *TEC1* are also important for maintaining maximal *FLO11* expression observed in *ssn8Δjhd2Δ* mutants. These results are in agreement with the partial suppression of pseudohyphal phenotypes observed in these triple-mutant yeast. Finally, *FLO11* expression was completely abrogated in yeast lacking *FLO8*, *MSS11*, or *MFG1*, which again agrees well with the phenotypic data (Figure 5F). Together, these data show that, while *FLO11* is not essential for the synthetic pseudohyphal phenotypes, its transcriptional regulators are important for both pseudohyphal induction and *FLO11* constitutive expression. This is in support of previous work characterizing the complex genetic networks underpinning the pseudohyphal dimorphic switch.

### *Histone H3 Lys4 trimethylation is repressed by SSN8 independently of JHD2 at the FLO11 promoter*

Since *FLO11* transcription is constitutively activated in rich media in yeast mutants that lack both *SSN8* and the histone H3 Lys4 demethylase *JHD2*, we wanted to understand if this transcriptional misregulation is the result of changes in the H3 Lys4 methylation levels at the *FLO11* promoter. To test this possibility, ChIP was performed in wild-type or mutant mid-logarithmic yeast cultures lacking *SSN8*, *JHD2*, or both, using antibodies directed toward trimethylated histone H3 Lys4 or an antibody that recognizes the C-terminal domain of histone H3. Histone H3 Lys4 trimethylation was determined at four independent genomic loci, *FLO11*, *SUC2*, *18S*, and *NUP85*. First, qPCR reactions were directed toward three regions of *FLO11*: two primer pairs were in the promoter, while one was in the coding region. Removal of either *SSN8* or *JHD2* did not significantly impact the levels of H3 Lys4 methylation at this locus relative to wild type (Figure 6, A–C and G). Interestingly, the levels of H3 Lys4 trimethylation were increased in yeast lacking both *SSN8* and *JHD2*. This enrichment was present at the promoter sequence most proximal to the ATG (*FLO11*-733), but not observed in qPCR reactions directed toward a region located only ~600 nucleotides upstream that encompasses the Flo8p binding site (Figure 6, A, B, and G). The enhanced methylation levels in the *FLO11* promoter region observed in *ssn8Δjhd2Δ* mutants was extended into the *FLO11*-coding region, consistent with constitutive *FLO11* expression in these mutants (Figure 6C). These increases were specific to the double-null yeast mutants, as each single mutation did not alter H3 Lys4 trimethylation.

Two independent models may explain the increased H3 Lys4 trimethylation levels at the FLO11 locus in *ssn8Δjhd2Δ* . First, these genes may be regulating histone methylation on a genome-wide basis. Alternatively, *SSN8* and *JHD2* may fine-tune locus-specific H3 Lys4 trimethylation levels. To discriminate between these two models, qPCR reactions were directed toward two control loci: the coding region of *NUP85*, a constitutively expressed gene that should contain high levels of H3 Lys4 trimethylation, or 18S rDNA, a genomic locus previously identified to contain low H3 Lys4 methylation levels (Bernstein *et al.* 2002). Unlike *FLO11*, H3 Lys4 trimethylation levels were similarly elevated at the *NUP85* locus in wild-type and *SSN8 JHD2* single and double mutants (Figure 6, D and G). Contrary to this, H3Lys4 trimethylation was nearly absent at the 18S rDNA locus in all genotypes examined (Figure 6, E and G). These data suggest that *SSN8* and *JHD2* are not globally repressing histone H3 Lys4 trimethylation.

Finally, H3 Lys4 trimethylation levels were examined at the *SUC2* promoter. *SUC2* encodes a sucrose invertase that is

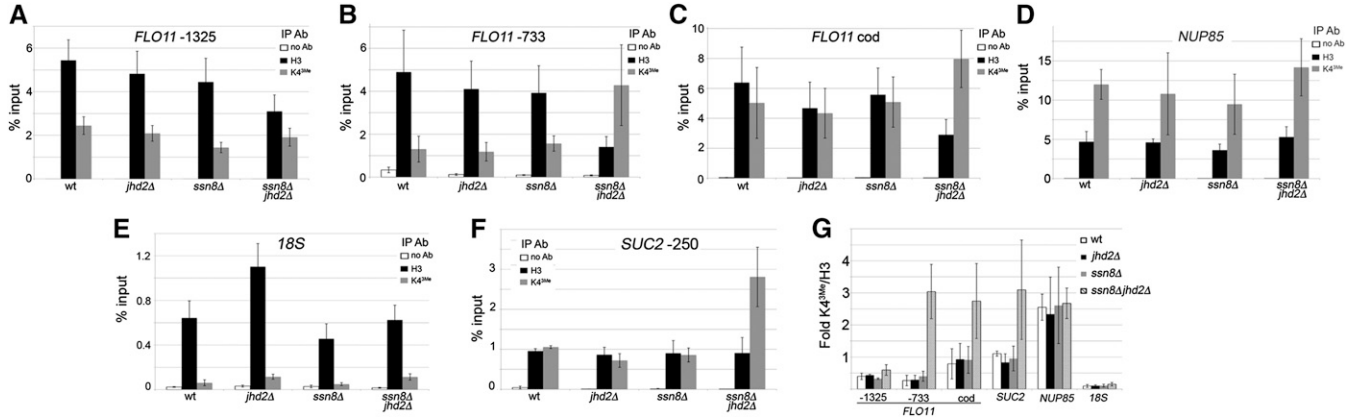


**Figure 6** Chromatin immunoprecipitations for H3 Lys4 trimethylation levels in *SSN8* and *JHD2* yeast mutants. (A–F) ChIP assays were performed on yeast harboring the indicated mutations grown to mid-logarithmic phase in rich media using antibodies directed toward the C-terminal domain of histone H3 or toward trimethylated histone H3 Lys4. No antibody (IgG alone) was used as a negative control. qPCR reactions were directed toward (A and B) *FLO11* promoter (numbers relative to ATG), (C) the *FLO11*-coding region, (D) the *NUP85*-coding region, (E) the 18S rDNA locus, or (F) the *SUC2* promoter. Enrichment values were calculated as the percentage input using a standard curve and represent the average for three independent biological repeats. Error bars show the standard deviation. (G) Ratio of trimethylated histone H3 Lys4 relative to histone H3 CTD. Error bars show the standard deviation.

not involved in pseudohyphal growth but is negatively regulated by *SSN8* (Carlson *et al.* 1984). ChIP experiments using qPCR primers directed toward the *SUC2* promoter revealed that H3 Lys4 trimethylation levels display a similar pattern to the *FLO11* promoter (Figure 6, F and G). While deleting either *SSN8* or *JHD2* resulted in minor impacts on H3 Lys4 trimethylation, deleting both genes resulted in elevated H3 Lys4 trimethylation. These results, combined with the direct regulatory role for *SSN8* at the *SUC2* locus, are consistent with a model in which *SSN8* represses locus-specific H3 Lys4 trimethylation independently of *JHD2*, raising the possibility that the CDK8 subcomplex may direct transcription by influencing chromatin modifications.

### *SSN8 and JHD2 repress H3 Lys4 trimethylation independently of FLO8*

Our genetic analyses have revealed that *ssn8Δjhd2Δ* yeast mutants enter pseudohyphal growth in rich media and constitutively express *FLO11* in a *FLO8*-, *MSS11*-, and *MFG1*-dependent manner. ChIP data show that yeast lacking both *SSN8* and *JHD2* display increased H3 Lys4 trimethylation levels at the *FLO11* promoter relative to either single mutant or wild type. These results suggest that the Flo8p/Mss11p/Mfg1p complex may be recruiting COMPASS to *FLO11*, resulting in increases in H3 Lys4 trimethylation. To test this possibility, ChIP experiments were performed in *ssn8Δjhd2Δflo8Δ* yeast mutants using immunoprecipitations specific for histone H3 or H3 Lys4 trimethylation. These experiments revealed that *SSN8* and *JHD2* repress H3 Lys4 trimethylation at the *FLO11* promoter independently of *FLO8* (Figure 7A). Comparing the ChIP data from *ssn8Δjhd2Δ* and *ssn8Δjhd2Δflo8Δ* mutants revealed similarities and differences between the H3 Lys4 trimethylation patterns at the *FLO11* locus. For example, primer pairs directed toward the upstream promoter region detected elevated H3 Lys4 trimethylation in *ssn8Δjhd2Δflo8Δ* yeast mutants, similar to those observed in *ssn8Δjhd2Δ* mutants (compare Figure 7, A and B, to Figure 6, B and G; *FLO11*-733 primers). Contrary to this, the elevated methylation observed in the *FLO11*-coding region in *ssn8Δjhd2Δ* mutants is dependent upon the presence of *FLO8* (compare Figure 7, A and B, to Figure 6, C and G). These results are consistent with the requirement of *FLO8* for the constitutive activation of *FLO11*; since H3 Lys4 trimethylation correlates with active transcription, loss of this transcription would result in losing elevated methylation. Interestingly, the opposite result is observed in qPCR primers that encompass the Flo8p-binding site (*FLO11*-1325). While *ssn8Δjhd2Δ* mutants display no change in methylation relative to wild type at this site, *ssn8Δjhd2Δflo8Δ* mutants show minor changes in the ratios of H3 Lys4 trimethylation relative to histone H3 (compare Figure 7B and Figure 6G), although the total enrichments are decreased. Importantly, the control qPCR reactions directed toward *NUP85* and *18S* rDNA display the same trends for H3 Lys4 trimethylation as all other genotypes (Figure 7, A and B). These data indicate that *SSN8* and *JHD2* repress H3 Lys4 trimethylation at the *FLO11* promoter independently of *FLO8* and support a direct regulation of histone methylation by *SSN8*. Additionally, they also show that *FLO8* acts downstream of histone H3 Lys4 trimethylation in stimulating *FLO11* transcriptional activation and pseudohyphal induction, similar to previous observations reporting this relationship between Flo8p and the Rpd3L HDAC complex (Bumgarner *et al.* 2009).

## Discussion

The results described here uncover a mechanism in which the CDK8 subcomplex of mediator may direct transcription by fine-tuning the H3 Lys4 trimethylation levels at specific

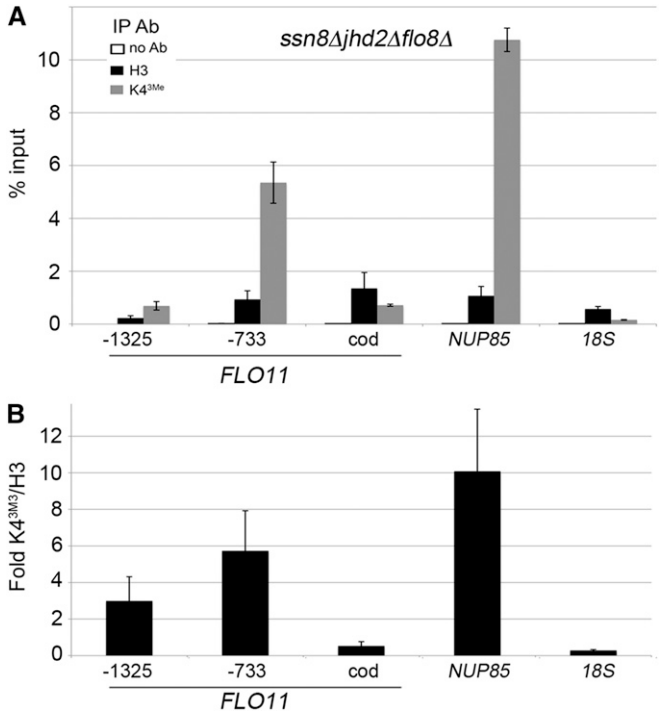


**Figure 7** ChIP assays in ssn8Δjhd2Δflo8Δ yeast mutants (A) % inputs for ChIPs performed on ssn8Δjhd2Δflo8Δ yeast mutants grown to mid-logarithmic phase in rich media. qPCR reactions were directed at the indicated regions as described in Figure 6. Values represent the average for three independent biological replicates and error bars are standard deviations. (B) Fold H3 Lys4 trimethylation relative to total H3 from assays reported in (A). Error bars show the standard deviations.

loci. Our data indicate that H3 Lys4 methylation and the CDK8 subcomplex play important roles in this decision-making process. We have shown that both *SSN8*/*SSN3* and *JHD2* are required to inhibit filamentous growth under rich conditions. Epistasis analyses determined that constitutive *FLO11* expression in yeast lacking both *SSN8* and *JHD2* requires *FLO8*, *MSS11*, and *MFG1* and that other transcriptional activators for *FLO11* including *TEC1*, *SNF1*, *KSS1*, *GCN4*, and *RTG3* play a partial role in this process. Using ChIP, we found that *SSN8* represses H3 Lys4 trimethylation independently of *JHD2* at both the *FLO11* and *SUC2* loci, but not at the *NUP85* and *18S* rDNA control loci. These studies have uncovered a previously undescribed function for the CDK8 subcomplex in regulating locus-specific transcription through modulating histone H3 Lys4 methylation levels.

Since its identification, the Ssn8p/Ssn3p (Cnc1p/Cdk8p) protein complex has been characterized as both an activator and a repressor of gene transcription, with many molecular targets identified including pol II CTD, other mediator subunits, and transcription factors (Hengartner *et al.* 1995; Chi *et al.* 2001; Nelson *et al.* 2003; Raithatha *et al.* 2012; Nemet *et al.* 2014). Microarray studies of yeast lacking the *SSN3* kinase showed that ~40% of the 173 genes that were upregulated were also increased during diauxic shift (Holstege *et al.* 1998). Two of the top hits for gene upregulation in this study were the pseudohyphal regulatory genes *FLO1* and *FLO11*. Our data show that *ssn8Δjhd2Δ* mutants can induce pseudohyphal growth independently of *FLO11*, but this induction requires the *FLO8*, *MSS11*, and *MFG1* activators. Both *FLO8* and *MSS11* are also required for *FLO1* transcription (Fichtner *et al.* 2007), making it likely that *ssn8Δjhd2Δ* mutants are upregulating this *FLO* gene family member as well. It is also possible that deleting *SSN8* and thereby eliminating Ssn3p kinase activity has broad impacts on many pseudohyphal regulators. For example, phosphorylation of Ste12p, Gcn4p, Msn2p, and Phd1p stimulates their proteasome-dependent degradation (Chi *et al.* 2001; Nelson *et al.* 2003; Raithatha *et al.* 2012). All of these targets are known to stimulate *FLO11* transcription, providing a mechanism in which *SSN8* may negatively coregulate multiple transcription factors that all function in the same biological process. Interestingly, our qPCR experiments measuring steady-state *FLO11* transcript levels show that deleting *TEC1* (the binding partner for Ste12p) and *GCN4* results in a partial reduction in *FLO11* transcript levels. These results suggest that, while protein upregulation for these transcriptional activators is important for maximal *FLO11* induction, they are not essential.

Our data support a model in which the Ssn8p/Ssn3p kinase complex regulates locus-specific H3 Lys4 methylation levels. A major question remains in how the Ssn8p/Ssn3p complex might be specifically targeting H3 Lys4 at *FLO11* and *SUC2*, but not other loci. Previous work identified a physical interaction between the transcriptional repressor Sfl1p (**S**uppressor gene for **fl**occulation) and Ssn8p (Song and Carlson 1998). Sfl1p and Flo8p antagonize each other to regulate *FLO11* transcription (Halme *et al.* 2004; Bumgarner *et al.* 2009, 2012). Since *FLO8* is also required for synthetic pseudohyphal growth (Figure 5), these data support a model in which Ssn8p may be recruited to the *FLO11* promoter by physically interacting with Sfl1p. In support of this hypothesis, Sfl1p and Ssn8p physically interact at the *SUC2* locus to inhibit its expression. Our ChIP experiments show that histone H3 Lys4 trimethylation at the *SUC2* promoter is also repressed by *SSN8* (Figure 6). This interaction would help to suppress aberrant *FLO11* (or *SUC2*) transcription by inhibiting H3 Lys4 methylation. Deleting *SSN8* would allow H3 Lys4 methylation, but in the presence of the demethylase Jhd2p these methylation marks would be quickly erased. In the absence of *JHD2*, enhanced methylation would be inhibited by the presence of Ssn8p. Therefore, we would observe increased H3 Lys4 methylation only in the absence of both *SSN8* and *JHD2*, supporting a dual regulatory mechanism for H3 Lys4 methylation targeting.

Our data showing elevated H3 Lys4 trimethylation only in yeast lacking both *SSN8* and *JHD2* are intriguing (Figure 6). The observation that the CDK8 submodule may regulate transcription by fine-tuning H3 Lys4 methylation levels provides another example for the intricacies of transcriptional regulation. Given the aforementioned diverse targets already identified for Ssn3p, we provide multiple models to explain how *SSN8* represses H3 Lys4 methylation. The

Ssn8p/Ssn3p complex may impact Set1p function directly via phosphorylation. As has been shown for other examples, Set1p phosphorylation would be followed by protein ubiquitination and degradation. Therefore, locus-specific Ssn8p/Ssn3p presence would prevent H3 Lys4 methylation by stimulating Set1p turnover. An alternative model involves histone crosstalk mechanisms. H3 Lys4 trimethylation requires H2B Lys123 ubiquitination, leading to COMPASS complex recruitment and H3 Lys4 methylation (Wood *et al.* 2003b). Given the extensive crosstalk between histone amino acids, it remains possible that Ssn8p/Ssn3p kinase phosphorylates a histone amino acid, which in turn prevents H2B Lys123 ubiquitination and/or H3 Lys4 methylation. Removal of this kinase activity would then result in enhancement of H3 Lys4 methylation. Alternatively, H2B Lys123 ubiquitination may be inhibited by Ssn8p/Ssn3p phosphorylating the E3 ligase Bre1p. Another potential model involves the PAF1 complex, which interconnects Bre1p, COMPASS, and RNA pol II CTD (Ng *et al.* 2003b). In this model, protein phosphorylation would inhibit complex formation or activity, resulting in low H3 Lys4 methylation levels independent of *JHD2*. It will be interesting to uncover the mechanism by which *SSN8* can inhibit H3 Lys4 trimethylation.

The evolutionary conservation of *SET1*, *JHD2*, *SSN8* (*CNC1*), and *SSN3* (*CDK8*) raises the possibility that their molecular function and regulation may also be conserved. For example, recent work in *Candida albicans* has shown that both *SSN8* and *SSN3* are required for biofilm formation in the presence of the biofilm inhibitor PYO (Lindsay *et al.* 2014). Additionally, *CDK8* knockout mice are embryonic lethal at embryonic day 2.5, which is prior to compaction and implantation (Westerling *et al.* 2007). This suggests that mammalian *CDK8* is critical for cell-fate determination during early embryogenesis. Further evidence for the crucial role played by *CNC1* and *CDK8* in cell-fate decisions comes from studies on cancer, where genetic evidence has shown that both genes are misregulated. Somewhat paradoxically, *CDK8* is found upregulated in colon cancers and melanoma, but is also reduced, mutated, or deleted in diverse cancer types, including esophageal squamous cell carcinoma and bladder cancers (Mitra *et al.* 2006; Greenman *et al.* 2007; Firestein *et al.* 2008; Chattopadhyay *et al.* 2010; Kapoor *et al.* 2010). Similarly, *CNC1* is upregulated in many cancers including colorectal cancer, lymphoblastic leukemia, and adenocarcinoma, but is frequently deleted in osteosarcomas and gastric cancers (reviewed in Xu and Ji 2011). These studies have suggested that both *CDK8* and *CNC1* play critical roles during oncogenesis, but the *in vivo* role for these genes remains elusive. How could amplifications and deletions of the same target genes lead to similar cell-fate decisions and cancer development? Our data indicate that these genes are important for repressing H3 Lys4 trimethylation at specific loci. Therefore, crippling *CNC1* or *CDK8* may lead to increased H3 Lys4 methylation levels at targeted loci, while overexpressing *CNC1* or *CDK8* may lead to decreased methylation at other loci. In support of this, locus-specific regulation of H3 Lys4 methylation is critical for cell-fate decisions. For example, misregulating the writing, erasing, or reading of H3 Lys4 methylation has been linked to multiple types of cancers including myeloid and lymphoid leukemias (reviewed in Chi *et al.* 2010). Therefore, future studies investigating the evolutionary conservation of *CNC1* and *CDK8* in regulating histone H3 Lys4 methylation will be of critical importance.

## Acknowledgments

The authors thank Eric G. Moss and Michael F. Henry for careful reading of the manuscript; Randy Strich and Katrina F. Cooper for reagents; Randy Strich for stimulating conversations; and Sarah Hatton, Joseph P. Sheehan, and Stephen K. Kim for providing technical assistance. Funding for this project was provided by New Jersey Health Foundation grant #PC 79-15.

## Literature Cited

Bardwell, L., J. G. Cook, D. Voora, D. M. Baggott, A. R. Martinez *et al.*, 1998 Repression of yeast Ste12 transcription factor by direct binding of unphosphorylated Kss1 MAPK and its regulation by the Ste7 MEK. Genes Dev. 12: 2887–2898.

Barrales, R. R., P. Korber, J. Jimenez, and J. I. Ibeas, 2012 Chromatin modulation at the FLO11 promoter of *Saccharomyces cerevisiae* by HDAC and Swi/Snf complexes. Genetics 191: 791–803.

Berger, S. L., 2007 The complex language of chromatin regulation during transcription. Nature 447: 407–412.

Bernstein, B. E., E. L. Humphrey, R. L. Erlich, R. Schneider, P. Bouman *et al.*, 2002 Methylation of histone H3 Lys 4 in coding regions of active genes. Proc. Natl. Acad. Sci. USA 99: 8695–8700.

Bester, M. C., D. Jacobson, and F. F. Bauer, 2012 Many *Saccharomyces cerevisiae* cell wall protein encoding genes are coregulated by Mss11, but cellular adhesion phenotypes appear only Flo protein dependent. G3 (Bethesda) 2: 131–141.

Boa, S., C. Coert, and H. G. Patterton, 2003 Saccharomyces cerevisiae Set1p is a methyltransferase specific for lysine 4 of histone H3 and is required for efficient gene expression. Yeast 20: 827–835.

Borde, V., N. Robine, W. Lin, S. Bonfils, V. Geli *et al.*, 2009 Histone H3 lysine 4 trimethylation marks meiotic recombination initiation sites. EMBO J. 28: 99–111.

Borggrefe, T., R. Davis, H. Erdjument-Bromage, P. Tempst, and R. D. Kornberg, 2002 A complex of the Srb8, -9, -10, and -11 transcriptional regulatory proteins from yeast. J. Biol. Chem. 277: 44202–44207.

Borneman, A. R., J. A. Leigh-Bell, H. Yu, P. Bertone, M. Gerstein *et al.*, 2006 Target hub proteins serve as master regulators of development in yeast. Genes Dev. 20: 435–448.

Borneman, A. R., T. A. Gianoulis, Z. D. Zhang, H. Yu, J. Rozowsky *et al.*, 2007a Divergence of transcription factor binding sites across related yeast species. Science 317: 815–819.

Borneman, A. R., Z. D. Zhang, J. Rozowsky, M. R. Seringhaus, M. Gerstein *et al.*, 2007b Transcription factor binding site identification in yeast: a comparison of high-density oligonucleotide and PCR-based microarray platforms. Funct. Integr. Genomics 7: 335–345.

Braus, G. H., O. Grundmann, S. Bruckner, and H. U. Mosch, 2003 Amino acid starvation and Gcn4p regulate adhesive

growth and FLO11 gene expression in Saccharomyces cerevisiae. Mol. Biol. Cell 14: 4272–4284.
Bruckner, S., and H. U. Mosch, 2012 Choosing the right lifestyle: adhesion and development in Saccharomyces cerevisiae. FEMS Microbiol. Rev. 36: 25–58.
Bryk, M., S. D. Briggs, B. D. Strahl, M. J. Curcio, C. D. Allis *et al.*, 2002 Evidence that Set1, a factor required for methylation of histone H3, regulates rDNA silencing in S. cerevisiae by a Sir2-independent mechanism. Curr. Biol. 12: 165–170.
Bumgarner, S. L., R. D. Dowell, P. Grisafi, D. K. Gifford, and G. R. Fink, 2009 Toggle involving cis-interfering noncoding RNAs controls variegated gene expression in yeast. Proc. Natl. Acad. Sci. USA 106: 18321–18326.
Bumgarner, S. L., G. Neuert, B. F. Voight, A. Symbor-Nagrabska, P. Grisafi *et al.*, 2012 Single-cell analysis reveals that noncoding RNAs contribute to clonal heterogeneity by modulating transcription factor recruitment. Mol. Cell 45: 470–482.
Carlson, M., 1997 Genetics of transcriptinal regulation in yeast: connection of the RNA polymerase II CTD. Annu. Rev. Cell Dev. 13: 1–23.
Carlson, M., B. C. Osmond, L. Neigeborn, and D. Botstein, 1984 A suppressor of *snf1* mutations causes constitutive high-level invertase synthesis in yeast. Genetics 107: 19–26.
Carrozza, M. J., B. Li, L. Florens, T. Suganuma, S. K. Swanson *et al.*, 2005 Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. Cell 123: 581–592.
Chattopadhyay, I., A. Singh, R. Phukan, J. Purkayastha, A. Kataki *et al.*, 2010 Genome-wide analysis of chromosomal alterations in patients with esophageal squamous cell carcinoma exposed to tobacco and betel quid from high-risk area in India. Mutat. Res. 696: 130–138.
Chavel, C. A., H. M. Dionne, B. Birkaya, J. Joshi, and P. J. Cullen, 2010 Multiple signals converge on a differentiation MAPK pathway. PLoS Genet. 6: e1000883.
Chen, R. E., and J. Thorner, 2007 Function and regulation in MAPK signaling pathways: lessons learned from the yeast Saccharomyces cerevisiae. Biochim. Biophys. Acta 1773: 1311–1340.
Chi, P., C. D. Allis, and G. G. Wang, 2010 Covalent histone modification: miswritten, misinterpreted and mis-erased in human cancers. Nat. Rev. Cancer 10: 457–469.
Chi, Y., M. J. Huddleston, X. Zhang, R. A. Young, R. S. Annan *et al.*, 2001 Negative regulation of Gcn4 and Msn2 transcription factors by Srb10 cyclin-dependent kinase. Genes Dev. 15: 1078–1092.
Chou, S., S. Lane, and H. Liu, 2006 Regulation of mating and filamentation genes by two distinct Ste12 complexes in Saccharomyces cerevisiae. Mol. Cell. Biol. 26: 4794–4805.
Cohen, T., M. Mallory, R. Strich, and T. Yao, 2008 Hos2p/Set3p deacetylase complex signals secretory stress through the Mpk1p cell integrity pathway. Eukaryot. Cell 7: 1191–1199.
Conlan, R. S., and D. Tzamarias, 2001 Sfl1 functions via the co-repressor Ssn6-Tup1 and the cAMP-dependent protein kinase Tpk2. J. Mol. Biol. 309: 1007–1015.
Cook, J. G., L. Bardwell, and J. Thorner, 1997 Inhibitory and activating functions for MAPK Kss1 in the S. cerevisiae filamentous-growth signalling pathway. Nature 390: 85–88.
Cooper, K. F., and R. Strich, 1999 Functional analysis of the yeast C-type cyclin Ume3p/Srb11p-RNA polymerase II holoenzyme interaction. Gene Expr. 8: 43–57.
Cooper, K. F., and R. Strich, 2002 Saccharomyces cerevisiae C-type cyclin Ume3p/Srb11p is required for efficient induction and execution of meiotic development. Eukaryot. Cell 1: 66–74.
Cooper, K. F., M. J. Mallory, J. S. Smith, and R. Strich, 1997 Stress and developmental regulation of the yeast C-type cyclin *UME3* (*SRB11/SSN8*). EMBO J. 16: 4665–4675.
Cooper, K. F., S. Khakhina, S. K. Kim, and R. Strich, 2014 Stress-induced nuclear-to-cytoplasmic translocation of cyclin C promotes mitochondrial fission in yeast. Dev. Cell 28: 161–173.
Crespo, J. L., and M. N. Hall, 2002 Elucidating TOR signaling and rapamycin action: lessons from Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. 66: 579–591.
Cullen, P. J., and G. F. Sprague, Jr.., 2000 Glucose depletion causes haploid invasive growth in yeast. Proc. Natl. Acad. Sci. USA 97: 13619–13624.
Cullen, P. J., and G. F. Sprague, Jr.., 2002 The Glc7p-interacting protein Bud14p attenuates polarized growth, pheromone response, and filamentous growth in Saccharomyces cerevisiae. Eukaryot. Cell 1: 884–894.
Cullen, P. J., and G. F. Sprague, Jr.., 2012 The regulation of filamentous growth in yeast. Genetics 190: 23–49.
Deutschbauer, A. M., R. M. Williams, A. M. Chu, and R. W. Davis, 2002 Parallel phenotypic analysis of sporulation and postgermination growth in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 99: 15530–15535.
Dietvorst, J., and A. Brandt, 2008 Flocculation in Saccharomyces cerevisiae is repressed by the COMPASS methylation complex during high-gravity fermentation. Yeast 25: 891–901.
Dranginis, A. M., J. M. Rauceo, J. E. Coronado, and P. N. Lipke, 2007 A biochemical guide to yeast adhesins: glycoproteins for social and antisocial occasions. Microbiol. Mol. Biol. Rev. 71: 282–294.
Edwards, C. R., W. Dang, and S. L. Berger, 2011 Histone H4 lysine 20 of Saccharomyces cerevisiae is monomethylated and functions in subtelomeric silencing. Biochemistry 50: 10473–10483.
Fichtner, L., F. Schulze, and G. H. Braus, 2007 Differential Flo8p-dependent regulation of FLO1 and FLO11 for cell-cell and cell-substrate adherence of S. cerevisiae S288c. Mol. Microbiol. 66: 1276–1289.
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.
Firestein, R., A. J. Bass, S. Y. Kim, I. F. Dunn, S. J. Silver *et al.*, 2008 CDK8 is a colorectal cancer oncogene that regulates beta-catenin activity. Nature 455: 547–551.
Frederiks, F., G. J. Heynen, S. J. van Deventer, H. Janssen, and F. van Leeuwen, 2009 Two Dot1 isoforms in Saccharomyces cerevisiae as a result of leaky scanning by the ribosome. Nucleic Acids Res. 37: 7047–7058.
Gagiano, M., D. van Dyk, F. F. Bauer, M. G. Lambrechts, and I. S. Pretorius, 1999 Msn1p/Mss10p, Mss11p and Muc1p/Flo11p are part of a signal transduction pathway downstream of Mep2p regulating invasive growth and pseudohyphal differentiation in Saccharomyces cerevisiae. Mol. Microbiol. 31: 103–116.
Gavrias, V., A. Andrianopoulos, C. J. Gimeno, and W. E. Timberlake, 1996 Saccharomyces cerevisiae TEC1 is required for pseudohyphal growth. Mol. Microbiol. 19: 1255–1263.
Gimeno, C. J., and G. R. Fink, 1992 The logic of cell division in the life cycle of yeast. Science 257: 626.
Gimeno, C. J., P. O. Ljungdahl, C. A. Styles, and G. R. Fink, 1992 Unipolar cell divisions in yeast S. cerevisiae lead to filamentous growth: regulation by starvation and RAS. Cell 68: 1077–1090.
Goldstein, A. L., and J. H. McCusker, 1999 Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. Yeast 15: 1541–1553.
Govind, C. K., H. Qiu, D. S. Ginsburg, C. Ruan, K. Hofmeyer *et al.*, 2010 Phosphorylated Pol II CTD recruits multiple HDACs, including Rpd3C(S), for methylation-dependent deacetylation of ORF nucleosomes. Mol. Cell 39: 234–246.
Green, E. M., G. Mas, N. L. Young, B. A. Garcia, and O. Gozani, 2012 Methylation of H4 lysines 5, 8 and 12 by yeast Set5

calibrates chromatin stress responses. Nat. Struct. Mol. Biol. 19: 361–363.
Greenman, C., P. Stephens, R. Smith, G. L. Dalgliesh, C. Hunter *et al.*, 2007 Patterns of somatic mutation in human cancer genomes. Nature 446: 153–158.
Guo, B., C. A. Styles, Q. Feng, and G. R. Fink, 2000 A Saccharomyces gene family involved in invasive growth, cell-cell adhesion, and mating. Proc. Natl. Acad. Sci. USA 97: 12158–12163.
Halme, A., S. Bumgarner, C. Styles, and G. R. Fink, 2004 Genetic and epigenetic regulation of the FLO gene family generates cell-surface variation in yeast. Cell 116: 405–415.
Hengartner, C. J., C. M. Thompson, J. Zhang, D. M. Chao, S.-M. Liao *et al.*, 1995 Association of an activator with an RNA polymerase II holoenzyme. Genes Dev. 9: 897–910.
Hengartner, C. J., V. E. Myer, S.-M. Liao, C. J. Wilson, S. S. Koh *et al.*, 1998 Temporal regulation of RNA Polymerase II by Srb10 and Kin28 cyclin-dependent kinases. Mol. Cell 2: 43–53.
Hinnebusch, A. G., 2005 Translational regulation of GCN4 and the general amino acid control of yeast. Annu. Rev. Microbiol. 59: 407–450.
Hirst, K., F. Fisher, P. C. McAndrew, and C. R. Godding, 1994 The transcription factor, the Cdk, its cyclin and their regulator: directing the transcriptional response to a nutritional signal. EMBO J. 13: 5410–5420.
Holstege, F. C., E. G. Jennings, J. J. Wyrick, T. I. Lee, C. J. Hengartner *et al.*, 1998 Dissecting the regulatory circuitry of a eukaryotic genome. Cell 95: 717–728.
Jenuwein, T., and C. D. Allis, 2001 Translating the histone code. Science 293: 1074–1080.
Jin, R., C. J. Dobry, P. J. McCown, and A. Kumar, 2008 Large-scale analysis of yeast filamentous growth by systematic gene disruption and overexpression. Mol. Biol. Cell 19: 284–296.
Kang, C. M., and Y. W. Jiang, 2005 Genome-wide survey of non-essential genes required for slowed DNA synthesis-induced filamentous growth in yeast. Yeast 22: 79–90.
Kapoor, A., M. S. Goldberg, L. K. Cumberland, K. Ratnakumar, M. F. Segura *et al.*, 2010 The histone variant macroH2A suppresses melanoma progression through regulation of CDK8. Nature 468: 1105–1109.
Kassir, Y., D. Granot, and G. Simchen, 1988 *IME1*, a positive regulator of meiosis in *S. cerevisiae*. Cell 52: 853–862.
Kassir, Y., N. Adir, E. Boger-Nadjar, N. G. Raviv, I. Rubin-Bejerano *et al.*, 2003 Transcriptional regulation of meiosis in budding yeast. Int. Rev. Cytol. 224: 111–171.
Kleinschmidt, M., O. Grundmann, N. Bluthgen, H. U. Mosch, and G. H. Braus, 2005 Transcriptional profiling of Saccharomyces cerevisiae cells under adhesion-inducing conditions. Mol. Genet. Genomics 273: 382–393.
Kohler, T., S. Wesche, N. Taheri, G. H. Braus, and H. U. Mosch, 2002 Dual role of the Saccharomyces cerevisiae TEA/ATTS family transcription factor Tec1p in regulation of gene expression and cellular development. Eukaryot. Cell 1: 673–686.
Krogan, N. J., J. Dover, S. Khorrami, J. F. Greenblatt, J. Schneider *et al.*, 2002 COMPASS, a histone H3 (Lysine 4) methyltransferase required for telomeric silencing of gene expression. J. Biol. Chem. 277: 10753–10755.
Krogan, N. J., J. Dover, A. Wood, J. Schneider, J. Heidt *et al.*, 2003a The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. Mol. Cell 11: 721–729.
Krogan, N. J., M. Kim, A. Tong, A. Golshani, G. Cagney *et al.*, 2003b Methylation of histone H3 by Set2 in Saccharomyces cerevisiae is linked to transcriptional elongation by RNA polymerase II. Mol. Cell. Biol. 23: 4207–4218.
Kuchin, S., P. Yeghiayan, and M. Carlson, 1995 Cyclin-dependent protein kinase and cyclin homologs *SSN3* and *SSN8* contribute to transcriptional control in yeast. Proc. Natl. Acad. Sci. USA 92: 4006–4010.
Li, B., M. Gogol, M. Carey, D. Lee, C. Seidel *et al.*, 2007 Combined action of PHD and chromo domains directs the Rpd3S HDAC to transcribed chromatin. Science 316: 1050–1054.
Liang, G., R. J. Klose, K. E. Gardner, and Y. Zhang, 2007 Yeast Jhd2p is a histone H3 Lys4 trimethyl demethylase. Nat. Struct. Mol. Biol. 14: 243–245.
Liao, S.-M., J. Zhang, D. A. Jeffery, A. J. Koleske, C. M. Thompson *et al.*, 1995 A kinase-cyclin pair in the RNA polymerase II holoenzyme. Nature 374: 193–196.
Lindsay, A. K., D. K. Morales, Z. Liu, N. Grahl, A. Zhang *et al.*, 2014 Analysis of Candida albicans mutants defective in the Cdk8 module of mediator reveal links between metabolism and biofilm formation. PLoS Genet. 10: e1004567.
Liu, H., Y. Chiang, J. Pan, J. Chen, C. Salvadore *et al.*, 2001 Characterization of CAF4 and CAF16 reveals a functional connection between the CCR4-NOT complex and a subset of SRB proteins of the RNA polymerase II holoenzyme. J. Biol. Chem. 276: 7541–7548.
Ljungdahl, P. O., C. J. Gimeno, C. A. Styles, and G. R. Fink, 1992 SHR3: a novel component of the secretory pathway specifically required for localization of amino acid permeases in yeast. Cell 71: 463–478.
Lorenz, M. C., and J. Heitman, 1998 The MEP2 ammonium permease regulates pseudohyphal differentiation in Saccharomyces cerevisiae. EMBO J. 17: 1236–1247.
Lorenz, M. C., N. S. Cutler, and J. Heitman, 2000 Characterization of alcohol-induced filamentous growth in Saccharomyces cerevisiae. Mol. Biol. Cell 11: 183–199.
Madhani, H. D., and G. R. Fink, 1997 Combinatorial control required for the specificity of yeast MAPK signaling. Science 275: 1314–1317.
Madhani, H. D., C. A. Styles, and G. R. Fink, 1997 MAP kinases with distinct inhibitory functions impart signaling specificity during yeast differentiation. Cell 91: 673–684.
Mayhew, D., and R. D. Mitra, 2014 Transcription factor regulation and chromosome dynamics during pseudohyphal growth. Mol. Biol. Cell 25(17): 2669–2676.
Meluh, P. B., and D. Koshland, 1997 Budding yeast centromere composition and assembly as revealed by in vivo cross-linking. Genes Dev. 11: 3401–3412.
Merker, J. D., M. Dominska, P. W. Greenwell, E. Rinella, D. C. Bouck *et al.*, 2008 The histone methylase Set2p and the histone deacetylase Rpd3p repress meiotic recombination at the HIS4 meiotic recombination hotspot in Saccharomyces cerevisiae. DNA Repair (Amst.) 7: 1298–1308.
Mitra, A. P., A. A. Almal, B. George, D. W. Fry, P. F. Lenehan *et al.*, 2006 The use of genetic programming in the analysis of quantitative gene expression profiles for identification of nodal status in bladder cancer. BMC Cancer 6: 159.
Morohashi, N., A. P. Mitchell, and M. Shimizu, 2005 Effect of histone methyltransferase gene mutations on sporulation in S. cerevisiae. Nucleic Acids Symp. Ser. (Oxf.) 49: 325–326.
Mosch, H. U., R. L. Roberts, and G. R. Fink, 1996 Ras2 signals via the Cdc42/Ste20/mitogen-activated protein kinase module to induce filamentous growth in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 93: 5352–5356.
Myers, L., C. Gustafsson, D. Bushnell, M. Lui, H. Erdjument-Bromage *et al.*, 1998 The Med proteins of yeast and their function through the RNA polymerase II carboxy-terminal domain. Genes Dev. 12: 45–54.
Nagy, S. R., and M. S. Denison, 2002 Specificity of nuclear protein binding to a CYP1A1 negative regulatory element. Biochem. Biophys. Res. Commun. 296: 799–805.
Nelson, C., S. Goto, K. Lund, W. Hung, and I. Sadowski, 2003 Srb10/Cdk8 regulates yeast filamentous growth by phosphorylating the transcription factor Ste12. Nature 421: 187–190.

Nemet, J., B. Jelicic, I. Rubelj, and M. Sopta, 2014 The two faces of Cdk8, a positive/negative regulator of transcription. Biochimie 97: 22–27.

Ng, H. H., S. Dole, and K. Struhl, 2003a The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. J. Biol. Chem. 278: 33625–33628.

Ng, H. H., F. Robert, R. A. Young, and K. Struhl, 2003b Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. Mol. Cell 11: 709–719.

Nislow, C., E. Ray, and L. Pillus, 1997 SET1, a yeast member of the trithorax family, functions in transcriptional silencing and diverse cellular processes. Mol. Biol. Cell 8: 2421–2436.

Pan, X., and J. Heitman, 1999 Cyclic AMP-dependent protein kinase regulates pseudohyphal differentiation in Saccharomyces cerevisiae. Mol. Cell. Biol. 19: 4874–4887.

Pan, X., and J. Heitman, 2002 Protein kinase A operates a molecular switch that governs yeast pseudohyphal differentiation. Mol. Cell. Biol. 22: 3981–3993.

Pringle, J. R., R. A. Preston, A. E. Adams, T. Stearns, D. G. Drubin *et al.*, 1989 Fluorescence microscopy methods for yeast. Methods Cell Biol. 31: 357–435.

Raithatha, S., T. C. Su, P. Lourenco, S. Goto, and I. Sadowski, 2012 Cdk8 regulates stability of the transcription factor Phd1 to control pseudohyphal differentiation of Saccharomyces cerevisiae. Mol. Cell. Biol. 32: 664–674.

Rando, O. J., and F. Winston, 2012 Chromatin and transcription in yeast. Genetics 190: 351–387.

Rea, S., F. Eisenhaber, D. O'Carroll, B. D. Strahl, Z. W. Sun *et al.*, 2000 Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature 406: 593–599.

Reynolds, T. B., and G. R. Fink, 2001 Bakers' yeast, a model for fungal biofilm formation. Science 291: 878–881.

Roberts, R. L., and G. R. Fink, 1994 Elements of a single MAP kinase cascade in Saccharomyces cerevisiae mediate two developmental programs in the same cell type: mating and invasive growth. Genes Dev. 8: 2974–2985.

Robertson, L. S., and G. R. Fink, 1998 The three yeast A kinases have specific signaling functions in pseudohyphal growth. Proc. Natl. Acad. Sci. USA 95: 13783–13787.

Ruthenburg, A. J., C. D. Allis, and J. Wysocka, 2007a Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. Mol. Cell 25: 15–30.

Ruthenburg, A. J., H. Li, D. J. Patel, and C. D. Allis, 2007b Multivalent engagement of chromatin modifications by linked binding modules. Nat. Rev. Mol. Cell Biol. 8: 983–994.

Ryan, F. J., 1950 Selected methods of Neurospora genetics. Methods Med. Res. 3: 51–75.

Ryan, O., R. S. Shapiro, C. F. Kurat, D. Mayhew, A. Baryshnikova *et al.*, 2012 Global gene deletion analysis exploring yeast filamentous growth. Science 337: 1353–1356.

San-Segundo, P. A., and G. S. Roeder, 2000 Role for the silencing protein Dot1 in meiotic checkpoint control. Mol. Biol. Cell 11: 3601–3615.

Santos-Rosa, H., R. Schneider, A. J. Bannister, J. Sherriff, B. E. Bernstein *et al.*, 2002 Active genes are tri-methylated at K4 of histone H3. Nature 419: 407–411.

Schaft, D., A. Roguev, K. M. Kotovic, A. Shevchenko, M. Sarov *et al.*, 2003 The histone 3 lysine 36 methyltransferase, SET2, is involved in transcriptional elongation. Nucleic Acids Res. 31: 2475–2482.

Seward, D. J., G. Cubberley, S. Kim, M. Schonewald, L. Zhang *et al.*, 2007 Demethylation of trimethylated histone H3 Lys4 in vivo by JARID1 JmjC proteins. Nat. Struct. Mol. Biol. 14: 240–242.

Sheu, Y. J., Y. Barral, and M. Snyder, 2000 Polarized growth controls cell shape and bipolar bud site selection in Saccharomyces cerevisiae. Mol. Cell. Biol. 20: 5235–5247.

Shively, C. A., M. J. Eckwahl, C. J. Dobry, D. Mellacheruvu, A. Nesvizhskii *et al.*, 2013 Genetic networks inducing invasive growth in *Saccharomyces cerevisiae* identified through systematic genome-wide overexpression. Genetics 193: 1297–1310.

Singer, M. S., A. Kahana, A. J. Wolf, L. L. Meisinger, S. E. Peterson *et al.*, 1998 Identification of high-copy disruptors of telomeric silencing in *Saccharomyces cerevisiae*. Genetics 150: 613–632.

Smith, E., and A. Shilatifard, 2010 The chromatin signaling pathway: diverse mechanisms of recruitment of histone-modifying enzymes and varied biological outcomes. Mol. Cell 40: 689–701.

Smukalla, S., M. Caldara, N. Pochet, A. Beauvais, S. Guadagnini *et al.*, 2008 FLO1 is a variable green beard gene that drives biofilm-like cooperation in budding yeast. Cell 135: 726–737.

Sollier, J., W. Lin, C. Soustelle, K. Suhre, A. Nicolas *et al.*, 2004 Set1 is required for meiotic S-phase onset, double-strand break formation and middle gene expression. EMBO J. 23: 1957–1967.

Song, W., and M. Carlson, 1998 Srb/mediator proteins interact functionally and physically with transcriptional repressor Sfl1. EMBO J. 17: 5757–5765.

Sopko, R., D. Huang, N. Preston, G. Chua, B. Papp *et al.*, 2006 Mapping pathways and phenotypes by systematic gene overexpression. Mol. Cell 21: 319–330.

Starovoytova, A. N., M. I. Sorokin, S. S. Sokolov, F. F. Severin, and D. A. Knorre, 2013 Mitochondrial signaling in Saccharomyces cerevisiae pseudohyphae formation induced by butanol. FEMS Yeast Res. 13: 367–374.

Strahl, B. D., and C. D. Allis, 2000 The language of covalent histone modifications. Nature 403: 41–45.

Strahl, B. D., P. A. Grant, S. D. Briggs, Z. W. Sun, J. R. Bone *et al.*, 2002 Set2 is a nucleosomal histone H3-selective methyltransferase that mediates transcriptional repression. Mol. Cell. Biol. 22: 1298–1306.

Strich, R., M. R. Slater, and R. E. Esposito, 1989 Identification of negative regulatory genes that govern the expression of early meiotic genes in yeast. Proc. Natl. Acad. Sci. USA 86: 10018–10022.

Strich, R., M. J. Mallory, M. Jarnik, and K. F. Cooper, 2004 Cyclin B-Cdk activity stimulates meiotic re-replication in budding yeast. Genetics 167: 1621–1628.

Strudwick, N., M. Brown, V. M. Parmar, and M. Schroder, 2010 Ime1 and Ime2 are required for pseudohyphal growth of Saccharomyces cerevisiae on nonfermentable carbon sources. Mol. Cell. Biol. 30: 5514–5530.

Trelles-Sticken, E., S. Bonfils, J. Sollier, V. Geli, H. Scherthan *et al.*, 2005 Set1- and Clb5-deficiencies disclose the differential regulation of centromere and telomere dynamics in Saccharomyces cerevisiae meiosis. J. Cell Sci. 118: 4985–4994.

Tu, S., E. M. Bulloch, L. Yang, C. Ren, W. C. Huang *et al.*, 2007 Identification of histone demethylases in Saccharomyces cerevisiae. J. Biol. Chem. 282: 14262–14271.

van de Peppel, J., N. Kettelarij, H. van Bakel, T. T. Kockelkorn, D. van Leenen *et al.*, 2005 Mediator expression profiling epistasis reveals a signal transduction pathway with antagonistic submodules and highly specific downstream targets. Mol. Cell 19: 511–522.

van Dyk, D., I. S. Pretorius, and F. F. Bauer, 2005 Mss11p is a central element of the regulatory network that controls FLO11 expression and invasive growth in *Saccharomyces cerevisiae*. Genetics 169: 91–106.

van Leeuwen, F., P. R. Gafken, and D. E. Gottschling, 2002 Dot1p modulates silencing in yeast by methylation of the nucleosome core. Cell 109: 745–756.

Van Nuland, A., P. Vandormael, M. Donaton, M. Alenquer, A. Lourenco *et al.*, 2006 Ammonium permease-based sensing mechanism for rapid ammonium activation of the protein kinase A pathway in yeast. Mol. Microbiol. 59: 1485–1505.

Verstrepen, K. J., T. B. Reynolds, and G. R. Fink, 2004 Origins of variation in the fungal cell surface. Nat. Rev. Microbiol. 2: 533–540.

Vincent, O., S. Kuchin, S. P. Hong, R. Townley, V. K. Vyas *et al.*, 2001 Interaction of the Srb10 kinase with Sip4, a transcriptional activator of gluconeogenic genes in Saccharomyces cerevisiae. Mol. Cell. Biol. 21: 5790–5796.

Voordeckers, K., D. De Maeyer, E. van der Zande, M. D. Vinces, W. Meert *et al.*, 2012 Identification of a complex genetic network underlying Saccharomyces cerevisiae colony morphology. Mol. Microbiol. 86: 225–239.

Walter, D., A. Matter, and B. Fahrenkrog, 2014 Loss of histone H3 methylation at lysine 4 triggers apoptosis in Saccharomyces cerevisiae. PLoS Genet. 10: e1004095.

Westerling, T., E. Kuuluvainen, and T. P. Makela, 2007 Cdk8 is essential for preimplantation mouse development. Mol. Cell. Biol. 27: 6177–6182.

Wood, A., N. J. Krogan, J. Dover, J. Schneider, J. Heidt *et al.*, 2003a Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. Mol. Cell 11: 267–274.

Wood, A., J. Schneider, J. Dover, M. Johnston, and A. Shilatifard, 2003b The Paf1 complex is essential for histone monoubiquitination by the Rad6-Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. J. Biol. Chem. 278: 34739–34742.

Xiao, T., H. Hall, K. O. Kizer, Y. Shibata, M. C. Hall *et al.*, 2003 Phosphorylation of RNA polymerase II CTD regulates H3 methylation in yeast. Genes Dev. 17: 654–663.

Xu, M., M. Soloveychik, M. Ranger, M. Schertzberg, Z. Shah *et al.*, 2012 Timing of transcriptional quiescence during gametogenesis is controlled by global histone H3K4 demethylation. Dev. Cell 23: 1059–1071.

Xu, W., and J. Y. Ji, 2011 Dysregulation of CDK8 and Cyclin C in tumorigenesis. J. Genet. Genomics 38: 439–452.

Zeitlinger, J., I. Simon, C. T. Harbison, N. M. Hannett, T. L. Volkert *et al.*, 2003 Program-specific distribution of a transcription factor dependent on partner transcription factor and MAPK signaling. Cell 113: 395–404.

*Communicating editor: M. Freitag*

GENETICS

**Supporting Information**
http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.172841/-/DC1

# Fine-Tuning of Histone H3 Lys4 Methylation During Pseudohyphal Differentiation by the CDK Submodule of RNA Polymerase II

**Michael J. Law and Kerri Ciccaglione**


DOI: 10.1534/genetics.114.172841

**Table S1 Doubling times, budding pattern quantifications, and length-to-width ratios**

| Histone target | genotype | *SSN8* | doubling time (h) | Budding pattern | | | length to width ratio | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | % Bipolar | % Unipolar | % Random | 25% | median | 75% |
| | wt | + | 1.5 ± 0.2 | 83 | 14 | 3 | 1.05 | 1.19 | 1.39 |
| | | Δ | 1.6 ± 0.1 | 43 | 52 | 5 | 1.09 | 1.18 | 1.30 |
| | | *ssn3Δ* | 2.3 ± 0.5 | 68 | 30 | 2 | 1.15 | 1.32 | 1.56 |
| H3 Lys4 | *set1Δ* | + | 1.7 ± 0.1 | 11 | 62 | 27 | 1.08 | 1.18 | 1.35 |
| | | Δ | 1.7 ± 0.3 | 15 | 83 | 2 | 1.11 | 1.27 | 1.50 |
| | | *ssn3Δ* | 2.1 ± 0.3 | 77 | 20 | 3 | 1.13 | 1.24 | 1.38 |
| | *jhd2Δ* | + | 1.7 ± 0.2 | 49 | 47 | 4 | 1.10 | 1.31 | 1.58 |
| | | Δ | 1.9 ± 0.6 | 9 | 90 | 1 | 1.23 | 1.53 | 1.87 |
| | | *ssn3Δ* | 2.5 ± 0.1 | 13 | 82 | 5 | 1.16 | 1.40 | 1.69 |
| H3 Lys36 | *set2Δ* | + | 1.9 ± 0.5 | 61 | 38 | 1 | 1.24 | 1.43 | 1.77 |
| | | Δ | 1.5 ± 0.2 | 89 | 9 | 2 | 1.16 | 1.38 | 1.62 |
| | *jhd1Δ* | + | 1.6 ± 0.2 | 78 | 17 | 5 | 1.10 | 1.21 | 1.41 |
| | | Δ | 2.1 ± 0.9 | 83 | 14 | 3 | 1.14 | 1.30 | 1.55 |
| | *rph1Δ* | + | 1.7 ± 0.1 | 53 | 44 | 3 | 1.14 | 1.34 | 1.57 |
| | | Δ | 1.7 ± 0.1 | 60 | 35 | 5 | 1.10 | 1.24 | 1.41 |
| | *gis1Δ* | + | 2.0 ± 0.2 | 58 | 40 | 2 | 1.14 | 1.37 | 1.64 |
| | | Δ | 1.8 ± 0.5 | 17 | 82 | 1 | 1.10 | 1.25 | 1.54 |
| H3 Lys79 | *dot1Δ* | + | 1.7 ± 0.1 | 59 | 40 | 1 | 1.13 | 1.29 | 1.59 |
| | | Δ | 1.5 ± 0.3 | 71 | 27 | 2 | 1.14 | 1.24 | 1.44 |
| H4 Lys5, 8, 12 | *set5Δ* | + | 1.6 ± 0.1 | 84 | 13 | 3 | 1.12 | 1.22 | 1.39 |
| | | Δ | 1.9 ± 0.1 | 60 | 38 | 2 | 1.18 | 1.36 | 1.61 |

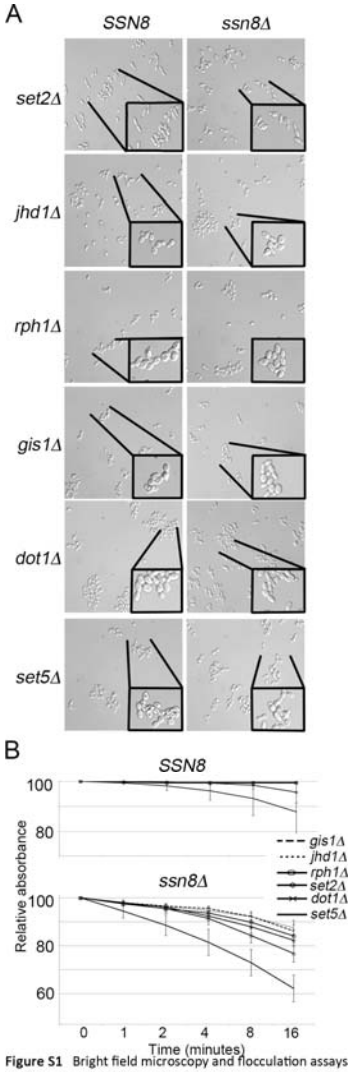


**Figure S1** Bright field microscopy and flocculation assays of histone methylation and *SSN8* yeast mutants (A) Bright field microscopy of yeast harboring the indicated mutations grown to mid-logarithmic phase in rich media. (B) Flocculation time course of yeast mutants with the indicated genotypes as described in Figure 1.

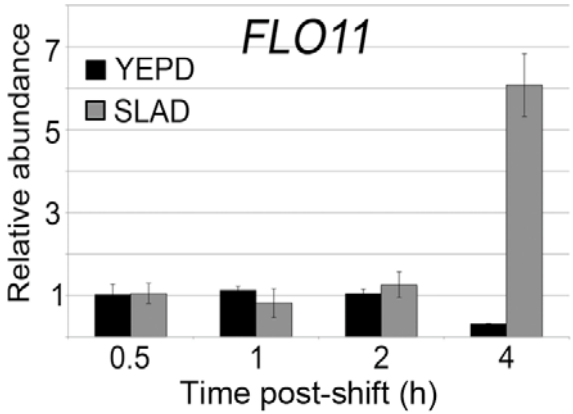


**Figure S2** *FLO11* SLAD time course optimization. *FLO11* mRNA was measured using RT-qPCR in a time course experiment. Wild type yeast cultured to mid-logarithmic phase in lowe peptone YEPD were harvested, washed, and shifted into either YEPD or SLAD media to monitor *FLO11* transcription kinetics. Results are from three independent biological replicates and error bars show SEM.