A search for proteins that interact genetically with histone H3 and H4 amino termini uncovers novel regulators of the Swe1 kinase in *Saccharomyces cerevisiae*

Xiao-Jun Ma, Quinn Lu,¹ and Michael Grunstein²

Department of Biological Chemistry, University of California at Los Angeles School of Medicine and the Molecular Biology Institute, University of California, Los Angeles, California 90095 USA

In a genetic screen for second-site mutations that are lethal in combination with a deletion of the amino terminus of histone H3, we have uncovered three new gene products that regulate the *Saccharomyces cerevisiae* Swe1 kinase. The Swe1 protein kinase phosphorylates tyrosine residue 19 of Cdc28 and inhibits its activity. One histone synthetic-lethal gene, *HSL1*, encodes a putative protein kinase that has high sequence and functional homology to fission yeast *cdrl/nim1*, an inhibitory kinase of *wee1*. Another gene, *HSL7*, is a novel negative regulator of Swe1 function. Sequences similar to *Hsl7* exist in *Caenorhabditis elegans* and humans. In addition, we have isolated a dosage-dependent suppressor, *OSS1*, of *hsl1* and *hsl7*. *OSS1* is important for the transcriptional repression of *SWE1* and *CLN2* in G₂. Mutations in *HSL1* and *HSL7* therefore cause hyperactivity of the Swe1 kinase, which in turn decreases mitotic Cdc28 kinase activity. Moreover, *HSL5* is identical to *CDC28*, further suggesting that it is the decreased Cdc28 kinase activity in these *hsl* mutants that causes lethality in the histone mutant background. Because neither *HSL1* nor *HSL7* is essential in yeast, and histone transcription is unaffected by the *hsl5/cdc28* mutation, it is unlikely that synthetic lethality results from reduced transcription of *HSL1* and *HSL7* caused by histone mutations, or from reduced histone transcription when Cdc28 kinase activity is compromised. We suggest that these cell cycle regulators function in a pathway upstream of both histones H3 and H4, thereby modulating histone function in the cell cycle.

[Key Words: Synthetic lethals; Swe1; *nim1*; *Hsl1*; *Hsl7*; *Oss1*]

Received February 20, 1996; revised version accepted April 11, 1996.

In studying the function of histones in *Saccharomyces cerevisiae* we have found that the highly conserved amino termini of histones H3 and H4 are nonessential for cellular viability but are important in gene regulation. Specific sequences within the H3 and H4 amino-terminal "tails" are involved in the assembly of heterochromatin-like structures at the silent mating loci (*HML* and *HMR*) and telomeres (Kayne et al. 1988; Johnson et al. 1990; Thompson et al. 1994). This occurs through their interactions with Sir3 and Sir4 repressor proteins of the silent mating loci and telomere-adjacent regions (Hecht et al. 1995). In addition, these histone amino termini are required for the proper activation or repression of a number of yeast genes (Durrin et al. 1991; Mann and

Grunstein 1992; Roth et al. 1992; Fisher-Adams and Grunstein 1995). Although deleting the amino terminus of H3 or H4 only causes moderate growth defects, simultaneous deletion of the H3 and H4 amino termini is lethal (Morgan et al. 1991). This suggests that these histone domains are redundant for one or more essential functions. To identify the essential functions of the H3 and H4 amino termini, we have carried out a synthetic-lethal screen for second-site mutations that cause the yeast cell to no longer tolerate the loss of the H3 amino terminus. This type of genetic screen has been used previously to identify new components of the nuclear pore complex (for review, see Doye and Hurt 1995) and to determine a role for Cln1 and Cln2 cyclins in cytokinesis (Cvrckova et al. 1995). Most of the *hsl* (for histone synthetic lethal) mutations that we have obtained have a similar phenotype, causing G₂ arrest in the histone mutant background. Their identification has uncovered a number of proteins that are intimately involved in reg-

¹Present address: Stratagene Cloning Systems, La Jolla, California USA.

²Corresponding author.

ulating the yeast cyclin-dependent protein kinase (CDK) known as Cdc28.

In the budding yeast *S. cerevisiae*, the Cdc28 gene product associates with multiple cyclins in a stage-specific manner to drive the major cell cycle transitions (Reed 1992; Nasmyth 1993). In addition, the mitotic Cdc28 kinase is subject to inhibitory tyrosine phosphorylation, a highly conserved mechanism of CDK regulation (for review, see Morgan 1995). In the fission yeast *Schizosaccharomyces pombe*, the tyrosine residue at position 15 (Y15) of cdc2 is phosphorylated by the wee1 protein kinase in G₂ (Russell and Nurse 1987b; Featherstone and Russell 1991; Parker et al. 1992). The cdc25 protein dephosphorylates this tyrosine before entry into mitosis (Russell and Nurse 1986; Dunphy and Kumagai 1991). In budding yeast, the analogous tyrosine (Y19) in Cdc28 is phosphorylated by Swe1 and dephosphorylated by Mhl1 (Russell et al. 1989; Booher et al. 1993). In *S. pombe*, mutations interfering with Y15 phosphorylation of cdc2 disrupt the cell cycle checkpoint function that prevents mitosis when DNA replication is incomplete or DNA damage is present (Enoch and Nurse 1990; Lundgren et al. 1991). Surprisingly, in *S. cerevisiae*, mutating Y19 of Cdc28 to the unphosphorylatable phenylalanine has no effect on this checkpoint function (Amon et al. 1992; Sorger and Murray 1992). However, Y19 phosphorylation is part of a novel morphogenesis checkpoint delaying mitosis when bud formation is impaired (Lew and Reed 1995).

The cell cycle-specific nature of CDK tyrosine phosphorylation and its importance in various checkpoint functions suggest that the wee1/Swe1 kinases and the cdc25/Mhl1 phosphatases themselves must be subject to regulation. In the fission yeast, the nim1 protein kinase phosphorylates the carboxy-terminal catalytic domain of wee1 and inhibits its activity (Russell and Nurse 1987a; Feilottter et al. 1991; Coleman et al. 1993; Parker et al. 1993; Wu and Russell 1993). Whether a nim1-like protein kinase regulates Swe1 in *S. cerevisiae* or the wee1 kinases in any other organism is presently unknown. However, this is not the only mechanism that regulates wee1-like proteins in eukaryotes. For example, specific degradation of the human wee1 kinase in M phase may also be an important mechanism in human cells (Watanabe et al. 1995).

Our analysis of *hsl* mutants has led to the identification of three new genes (*HSL1*, *HSL7*, and *OSS1*) that regulate the Swe1 kinase. Hsl1 has strong sequence and functional homology to cdr1/nim1, although it is much larger in size. Hsl7 is a novel negative regulator of Swe1. Oss1 is a transcriptional repressor of both *SWE1* and *CLN2*. A fourth gene, *HSL5*, is identical to *CDC28* itself. These data suggest a strong link between the cell cycle machinery and histones, which are assembled during S phase and are required for chromosomal segregation in mitosis.

Results

Isolation of *hsl* mutants

The highly conserved amino termini of histones H3 and

H4 are functionally redundant for mitotic growth in *S. cerevisiae* because cellular viability is lost only when both of these amino-terminal tails are deleted (Morgan et al. 1991). However, it is unclear which essential functions in yeast cells require these amino-terminal tails and which gene products interact with these amino-terminal tails directly or indirectly to carry out these functions. To address these questions, we screened for second-site mutations that prevent cellular viability when the H3 amino terminus is deleted (i.e., a synthetic-lethal screen).

The design of our genetic screen is depicted in Figure 1. In *S. cerevisiae*, there are two copies of the H3 and H4 gene pair (designated as *HHT1-HHF1* and *HHT2-HHF2*, respectively), which are arranged such that the H3 and H4 mRNAs are transcribed divergently from each pair (Smith and Murray 1983). To screen for mutations (*hsl*) that are lethal in combination with the deletion of the H3 amino terminus, we used the yeast strain RMY430 (Mann and Grunstein 1992). In this strain, both chromosomal copies of the H3 and H4 gene pair are deleted, and histones are supplied from two low-copy centromeric plasmids (Fig. 1). Plasmid A carries wild-type H3 and H4 genes under the control of the *GAL1-10* promoter and has the *URA3* gene as a selectable marker. Plasmid B carries the wild-type H4 gene and a mutant H3 gene, both under the control of their native promoters; the mutation in H3 (*H3Δ4-30*) is a deletion of residues 4–30. This strain grows well when *GAL*-driven transcription of wild-type H3 is repressed in glucose medium or when plasmid A is lost (Mann and Grunstein 1992). Therefore, viability of this strain does not depend on plasmid A.

After mutagenesis of this strain by ethylmethanesulfonate (EMS), we searched for mutants that had be-

Figure 1. Strain (RMY430) used for the histone synthetic-lethal screen. Chromosomal copies of the H3 and H4 genes (*HHT1-HHF1* and *HHT2-HHF2*) are deleted and marked with auxotrophic markers as indicated. Two plasmids, one carrying wild-type H3 and H4 under the control of the *GAL1-10* promoter (plasmid A), and the other carrying wild-type H4 and a mutant H3 (deletion of residues 4–30) under their respective native promoter control (plasmid B), are shown. Construction of this strain and the histone plasmids has been described (Mann and Grunstein 1992). Second-site mutations that are lethal in combination with the H3 mutation are designated *hsl*.

come dependent on plasmid A, that is, on the presence of wild-type histone H3 for viability. This was tested by two methods. Because the wild-type H3 gene on plasmid A is under the control of the *GAL1-10* promoter, *hsl* mutants should be sensitive to growth in glucose, which represses the *GAL1-10* promoter. Second, *hsl* mutants cannot lose plasmid A and therefore should be sensitive to the drug 5-fluoro-orotic acid (5-FOA), which selects against cells synthesizing Ura3 (Boeke et al. 1987). After screening some 12,000 mutagenized colonies by a combination of these two methods, a total of six *hsl* mutants were isolated and characterized. By crossing each mutant to the parental strain, we found that all were recessive *hsl* mutations. Of these, *hsl3* was represented twice. A second round of screening of 80,000 EMS-mutagenized colonies yielded only two new *hsl* mutants despite the much wider scope of the second screen. This suggests that although we may not yet have saturated the screen, there are a very limited number of *HSL* genes. Two recessive mutants (designated *hsl1-1* and *hsl7-1*) were found to have unusual morphological defects. Most cells are large and bear elongated buds. In the synthetic-lethal state, >90% of the cells have an undivided nucleus at the neck of the long buds, similar to a G₂/M arrest (data not shown). We also tested whether *hsl1-1* and *hsl7-1* mutations are lethal in the H4 amino-terminal deletion background. To this end, plasmid B was lost from these strains, which were then transformed with a similar plasmid except that it contained a wild-type H3 gene and a mutant H4 gene. The mutant H4 tested (H4Δ4-23) contained a deletion of residues 4–23, a viable mutation described previously (Kayne et al. 1988). The resulting strains were sensitive to 5-FOA, indicating that both *hsl1-1* and *hsl7-1* mutants were dependent on the H4 amino terminus for viability. Therefore, these two *hsl* mutants require both H3 and H4 amino termini for mitotic growth.

HSL1 encodes a nim1-like protein kinase

The synthetic lethality phenotype of *hsl1-1* in the presence of the H3 amino-terminal deletion (H3Δ4-30) was used to clone the *HSL1* gene by transformation with a wild-type genomic library. A single plasmid (pE14R1) that fully complemented *hsl1-1* was recovered. Partial sequences of the insert fragment in this plasmid were obtained and used to search the data bases using the BLAST (Altschul et al. 1990) service at the National Center for Biotechnology Information (NCBI). These sequences were found to be within chromosome XI, which had been sequenced completely by the yeast genome project (Dujon et al. 1994). Deletion analysis indicated that the complementing open reading frame (ORF) corresponds to YKL101w (GenBank accession no. Z28101; Fig. 2A). To ascertain that this ORF corresponds to *HSL1*, a gapped plasmid containing a large deletion within this ORF (pHSL1-gap; Fig. 2A) was transformed into *hsl1-1* and *HSL*⁺ cells. The gap-repaired plasmids (Orr-Weaver and Szostak 1983) recovered from the *hsl1-1* strain were unable to complement *hsl1-1*, whereas those

recovered from the *HSL1*⁺ strain were fully active. We conclude that the chromosomal locus YKL101w corresponds to *HSL1*.

To determine the null phenotype of *hsl1*, most of the coding region was deleted and replaced with *URA3*, creating a deletion/replacement allele *hsl1Δ1* (Fig. 2A). A wild-type diploid strain was transformed with this deletion allele. Diploids heterozygous for *hsl1Δ1* were identified as described in Materials and methods, and tetrad analysis was carried out. Each of the 11 complete tetrads dissected gave four viable spores, indicating that *HSL1* is not essential for viability. However, microscopic examination indicated that *hsl1Δ1* cells are highly elongated (Fig. 2B, left), a phenotype that is similar to, but more severe than, the original *hsl1-1* cells. DNA staining with DAPI (4',6-diamidino-2-phenylindole) indicated that most cells contain DNA at the mother bud neck and so are delayed in nuclear division (Fig. 2B, right). A second deletion (*hsl1Δ2*) removing only the amino-terminal third portion of *HSL1* causes similar morphological defects (data not shown).

The predicted Hsl1 protein is 1518 amino acids in length, and has a molecular mass of 169 kD. The protein sequence of Hsl1 was compared with sequences in the available data bases. We found that the amino-terminal portion of Hsl1 showed the highest homology (48% identical in 304 residues, see Fig. 3A) to the cdr1/nim1 protein kinase of *S. pombe* (Russell and Nurse 1987a; Feilolter et al. 1991). The region most similar between Hsl1 and Cdr1/Nim1 contains all the subdomains identified previously among serine/threonine protein kinases (Hanks et al. 1988). It is notable, however, that the long carboxyl terminus of Hsl1 is not homologous to any known proteins and that Hsl1 is almost three times as large as cdr1/nim1.

Hsl1 negatively regulates the Swe1 kinase

The high sequence homology of Hsl1 with nim1 suggests that Hsl1 may carry out analogous functions in *S. cerevisiae*. In *S. pombe*, nim1 inhibits the wee1 kinase by phosphorylating the catalytic domain of wee1 (Coleman et al. 1993; Parker et al. 1993; Wu and Russell 1993). Therefore, *nim1* deletion causes a G₂-delay attributable to increased wee1 protein kinase activity, which inhibits the mitotic cdc2–cyclin B kinase (Russell and Nurse 1987a; Feilolter et al. 1991). We analyzed the *hsl1Δ1* cells for DNA content by fluorescence-activated cell sorting (FACS). *hsl1Δ1* cells exhibit a significant delay in G₂ as indicated by the much reduced G₁ peak and increased G₂ peak, when compared with the isogenic wild-type strain (Fig. 3B). If this G₂-delay is attributable to a loss of inhibition of the Swe1 kinase in *hsl1Δ* cells, simultaneous deletion of the *SWE1* gene should suppress this G₂ delay. FACS analysis of the *hsl1Δ1 swe1Δ* cells indicates that this is the case (Fig. 3B). Therefore, these genetic data suggest that Hsl1 is a negative regulator of the Swe1 protein kinase.

In our attempts to clone the *HSL1* gene by complementing the histone synthetic-lethal phenotype of *hsl1-*

Figure 2. Cloning and disruption of *HSL1*. (A) Restriction map of the insert on plasmid pE14R1, which fully complemented the synthetic lethality of *hsl1-1* with H3Δ430. (Bold arrows) The location of a previously uncharacterized ORF, YKL101w (Dujon et al. 1994). The + and – signs indicate the complementation activity of the corresponding fragments shown at left. Digestion of this plasmid with *Nru*I and *Mlu*I creates a gapped plasmid (pHSL1-gap), which was used to recover the mutant allele in *hsl1-1* by the technique of gap repair (Orr-Weaver and Szostak 1983). Two deletion alleles are shown to indicate the regions deleted. (B) Morphological defects of *hsl1Δ1* cells. *hsl1Δ1* cells were grown in SD–Ura at 30°C. After staining with DAPI, cells were examined microscopically. (Left) Phase contrast microscopy (right) DNA staining with DAPI.

1, we also repeatedly obtained the *MIH1* gene (Russell et al. 1989) as an extra-copy suppressor of *hsl1-1* (data not shown). This suggests that *hsl1-1* mutant cells have excessive amounts of active Swel kinase, a situation that can be counteracted by increased dosages of the Mih1 phosphatase. Moreover, deleting the *SWE1* gene in the *hsl1-1* mutant completely suppresses the synthetic lethality of *hsl1-1* with H3Δ4-30. These results suggest that the hyperactive Swel kinase in *hsl1* mutant strains causes lethality in the presence of the H3 amino-terminal deletion.

To further confirm that Hsl1 acts to negatively regulate the Swel kinase, which in turn inhibits the Cdc28–Clb kinase by Y19 phosphorylation, we deleted *HSL1* in a strain carrying the *cdc28* mutant whose Y19 is mutated to the unphosphorylatable phenylalanine residue (*cdc28-F*) (Sorger and Murray 1992) and in an isogenic *CDC28* wild-type strain. As expected, *hsl1 CDC28* mutants have large buds and are elongated, whereas the *hsl1 cdc28-F* double mutant exhibits wild-type morphology (data not shown). Therefore, preventing the inhibitory phosphorylation of Cdc28 suppresses the *hsl1* phenotype, arguing that Hsl1 is a negative regulator of Swel,

which in turn inhibits Cdc28. These data argue strongly that in *S. cerevisiae*, Hsl1 is both structurally and functionally homologous to the fission yeast *cdrl/nim1* gene product.

HSL7 encodes a novel negative regulator of the Swel kinase

The *hsl7-1* mutant is phenotypically similar to *hsl1-1*; however, it is not complemented by *HSL1* on a low-copy plasmid. Complementation cloning of *hsl7-1* resulted in the isolation of a single plasmid (pBB311) that fully complemented the synthetic lethality of *hsl7-1* carrying the H3 mutation H3Δ4-30. Sequencing of both ends of the insert in this plasmid indicated that this fragment resides in the right arm of chromosome II, which had been sequenced completely by the yeast genome project (Feldmann et al. 1994). The complete sequence of this fragment was retrieved from the EMBL data base library (accession no. X75891), and analyzed for potential ORF. As indicated in Figure 4A, two complete ORFs (YBR132c and YBR133c) and an amino-terminally truncated ORF (YBR131w) exist within this region. YBR132c encodes a protein highly similar to amino-acid permeases, whereas

Figure 3. Hsl1 is both structurally and functionally similar to the *cdl1/nim1* protein kinase. (A) Homology between Hsl1 (top) and the fission yeast *cdl1/nim1* protein kinase (bottom). Subdomains conserved among protein kinases are indicated by brackets. Boldface residues are most conserved among serine–threonine protein kinases. (B) FACS analysis. Wild type (YDS2), *hsl1Δ* (MAY1), and *hsl1Δ swe1Δ* (MAY2) cells were grown logarithmically in YEPD at 30°C and prepared for FACS analysis.

the other two ORFs are not similar to any known genes in the data bases.

To determine which ORF is responsible for the *hsl7* complementation activity, we used PCR to clone YBR131w and YBR133c and their immediate flanking sequences. Multiple independent PCR clones were transformed into *hsl7-1*. Clones containing YBR133c had full complementation activity, whereas those containing YBR131w failed to complement. To determine whether YBR133c contained the *hsl7-1* mutation in the genome, we used PCR to clone the region containing YBR133c using genomic DNA isolated from the *hsl7-1* mutant. Five independent PCR clones derived from the *hsl7-1* genomic DNA were transformed into *hsl7-1*, and none of them complemented *hsl7-1*. As control, two independent clones derived from *HSL7*⁺ genomic DNA were isolated, and both fully complemented *hsl7-1*. This demonstrates that the chromosomal locus containing YBR133c corresponds to the *HSL7* gene.

To analyze the null phenotype of *hsl7*, we constructed an *hsl7Δ* allele, *hsl7Δ1::URA3*, in which a large portion of the coding sequence was deleted and marked with the *URA3* gene (Fig. 4A). This *hsl7* deletion allele could be introduced with high frequency into wild-type haploid cells, suggesting that *HSL7* is not essential for mitotic growth. However, a large fraction of *hsl7Δ* cells have elongated buds (Fig. 4B, left) and an undivided nucleus (Fig. 4B, right), a phenotype similar to that of the original *hsl7-1* mutant. As with *hsl1-1*, the synthetic lethality of *hsl7-1* with the H3 mutation H3Δ4-30 can be fully suppressed by *swe1Δ* and partially suppressed by extra copies of the *MIH1* gene (data not shown). Moreover, FACS

analysis indicated that *hsl7Δ* cells are delayed in G₂, and this delay was suppressed completely by *swe1Δ* (Fig. 4C). Therefore, like *HSL1*, *HSL7* is also a negative regulator of the Swe1 kinase.

A data base search using the encoded amino acid sequence of Hsl7 revealed no significant similarity to known genes. However, an uncharacterized ORF in *Caenorhabditis elegans* (GenBank accession no. U10402; Wilson et al. 1994) showed significant homology to Hsl7 along most of its length (Fig. 5). A search against the Database for Expressed Sequence Tags (dbEST) (Boguski et al. 1994) carried out at XREFdb (Bassett et al. 1995) revealed significant similarities to the encoded amino acid sequences of two human cDNA clones (R65681, deposited to the data bases by the WashU–Merck EST project, Washington University, St. Louis, MO; F12149, deposited to the data bases by the Genexpress cDNA program and Auffray et al. 1995). Alignment of Hsl7 with these sequences is also shown in Figure 5. The two human EST peptides are highly similar to both Hsl7 and the *C. elegans* ORF U10402 in two adjacent regions. Strong similarities among these sequences suggest that Hsl7 may be a conserved protein from yeast to man.

Isolation of OSS1, an extra-copy suppressor of *hsl1-1* and *hsl7-1*

During the cloning of *HSL1* and *HSL7*, we isolated two other genes capable of suppressing *hsl1-1* and *hsl7-1*. One of these genes is *MIH1* (data not shown), as mentioned above. The analysis of the second gene is shown in Figure 6A. Deletion analysis indicated that the 1.3-kb

Figure 4. Cloning and analysis of *HSL7*. (A) Restriction map of the insert in plasmid pBB311, which fully complemented *hsl7-1*. Sequence analysis indicated that the complete sequence of this fragment had been deposited to the data bases by the Yeast Genome Project (accession no. X75891). Three potential ORFs are indicated. Fragments containing YBR131w and YBR133c were cloned into a low-copy yeast vector to test for complementation of *hsl7-1* [(+), full complementation; (–) no complementation]. An internal deletion of the *NarI* fragment within YBR133c was made and marked with the *URA3* gene to create the deletion allele *hsl7Δ1*. (B) Morphology of *hsl7Δ1* cells. Strain MAY3 (*hsl7Δ1*) was grown in YEPD at 30°C, and the cells were examined microscopically after staining with DAPI. (Left) Phase contrast microscopy; (right) DNA staining. (C) FACS analysis. Wild-type (YDS2), *hsl7Δ1* (MAY3), and *hsl7Δ1 swe1Δ* (MAY4) cells were grown logarithmically in YEPD at 30°C and prepared for FACS analysis.

SacI–*SacII* fragment was sufficient for suppression. Sequencing of this fragment revealed that it contained only the carboxy-terminal portion of a large ORF present in the 3.5-kb *EcoRI*–*Sall* fragment. The complete ORF encodes a 103-kD protein with no significant similarity to any known proteins but contains several potential phosphorylation sites for the Cdc28 kinase (Fig. 6B). Later data base searches indicated that it is identical to an ORF designated YM8156.15c (accession no. Z49260), deposited to the data bases by the yeast genome project (G. Lye and C.M. Church; B. Barrell and M.A. Rajandream; both unpubl.). The *EcoRI*–*Sall* region cloned by PCR using genomic DNA derived from *hsl1-1* and *hsl7-1* mutants can suppress both *hsl1* and *hsl7* to the same extent as the original clone pE148. Therefore, we conclude that this ORF represents a dosage-dependent suppressor of both *hsl1-1* and *hsl7-1* mutations, and was designated *OSS1*, for *hsl* one and seven suppressor.

An *oss1* deletion allele removing most of the coding sequence in the *SacI*–*SacII* fragment (*oss1Δ1::TRP1*, Fig. 6A) or a larger deletion allele removing most of the coding sequence in the *EcoRI*–*Sall* fragment (*oss1Δ5::TRP1*, Fig. 6A) was readily introduced into wild-type haploid cells, indicating that *OSS1* is not essential for mitotic

growth. However, microscopic examination of the *oss1Δ* cells revealed that most cells have elongated buds (Fig. 6C, left), similar to those of *hsl1Δ* and *hsl7Δ* cells. Moreover, deleting the *SWE1* gene in *oss1Δ* cells completely suppresses this abnormal morphology (Fig. 6C, right). Therefore, *OSS1* may suppress *hsl1-1* and *hsl7-1* by negatively regulating the Swel kinase.

We then asked whether *OSS1* might also be an *HSL* gene. We found that *oss1Δ* has a synthetic-lethal interaction with H3Δ430 only in diploid cells. This may reflect that diploids are more sensitive to compromised CDK activity, as suggested from earlier observations that loss of Swi4, a transcription activator for G₁ cyclins, is lethal in diploid but not in haploid cells (Ogas et al. 1991).

Regulation of *SWE1* and *CLN2* transcription by *OSS1*

Oss1 does not have any homology to known kinases or phosphatases but is rich in glutamine residues, similar to those of certain known transcription factors (Mitchell and Tjian 1989). Therefore, we asked whether *OSS1* might be a negative regulator of *SWE1* transcription. Because the level of Y19 phosphorylation on Cdc28 is reg-

Figure 5. Homology of Hsl7 with a *C. elegans* ORF (accession no. U10402) and two human EST-encoded peptides (accession nos. F12149 and R65681). Conserved residues are boxed and shaded.

et al. 1990). When *oss1Δ* cells were analyzed in parallel, we found that the cell cycle regulation of *SWE1* and *CLN2* was disrupted in *oss1Δ* cells; activation of *SWE1* and *CLN2* transcription was slower by ~15 min, and their repression in G₂ was delayed and incomplete (Fig. 7A,B). Therefore, *OSS1* is necessary for the repression of both *SWE1* and *CLN2* transcription during G₂ and M phases. It is notable, however, that the peak level of *SWE1* and *CLN2* transcription in *oss1Δ* cells was similar to that in wild-type cells, suggesting that *OSS1* primarily regulates the timing of cell cycle-specific activation and repression of *SWE1* and *CLN2* transcription rather than the maximal amounts of their mRNAs.

GENES & DEVELOPMENT 1333

strate that Hsl1 and Hsl7 are negative regulators of Swe1, an inhibitory protein kinase that phosphorylates Cdc28 at tyrosine 19. This conclusion is based on the following. (1) All the phenotypes of *hsl1* and *hsl7* are suppressed completely by deletion of *SWE1*. (2) *MIH1* was isolated as a dosage-dependent suppressor of both *hsl1* and *hsl7*. The fact that Mih1 dephosphorylates CDC28 Y19, which SWE1 phosphorylates, argues strongly that *hsl1* and *hsl7* mutant cells have excessive Swe1 activity. (3) *OSS1*, a second dosage-dependent suppressor of *hsl1* and *hsl7*, represses *SWE1* transcription in G₂. (4) A mutation at Y19 (Y19F) suppresses *hsl1* phenotypes completely, suggesting that *HSL1* functions in the pathway leading to Y19 phosphorylation by Swe1. (5) There is strong homology between Hsl1 and nim1, the inhibitory kinase of weel. These data argue that the decreased activity of Cdc28 caused by mutations in *HSL1* and *HSL7* results in lethality in the presence of the H3 amino-terminal deletion (H3Δ4-30) or the H4 amino-terminal deletion (H4Δ4-23). In support of this, we have recently found

Figure 7. *OSS1* is required for the periodic transcription of *SWE1* and *CLN2* transcription during the cell cycle. Logarithmically growing cultures of wild-type (YDS2) and *oss1Δ5* (MAY7) cells were synchronized with α -factor, and aliquots of the cultures were taken at 15-min intervals after release from the α -factor-induced arrest. Total RNA was extracted from cells taken at the indicated time points, and Northern analysis was carried out. (A) An RNA blot was hybridized with probes for *SWE1* and *CDC28* mRNA, and the signals on the autoradiograph were scanned by densitometry. The *SWE1* mRNA levels were normalized to that of the *CDC28* mRNA (top). (B) The same RNA samples analyzed in A were analyzed with probes against *CLN2*, *CLB5*, and *CDC28*.

that *HSL5* is identical to *CDC28*, and the *hsl5* mutation also causes cells to accumulate at the G_2/M boundary (data not shown).

We have determined that the amino-terminal deletions of H3 and H4 do not reduce transcription of *CDC28* (data not shown). Conversely, the *hsl5/cdc28* mutation does not deregulate histone transcription during the cell cycle (data not shown). In addition, because *HSL1*, *HSL7*, and *OSS1* all are nonessential genes, it is unlikely that synthetic lethality is attributable to reduced transcription of these genes in the histone amino-terminal deletions background. Therefore, it is possible that Cdc28 functions upstream of the histone H3 and H4 amino termini for the G_2 -M transition. However, other possibilities exist. First, it has been shown that H4 amino-terminal mutations can cause G_2 delay by activating a *RAD9*-dependent checkpoint that monitors genome integrity (Megee et al. 1995). Moreover, Swel may be involved in a checkpoint that monitors bud formation, delaying cells in G_2 when bud formation is impaired (Lew and Reed 1995). Therefore, the activation of the *RAD9* checkpoint in combination with Swel hyperactivation attributable to *hsl* mutations may cause G_2 arrest. Second, histone amino-terminal mutations may deregulate other genes, which in turn causes lethality in combination with a decrease in Cdc28 function. At present we are unable to distinguish among these possible models of synthetic lethality. However, the high degree of specificity of our

genetic screen, as highlighted by the fact that mutations in five of the seven *HSL* genes that we isolated after extensive searches all result in decreased Cdc28 function, is striking. Therefore, we favor the idea that Cdc28 may function upstream of histones, thereby regulating chromosome dynamics during the cell cycle.

To date, the only regulator of weel identified and characterized so far is the *cdr1/nim1* kinase in *S. pombe* (Russell and Nurse 1987a; Feilottter et al. 1991). A prior attempt to clone the *Drosophila nim1* homolog by complementation of a *cdr1/nim1* mutant in fission yeast was not successful (Warbrick and Glover 1994). It is interesting to note that Hsl1 is highly similar to *cdr1/nim1* both in sequence and function. Because *hsl7Δ* cells grow much slower than *hsl1Δ* cells, it is likely that Hsl7, a novel protein, regulates Swel by a different mechanism. We have determined that Hsl7 does not regulate *SWE1* mRNA levels during the cell cycle (data not shown). The existence of similar proteins in *C. elegans* and humans suggests that it may also be a highly conserved regulatory protein. The fact that both Hsl1 and Hsl7 are conserved in sequence in evolution suggests that not only is CDK function highly conserved, but the regulatory mechanisms upstream of Swel/weel are also conserved. This conservation is particularly striking in light of the fact that the budding yeast uses the Swel pathway in a different checkpoint function from other organisms (Lew and Reed 1995).

In addition, we have found that transcription of *SWE1* is regulated coordinately with *CLN2*, a G_1 cyclin, during the cell cycle. The mechanism for the coordinate control is suggested from an examination of the promoter regions of both *SWE1* and *CLN2*. The *SWE1* promoter contains two exact copies of the sequence 5'-CGACGC-GAAAAA-3' (Booher et al. 1993). The CGCGAAA sequence is also present in the regulatory sequence of *CLN1* and *CLN2* (Ogas et al. 1991) and is highly similar (1-bp mismatch) to the consensus sequence of the Swi4, 6-dependent cell cycle box (SCB) (CACGAAA) (Koch and Nasmyth 1994). This similarity in promoter sequence among *SWE1*, *CLN1*, and *CLN2* suggests that they may share regulatory mechanisms. *OSS1* is important for the cell cycle periodicity of both *SWE1* and *CLN2* transcription. Although there is also a delay in the transcriptional activation of *CLB5* in *oss1Δ* cells, it remains to be determined whether this occurs indirectly because of the delayed accumulation of Cln2 or reflects a more direct role for *OSS1* in *CLB5* transcription.

It has been shown previously that G_2 repression of *CLN2* requires mitotic cyclins, especially Clb1 and Clb2 (Amon et al. 1993). However, it is unclear how Clbs repress *CLN2* transcription and whether additional repressors are also required. Whether *Oss1* is part of the Clb-dependent repression mechanism remains to be determined. However, the presence of multiple potential phosphorylation sites for Cdc28 within *Oss1* would suggest that the activity of *Oss1* may be subject to regulation by the Cdc28 protein kinase. Preliminary experiments indicated that some of these potential phosphorylation sites are critical for the suppressor activity of *Oss1* (X. Ma and M. Grunstein, unpubl.). Therefore, it will be interesting to determine whether *Oss1* activity is dependent on Cdc28 and mitotic cyclins.

Our results indicate that compromised mitotic Cdc28 activity, caused by nonlethal mutations in *HSL1*, *HSL7*, and *CDC28*, results in lethality of cells carrying viable H3 or H4 amino-terminal deletions. These cell cycle regulators are all important for the G_2 /M transition. Significantly, histone H3 and H4 amino-terminal deletions also cause cells to accumulate in G_2 /M (Kayne et al. 1988; Morgan et al. 1991), suggesting that the histone amino termini have important roles in allowing cells to progress through G_2 . One possible role may be in replication-coupled nucleosome assembly during S phase (Kaufman and Botchan 1994; Kaufman et al. 1995). A close temporal link between DNA synthesis and nucleosome assembly is reflected by the fact that histone synthesis takes place in S phase (Hereford et al. 1981; Cross and Smith 1988). We have demonstrated recently that the amino-termini of H3 and H4 are required for nucleosome assembly in a redundant fashion both in vivo and in vitro (Ling et al. 1996). Because both H3 and H4 amino termini are phosphorylated in eukaryotic cells (Van Holde 1989), it is possible that a cascade of protein kinases controlled by Cdc28 may lead to the phosphorylation of histones and regulate their assembly into nucleosomes.

A second role for the H3 and H4 amino termini may be in the process of mitotic chromosome condensation

(Guacci et al. 1994), which is triggered by the activation of mitotic CDKs (for review, see Yanagida 1995). A function for the H3 amino terminus during chromosome condensation has been suggested by earlier observations that phosphorylation of the serine residue at position 10 (S10) accompanies chromosome condensation (Gurley et al. 1978). However, the fact that the H3 mutant carrying a deletion of residues 4–30 including S10 is viable suggests that S10 phosphorylation alone is not essential for mitosis. Rather, the structure and phosphorylation state of other histone amino termini may also contribute to chromosome condensation. Consistent with this, removal of the amino-terminal tails of all four core histones by trypsin digestion prevents salt-induced condensation of polynucleosomes in vitro (Garcia-Ramirez et al. 1992). Moreover, deletion of the H4 amino terminus in vivo not only causes a delay of cell cycle progression in G_2 but increased sensitivity of chromatin to micrococcal nuclease, suggesting a decondensed state (Kayne et al. 1988).

In conclusion, the ultimate result of a cell-division cycle is the accurate duplication and segregation of chromosomes. Our data have not only uncovered new regulators of Cdc28 but argue that the functional interactions between Cdc28 and histones are essential for the progression through the G_2 /M transition. The mechanism by which Cdc28 may regulate nucleosome assembly or chromosomal condensation is a mystery at present. The discovery that Nap1, a nucleosome assembly protein conserved throughout evolution, is associated with Clb2 in *S. cerevisiae* may provide one missing link between the cell cycle and chromatin assembly (Kellogg et al. 1995; Kellogg and Murray 1995). How the CDK complex interacts with such proteins to coordinate nucleosome assembly and chromosomal condensation with other cell cycle events remains to be determined.

Materials and methods

Strains and media

Strains used in this study are listed in Table 1. Standard yeast growth media were used, and standard genetic techniques were followed (Ausubel et al. 1987). Rich medium contains YEP (1% yeast extract, 2% peptone) plus 2% glucose (YEPD) or 2% galactose (YEPG). Synthetic complete media (SD or SG, containing 2% glucose or galactose, respectively) lacking uracil or specified amino acids were prepared as described (Ausubel et al. 1987). 5-FOA was added to synthetic selective media at the concentration of 1 gram/liter. Yeast transformations were carried out as described (Gietz and Schiestl 1991).

Isolation of *hsl* mutants

The strain RMY430 was transformed with pRM102 (Mann and Grunstein 1992). The resulting strain was grown in SG – Ura – Trp to mid-log phase. A suspension of these cells was incubated with 3% EMS for 1 hr at 30°C. Surviving cells (10%–20% of the starting cells) were grown on SG – Ura – Trp plates for 3 days at 30°C. Colonies (~12,000) were replica-plated onto SD – Ura – Trp or SG – Trp + 5-FOA for 2 days at 30°C. Colonies on the master plates and the replica plates were compared, and

Table 1. Strains used in this study

Strain	Genotype	Origin/ reference
RM430	<i>MATa ade2-101 his3-Δ201 lys2-801 trp1-Δ901 ura3-52 hht1 hhf1::LEU2 hht2hhf2::HIS3</i> plus pRM430	Mann and Grunstein (1992)
YDS2	<i>MATa ade2-1 trp1 can1-100 leu2-3,112 his3-11,15 ura3-23</i>	David Shore (Columbia University, New York, NY)
YDS3	<i>MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-23</i>	David Shore
YDS23	<i>MATa/α ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-23</i>	this study
MAY1	<i>MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-23 hsl1Δ1::URA3</i>	this study
MAY2	<i>MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-23 hsl1Δ1::URA3 swe1Δ::LEU2</i>	this study
MAY3	<i>MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-23 hsl7Δ1::URA3</i>	this study
MAY4	<i>MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-23 hsl7Δ1::URA3 swe1Δ::LEU2</i>	this study
MAY5	<i>MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-23 oss1Δ1::TRP1</i>	this study
MAY6	<i>MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-23 oss1Δ1::TRP1 swe1Δ::LEU2</i>	this study
MAY7	<i>MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-23 oss1Δ5::TRP1</i>	this study
PS692	<i>MATα ura3-52 leu2 trp1-1 CDC28::TRP1</i>	Sorger and Murray (1992)
PS694	<i>MATα ura3-52 leu2 trp1-1 cdc28-F::TRP1</i>	Sorger and Murray (1992)

those failing to grow on the glucose and 5-FOA plates were identified as candidate *hsl* mutants whose viability became dependent on the *GAL*-controlled wild-type *H3* gene present on pRM102 (plasmid A; see Fig. 1). To eliminate those mutants that became dependent on plasmid A because of inviable mutations on the *H3* or *H4* genes on pRM430 (plasmid B), all candidates were grown nonselectively on YEPG to lose plasmid B. These strains were retransformed with plasmid B or pRM200, which carries both wild-type *H3* and *H4* with their native promoters (Mann and Grunstein 1992). The resulting transformants were re-tested for 5-FOA sensitivity. Mutants that remained sensitive to 5-FOA with the newly introduced plasmid B, but became 5-FOA resistant when containing pRM200, were selected as final *hsl* mutants.

To determine for dominance of the *hsl* mutations, the collection of *hsl* mutants were crossed to the parental strain of the opposite mating type (*MATα*). The resulting diploids were tested for histone synthetic lethality on 5-FOA plates. Recessive *hsl* mutations were indicated by the 5-FOA resistance of the heterozygous diploids.

Cloning of *HSL1*, *HSL7*, and *OSS1*

Wild-type genomic libraries were constructed by partial digestion of genomic DNA with *Sau3AI* or a combination of *Bam*HI and *Bgl*II, and ligating fragments of 8–12 kb into pRS200 (Sikorski and Hieter 1989) digested with *Bam*HI and *Bgl*II. A genomic library on pRS200 purchased from American Type Culture Collection (Rockville, MD) was also used in some of the cloning experiments.

For the purpose of transformation with the *TRP1*-marked genomic libraries, pRM430 (plasmid B) in *hsl* mutants was exchanged for pMX430. pMX430 was constructed by cloning the *Eco*RI–*Sal*I fragment containing the *H3* and *H4* genes from pRM430 into the vector pRS317, a *LYS2*-marked centromeric vector (Sikorski and Hieter 1989). Mutants were grown in SG–Ura–Lys medium and transformed with genomic library DNA. Transformants were replica-plated onto SD–Lys–Trp + 5-FOA plates. Colonies from 5-FOA plates were regrown in SD–Lys–Trp medium, and total DNA extracted. Plasmids

were recovered after transformation of total yeast DNA into *Escherichia coli* (DH5α).

For the *hsl1-1* mutant, a total of four plasmids were found to rescue the *hsl* phenotype. One clone (pE14R1) fully rescued this mutant, and three other plasmids (pE147, pE148, and pE149) only allowed slow growth on 5-FOA plates. pE147 and pE149 had overlapping inserts based on restriction analyses. The overlapped region was partially sequenced and found to contain the *MIH1* gene, which was responsible for the rescue activity. Deletion analysis of pE148 was performed, and the 3.5-kb *Eco*RI–*Sal*I fragment containing full activity was sequenced completely using the Erase-a-Base system (Promega, Madison, WI) and synthetic oligonucleotides. Partial sequencing of the clone pE14R1 indicated that it contained an 8-kb fragment sequenced completely by the *S. cerevisiae* sequencing project (Dujon et al. 1994). Deletion analysis using restriction sites was carried out to determine the gene responsible for *hsl1* complementation.

To determine whether pE14R1 contained the *HSL1* gene as opposed to a copy-dependent suppressor of *hsl1-1*, the gap-repair technique (Orr-Weaver and Szostak 1983) was used to show that the corresponding chromosomal region in *hsl1-1* mutant was incapable of complementing *hsl1*. As control, similarly gap-repaired plasmids from *HSL1* wild-type strain were fully active in *hsl1* complementation. Therefore, the pE14R1 contained the *HSL1* gene. In contrast, the gene present in pE148, capable of rescuing *hsl1-1* and *hsl7-1*, was phenotypically wild type in either the *hsl1-1* or the *hsl7-1* genome. This was determined as follows: Genomic DNAs derived from *hsl1-1*, *hsl7-1*, and *HSL*⁺ strains were used to amplify the 3.5-kb *Eco*RI–*Sal*I region by PCR, and the resulting PCR products were cloned into pRS200. The primers used are 5' primer, AGATCTGAATTCTATC-GAGCGACCG, and 3' primer, AGCAGTCATTGGCAGTAT-TGTG. Multiple clones derived from each strain background were tested for rescuing activity of *hsl1-1* and *hsl7-1*, and all clones derived from both the mutants and the wild type were identical in complementation activity. Therefore, we conclude that the 3.5-kb *Eco*RI–*Sal*I region contained a dosage-dependent suppressor, which was designated as *OSS1*.

Complementation cloning of *hsl7-1* yielded two complementing plasmids. pBB311 fully complemented *hsl7-1*, whereas

p311 only allowed slow growth of the *hsl7* mutant on a 5-FOA plate. Restriction and sequence analyses indicated that p311 overlapped with pE148 in the *OSS1* region. The insert in pBB311 was sequenced from both ends and found to contain an 8-kb region completely sequenced by the yeast genome project (Feldmann et al. 1994). Regions containing YBY133c and YBR131w were cloned individually into pRS200 after PCR amplification using wild-type genomic DNA as template. The primers for cloning YBR133c were 5' primer, CTTCGAGCTCTCGTTT-TGCACTGAG (the *SacI* site is underlined), and 3' primer, GACTTGTGGTACCTCAGGACGTAT (the *KpnI* site is underlined). The primers used for PCR YBR131w were 5' primer, GTTAGTAATGAGCTCTCGCCCGAA (the *SacI* site is underlined), and 3' primer, ATTCTATGTCGACCAAATGCTA-AACG (the *SalI* site is underlined). Unique restriction enzyme sites, as indicated above, were engineered into each primer to facilitate cloning. To recover the mutant allele in the *hsl7-1* mutant, the same primers used to amplify YBR133c by PCR, as described above, were used to amplify and clone the corresponding region from the *hsl7-1* genome.

Construction of null alleles of *hsl1*, *hsl7*, and *oss1* and *swe1*

To construct plasmids for one-step gene disruption of *HSL1*, the 6-kb *KpnI*–*SacI* fragment from pE14R1 was cloned into pBlueScript SK. The resulting plasmid was digested with *NruI* and *MluI* (*hsl1Δ1*) or *NruI* and *StuI* (*hsl1Δ2*) to remove *HSL1*-coding sequences, which were replaced by the *URA3* gene isolated from YE24.

The *SacI*–*KpnI* fragment containing *HSL7* obtained by PCR using primers described in the last section was subcloned into pBlueScript SK. The internal *NarI* fragment was deleted from the insert and replaced with the *URA3* gene.

The *oss1Δ1* allele was constructed as follows: The 1.3-kb *SacI*–*SacII* fragment was subcloned into pBlueScript SK, and resulting plasmid was cut with *HindIII* to delete the *OSS1*-coding sequence, which was replaced by *TRP1*. The *oss1Δ5* allele was constructed as follows: The 3.5-kb *EcoRI*–*SalI* fragment was subcloned into pBlueScript, the resulting plasmid was cut with *Eco47III* and *StuI*, and the *OSS1*-coding sequence was replaced with the *TRF* gene.

A *swe1* disruption plasmid *swe1Δ::LEU2* was kindly provided by R. Booher (Booher et al. 1993).

Plasmids containing disruption alleles described above were cut to generate the gene disruption fragments, which were used to transform haploid and diploid cells. Correct gene replacements were confirmed by Southern blotting and PCR analysis.

Cell culture synchronization, RNA isolation, and Northern analysis

Exponentially growing cultures (OD₆₀₀ of 0.2–0.3) of appropriate strains were incubated with two doses of α -factor (5 μ g/ml) in YPD to arrest cells in G₁ for a total of 160 min. After washing the cells with water to remove α -factor, the cells were returned to growth in fresh YPD at 30°C. Portions of the culture were taken at 15-min intervals, and total RNA isolated as described (Cross and Tinkelenberg 1991). RNA was separated on a formaldehyde–agarose gel and transferred onto Nytran Plus membrane (Schleicher & Schuell). The RNA blot was hybridized with probes labeled with ³²P by the random prime labeling technique (Feinberg and Vogelstein 1984). The probes for *SWE1*, *CLB5*, and *CDC28* were obtained by amplifying the coding region of the respective genes by PCR. The *CLN2* probe was a gift from Steve Reed (Scripps Research Institute, La Jolla, CA).

FACS analysis

Approximately 1×10⁷ cells from exponentially growing cultures were sonicated to disrupt cell aggregates and fixed with 70% ethanol overnight at 4°C. The fixed cells were washed with 50 mM sodium citrate (pH 7) and resuspended in the same buffer containing RNase A at 10 μ g/ml. RNase digestion was carried out for 3 hr at 37°C. The resulting cells were stained with propidium iodide (50 μ g/ml) in 180 mM Tris at pH 7.5 190 mM NaCl, and 70 mM MgCl₂ overnight. The cells were analyzed on Beckton-Dickson FACScan. Ten thousand events were analyzed for each sample.

DNA sequencing and sequence analysis

All sequencing reactions were carried out with double-stranded plasmid DNA as template using Sequenase version 2.0 (U.S. Biochemical-Amersham) according to the manufacturer's recommendations. The Erase-a-Base system (Promega) was used to create sequential deletions for sequencing *OSS1*. Synthetic oligonucleotides were also used to obtain the complete sequence of *OSS1*.

DNA sequences were analyzed by DNA Strider and the Wisconsin GCG package (Madison, WI). Homology searches were carried out using the BLAST (Altschul et al. 1990) service at NCBI.

Acknowledgments

We especially thank Steve Reed for helpful suggestions during the course of this work and for gifts of plasmids, and Robert Booher and Peter Sorger for strains and plasmids. We also acknowledge the excellent technical assistance of Hana Kim. This work was funded by a U.S. Public Health Service grant (GM23674) from the National Institutes of Health and by grants from the Margaret E. Early Medical Research Trust and the Jonsson Cancer Foundation, which provided postdoctoral support.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**: 403–410.
- Amon, A., U. Surana, I. Muroff, and K. Nasmyth. 1992. Regulation of p34^{CDC28} tyrosine phosphorylation is not required for entry into mitosis in *S. cerevisiae*. *Nature* **355**: 368–371.
- Amon, A., M. Tyers, B. Futcher, and K. Nasmyth. 1993. Mechanisms that help the yeast cell cycle clock tick: G2 cyclins transcriptionally activate G2 cyclins and repress G1 cyclins. *Cell* **74**: 993–1007.
- Auffray, C., G. Behar, F. Bois, C. Bouchier, C. Da Silva, M.D. Devignes, S. Duprat, R. Houlgatte, M.N. Jumeau, B. Lamy, et al. 1995. IMAGE: Molecular integration of the analysis of the human genome and its expression. *C. R. Acad. Sci.* **318**: 263–272.
- Ausubel, F.M., R. Bent, R.E. Kingston, D. Moore, J.A. Smith, J.G. Seidman, and K. Struhl, ed. 1987. *Current protocols in molecular biology*. Wiley, New York, NY.
- Bassett Jr., D., M.S. Boguski, F. Spencer, R. Reeves, M. Goebel, and P. Hieter. 1995. Comparative genomics, genome cross-

- referencing and XREFdb. *Trends Genet.* **11**: 372–373.
- Boeke, J.D., J. Trueheart, G. Natsoulis, and G.R. Fink. 1987. 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. *Methods Enzymol.* **154**: 164–175.
- Boguski, M.S., C.M. Tolstoshev, and D. Bassett Jr. 1994. Gene discovery in dbEST [letter]. *Science* **265**: 1993–1994.
- Booher, R.N., R.J. Deshaies, and M.W. Kirschner. 1993. Properties of *Saccharomyces cerevisiae* wee1 and its differential regulation of p34CDC28 in response to G1 and G2 cyclins. *EMBO J.* **12**: 3417–3426.
- Coleman, T.R., Z. Tang, and W.G. Dunphy. 1993. Negative regulation of the wee1 protein kinase by direct action of the nim1/cdr1 mitotic inducer. *Cell* **72**: 919–929.
- Cross, F.R. and A.H. Tinkelenberg. 1991. A potential positive feedback loop controlling CLN1 and CLN2 gene expression at the start of the yeast cell cycle. *Cell* **65**: 875–883.
- Cross, S.L. and M.M. Smith. 1988. Comparison of the structure and cell cycle expression of mRNAs encoded by two histone H3-H4 loci in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **8**: 945–954.
- Cvrckova, F., C. De Virgilio, E. Manser, J.R. Pringle, and K. Nasmyth. 1995. Ste20-like protein kinases are required for normal localization of cell growth and for cytokinesis in budding yeast. *Genes & Dev.* **9**: 1817–1830.
- Doye, V. and E.C. Hurt. 1995. Genetic approaches to nuclear pore structure and function. *Trends Genet.* **11**: 235–241.
- Dujon, B., D. Alexandraki, B. Andre, W. Ansorge, V. Baladron, J.P. Ballesta, A. Banrevi, P.A. Bolle, M. Bolotin-Fukuhara, P. Bossier, et al. 1994. Complete DNA sequence of yeast chromosome XI. *Nature* **369**: 371–378.
- Dunphy, W.G. and A. Kumagai. 1991. The cdc25 protein contains an intrinsic phosphatase activity. *Cell* **67**: 189–196.
- Durrin, L.K., R.K. Mann, P.S. Kayne, and M. Grunstein. 1991. Yeast histone H4 N-terminal sequence is required for promoter activation in vivo. *Cell* **65**: 1023–1031.
- Enoch, T. and P. Nurse. 1990. Mutation of fission yeast cell cycle control genes abolishes dependence of mitosis on DNA replication. *Cell* **60**: 665–673.
- Featherstone, C. and P. Russell. 1991. Fission yeast p107wee1 mitotic inhibitor is a tyrosine/serine kinase. *Nature* **349**: 808–811.
- Feilolter, H., P. Nurse, and P.G. Young. 1991. Genetic and molecular analysis of cdr1/nim1 in *Schizosaccharomyces pombe*. *Genetics* **127**: 309–318.
- Feinberg, A.P. and B. Vogelstein. 1984. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity [addendum]. *Anal. Biochem.* **137**: 266–267.
- Feldmann, H., M. Aigle, G. Aljinovic, B. Andre, M.C. Baclet, C. Barthe, A. Baur, A.M. Becam, N. Biteau, E. Boles, et al. 1994. Complete DNA sequence of yeast chromosome II. *EMBO J.* **13**: 5795–5809.
- Fisher-Adams, G. and M. Grunstein. 1995. Yeast histone H4 and H3 N-termini have different effects on the chromatin structure of the GAL1 promoter. *EMBO J.* **14**: 1468–1477.
- Garcia-Ramirez, M., F. Dong, and J. Ausio. 1992. Role of the histone “tails” in the folding of oligonucleosomes depleted of histone H1. *J. Biol. Chem.* **267**: 19587–19595.
- Gietz, R.D. and R.H. Schiestl. 1991. Applications of high efficiency lithium acetate transformation of intact yeast cells using single-stranded nucleic acids as carrier. *Yeast* **7**: 253–263.
- Guacci, V., E. Hogan, and D. Koshland. 1994. Chromosome condensation and sister chromatid pairing in budding yeast. *J. Cell Biol.* **125**: 517–530.
- Gurley, L.R., J.A. D’Anna, S.S. Barham, L.L. Deaven, and R.A. Tobey. 1978. Histone phosphorylation and chromatin structure during mitosis in Chinese hamster cells. *Eur. J. Biochem.* **84**: 1–15.
- Hanks, S.K., A.M. Quinn, and T. Hunter. 1988. The protein kinase family: Conserved features and deduced phylogeny of the catalytic domains. *Science* **241**: 42–52.
- Hecht, A., T. Laroche, S. Strahl-Bolsinger, S.M. Gasser, and M. Grunstein. 1995. Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: A molecular model for the formation of heterochromatin in yeast. *Cell* **80**: 583–592.
- Hereford, L.M., M.A. Osley, T.R. Ludwig II, and C.S. McLaughlin. 1981. Cell-cycle regulation of yeast histone mRNA. *Cell* **24**: 367–375.
- Johnson, L.M., P.S. Kayne, E.S. Kahn, and M. Grunstein. 1990. Genetic evidence for an interaction between SIR3 and histone H4 in the repression of the silent mating loci in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **87**: 6286–6290.
- Kaufman, P.D. and M.R. Botchan. 1994. Assembly of nucleosomes: Do multiple assembly factors mean multiple mechanisms? *Curr. Opin. Genet. Dev.* **4**: 229–235.
- Kaufman, P.D., R. Kobayashi, N. Kessler, and B. Stillman. 1995. The p150 and p60 subunits of chromatin assembly factor I: A molecular link between newly synthesized histones and DNA replication. *Cell* **81**: 1105–1114.
- Kayne, P.S., U.J. Kim, M. Han, J.R. Mullen, F. Yoshizaki, and M. Grunstein. 1988. Extremely conserved histone H4 N terminus is dispensable for growth but essential for repressing the silent mating loci in yeast. *Cell* **55**: 27–39.
- Kellogg, D.R. and A.W. Murray. 1995. NAP1 acts with Clb1 to perform mitotic functions and to suppress polar bud growth in budding yeast. *J. Cell Biol.* **130**: 675–685.
- Kellogg, D.R., A. Kikuchi, T. Fujii-Nakata, C.W. Turck, and A.W. Murray. 1995. Members of the NAP/SET family of proteins interact specifically with B-type cyclins. *J. Cell Biol.* **130**: 661–673.
- Koch, C. and K. Nasmyth. 1994. Cell cycle regulated transcription in yeast. *Curr. Opin. Cell Biol.* **6**: 451–459.
- Lew, D.J. and S.I. Reed. 1995. A cell cycle checkpoint monitors cell morphogenesis in budding yeast. *J. Cell Biol.* **129**: 739–749.
- Ling, X., T.A. Harkness, M.C. Schultz, G. Fisher-Adams, and M. Grunstein. 1996. Yeast histone H3 and H4 amino termini are important for nucleosome assembly in vivo and in vitro: Redundant and position-independent functions in assembly but not in gene regulation. *Genes & Dev.* **9**: 686–699.
- Lundgren, K., N. Walworth, R. Booher, M. Dembski, M. Kirschner, and D. Beach. 1991. mik1 and wee1 cooperate in the inhibitory tyrosine phosphorylation of cdc2. *Cell* **64**: 1111–1122.
- Mann, R.K. and M. Grunstein. 1992. Histone H3 N-terminal mutations allow hyperactivation of the yeast GAL1 gene in vivo. *EMBO J.* **11**: 3297–3306.
- Megee, P.C., B.A. Morgan, and M.M. Smith. 1995. Histone H4 and the maintenance of genome integrity. *Genes & Dev.* **9**: 1716–1727.
- Mitchell, P.J. and R. Tjian. 1989. Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. *Science* **245**: 371–378.
- Morgan, B.A., B.A. Mittman, and M.M. Smith. 1991. The highly conserved N-terminal domains of histones H3 and H4 are required for normal cell cycle progression. *Mol. Cell. Biol.* **11**: 4111–4120.
- Morgan, D.O. 1995. Principles of CDK regulation. *Nature* **374**: 131–134.
- Nasmyth, K. 1993. Control of the yeast cell cycle by the Cdc28 protein kinase. *Curr. Opin. Cell Biol.* **5**: 166–179.

- Ogas, J., B.J. Andrews, and I. Herskowitz. 1991. Transcriptional activation of CLN1, CLN2, and a putative new G1 cyclin (HCS26) by SWI4, a positive regulator of G1-specific transcription. *Cell* **66**: 1015–1026.
- Orr-Weaver, T.L. and J.W. Szostak. 1983. Yeast recombination: The association between double-strand gap repair and crossing-over. *Proc. Natl. Acad. Sci.* **80**: 4417–4421.
- Parker, L.L., S. Atherton-Fessler, and H. Piwnica-Worms. 1992. p107wee1 is a dual-specificity kinase that phosphorylates p34cdc2 on tyrosine 15. *Proc. Natl. Acad. Sci.* **89**: 2917–2921.
- Parker, L.L., S.A. Walter, P.G. Young, and H. Piwnica-Worms. 1993. Phosphorylation and inactivation of the mitotic inhibitor Wee1 by the nim1/cdr1 kinase. *Nature* **363**: 736–738.
- Reed, S.I. 1992. The role of p34 kinases in the G1 to S-phase transition. *Annu. Rev. Cell Biol.* **8**: 529–561.
- Roth, S.Y., M. Shimizu, L. Johnson, M. Grunstein, and R.T. Simpson. 1992. Stable nucleosome positioning and complete repression by the yeast $\alpha 2$ repressor are disrupted by amino-terminal mutations in histone H4. *Genes & Dev.* **6**: 411–425.
- Russell, P. and P. Nurse. 1986. cdc25⁺ functions as an inducer in the mitotic control of fission yeast. *Cell* **45**: 145–153.
- . 1987a. The mitotic inducer nim1⁺ functions in a regulatory network of protein kinase homologs controlling the initiation of mitosis. *Cell* **49**: 569–576.
- . 1987b. Negative regulation of mitosis by wee1⁺, a gene encoding a protein kinase homolog. *Cell* **49**: 559–567.
- Russell, P. S. Moreno, and S.I. Reed. 1989. Conservation of mitotic controls in fission and budding yeasts. *Cell* **57**: 295–303.
- Sikorski, R.S. and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122**: 19–27.
- Smith, M.M. and K. Murray. 1983. Yeast H3 and H4 histone messenger RNAs are transcribed from two non-allelic gene sets. *J. Mol. Biol.* **169**: 641–661.
- Sorger, P.K. and A.W. Murray. 1992. S-phase feedback control in budding yeast independent of tyrosine phosphorylation of p34cdc28. *Nature* **355**: 365–368.
- Thompson, J.S., X. Ling, and M. Grunstein. 1994. Histone H3 amino terminus is required for telomeric and silent mating locus repression in yeast. *Nature* **369**: 245–247.
- Van Holde, K.E. 1989. *Chromatin*. Springer-Verlag, New York, NY.
- Warbrick, E. and D. Glover. 1994. A *Drosophila melanogaster* homolog of the TIS11 family of immediate early genes that can rescue a cdr1 cdc25 mutant strain of fission yeast. *Gene* **151**: 243–246.
- Watanabe, N., M. Broome, and T. Hunter. 1995. Regulation of the human WEE1Hu CDK tyrosine 15-kinase during the cell cycle. *EMBO J.* **14**: 1878–1891.
- Wilson, R., R. Ainscough, K. Anderson, C. Baynes, M. Berks, J. Bonfield, J. Burton, M. Connell, T. Copsey, J. Cooper, et al. 1994. 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans* [see comments]. *Nature* **368**: 32–38.
- Wittenberg, C., K. Sugimoto, and S.I. Reed. 1990. G1-specific cyclins of *S. cerevisiae*: Cell cycle periodicity, regulation by mating pheromone, and association with the p34CDC28 protein kinase. *Cell* **62**: 225–237.
- Wu, L. and P. Russell. 1993. Nim1 kinase promotes mitosis by inactivating Wee1 tyrosine kinase. *Nature* **363**: 738–741.
- Yanagida, M. 1995. Frontier questions about sister chromatid separation in anaphase. *BioEssays* **17**: 519–526.

A search for proteins that interact genetically with histone H3 and H4 amino termini uncovers novel regulators of the Swe1 kinase in *Saccharomyces cerevisiae*.

X J Ma, Q Lu and M Grunstein

Genes Dev. 1996, **10**:

Access the most recent version at doi:[10.1101/gad.10.11.1327](https://doi.org/10.1101/gad.10.11.1327)

References

This article cites 65 articles, 17 of which can be accessed free at:
<http://genesdev.cshlp.org/content/10/11/1327.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).