Yng2p-dependent NuA4 histone H4 acetylation activity is required for mitotic and meiotic progression

John S. Choy\textsuperscript{1,3}, Brian T.D. Tobe\textsuperscript{1,2}, Joon H. Huh\textsuperscript{1} and Stephen J. Kron\textsuperscript{1,2,3,4}

1. Center for Molecular Oncology, 2. Committee on Cancer Biology and 3. Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637

4. Corresponding author at:
   Center for Molecular Oncology
   University of Chicago
   Donnelly Biological Sciences Learning Center
   924 E. 57th Street Room R320
   Chicago, IL 60637
   Office - (773) 834-0250 Fax - (773) 702-4394
   Email - skron@midway.uchicago.edu

**Running Title:** Yng2p-dependent NuA4 activity in mitosis and meiosis
Summary

In all eukaryotes, multi-subunit histone acetyltransferase (HAT) complexes acetylate the highly conserved lysine residues in the amino terminal tails of core histones to regulate chromatin structure and gene expression. One such complex in yeast, NuA4, specifically acetylates nucleosome-associated histone H4. Recent studies have revealed that NuA4 is comprised of at least 11 subunits, including Yng2p, a yeast homolog of the candidate human tumor suppressor gene ING1. Consistent with prior data, we find that cells lacking Yng2p are deficient for NuA4 activity and are ts. Furthermore, we show that the NuA4 complex is present in the absence of Yng2p suggesting that Yng2p functions to maintain or activate NuA4 HAT activity. Sporulation of diploid yng2 mutant cells reveals a defect in meiotic progression while synchronized yng2 mutant cells display a mitotic delay. Surprisingly, genome-wide expression analysis revealed little change from wildtype. Nocodazole arrest and release relieves the mitotic defects, suggesting that Yng2p may have a critical function prior to or during metaphase. Rather then a uniform decrease in acetylated forms of histone H4, we find striking cell-to-cell heterogeneity in the loss of acetylated histone H4 in yng2 mutant cells. Treating yng2 mutants with the histone deacetylase inhibitor trichostatin A suppressed the mitotic delay and
restored global histone H4 acetylation, arguing that reduced H4 acetylation may underlie the cell cycle delay.
**Introduction**

Histone acetyltransferases (HATs) are related enzymes that mediate regulated acetylation of histones on their highly conserved amino-terminal lysines. HATs have been isolated from yeast, nematode, Drosophila, mouse and human (1,2). Recent work in budding yeast has demonstrated that multi-protein HAT containing complexes acetylate chromatin-associated histones (3). Histone acetylation has important roles in both growth and cell division. Mutations in the HAT catalytic subunits, like mutant histones lacking their amino-terminal tails, confer marked cell cycle delays in mitosis and other growth defects (4-6).

In yeast, the biochemically defined high molecular weight multi-subunit HAT complexes include ADA, SAGA, NuA3 and NuA4 (7). The former preferentially acetylate histones H3 and H2B whereas the NuA4 complex acetylates histone H4 and to a lesser extent H2A (8-10). The prototypic HAT, Gcn5, is the catalytic subunit of both ADA and SAGA, whereas Sas3 and Esa1p perform that role in NuA3 and NuA4, respectively (8-10).

NuA4 (Nucleosomal Acetyltransferase of H4) is likely responsible for most, if not all, histone H4 acetylation in chromatin during vegetative growth (9,11). This complex consists of at least 10 subunits in addition to Esa1p, virtually all of which are essential for life (4,5,11,12). Although biochemical
properties of NuA4 are well characterized, its regulation and function in cell division is less well understood. In wildtype yeast cells, most histone H4 is fully or partially acetylated, via modification of lysines at positions 5, 8, 12, and/or 16 in the histone H4 amino-terminus (13). While mutating the four histone H4 lysines to arginine confers dramatic growth defects (14), a single lysine is sufficient to restore regulated growth (15). Chromatin containing a high proportion of poly-acetylated histones is considered to be more "open" or accessible to regulatory factors while unacetylated chromatin is more compact and may be less active (16). In turn, acetylation may facilitate large-scale conformational change that is impaired by the tighter packing of unacetylated chromatin (17). Recent data that Esa1p has a relatively limited role in transcriptional regulation at specific loci but is important for large scale acetylation of chromatin (18,19) suggest that NuA4 has a primary role in global histone H4 acetylation and thereby in regulating changes in large-scale chromatin structure and function.

That recombinant Esa1p alone acetylates only free histone H4 suggests that other NuA4 subunits target this HAT to its chromatin-associated nucleosomal substrates (9). Thus, further insight into the function of NuA4 in cellular processes may be provided by genetic and biochemical analysis of the non-catalytic subunits. In this report, we further characterize the NuA4 subunit
Yng2p, previously identified as a yeast homolog of the candidate human tumor suppressor gene ING1 (12,20,21).
Experimental Procedures

Yeast Strains and Manipulations

All yeast strains used in this study derive from W303-1A (22). Yeast culture and genetic techniques were essentially as described (23). Media were obtained from US Biological, molecular biology reagents from New England BioLabs, and chemical reagents from Sigma, unless otherwise noted. Yeast were cultured in YPD (1% yeast extract, 2% peptone, 0.3 mM adenine and 2% glucose) or SC (synthetic complete media with 2% glucose) lacking the appropriate amino acids.

Plasmid and strain construction

A diploid strain heterozygous for a knockout of YNG2 (SKY2302) was constructed by PCR-based gene disruption as described (24) to replace the entire coding sequence of one allele of YNG2 in a wildtype diploid with the kanMX6 gene conferring G418 resistance. Transformants were sporulated and dissected to isolate YNG2 disruptants (yng2::kanMX6). YNG2 and EPL1 were epitope tagged at their carboxyl-terminus with 13-Myc or 3-Ha respectively, using a PCR-based strategy (24). All strain constructions were confirmed by
A unique Bgl II site was introduced immediately after the start codon of \textit{YNG2} to construct GFP (see below) fusions with \textit{YNG2}. The \textit{YNG2::Bgl II} construct inserts Arg and Ser directly after the first ATG but complements a \textit{YNG2} disruption. The \textit{YNG2::Bgl II} mutant was constructed in a centromeric \textit{LEU2} (pRS315, (25)) or integrating \textit{URA3} vector (pRS306, (25)), yielding pRS315-\textit{YNG2::Bgl II} (SKB4302) and pRS306-\textit{YNG2::Bgl II} (SKB4303). A mutant GFP, mgfp6 (26), a kind gift of J. Haseloff, was amplified and cloned via in-frame Bam HI sites into Bgl II-digested SKB4303 to construct pRS306-GFP-\textit{YNG2} (SKB4308). This GFP fusion complements a \textit{YNG2} disruption.

\textbf{Cell cycle analysis}

Cells of the relevant genotypes were grown in YPD overnight at 22°C then diluted to an O.D.600nm of ~0.05-0.1 in fresh YPD and allowed to grow for 3-4 h in the presence of 5 \textmu M alpha mating peptide (\textit{\alpha f}, Research Genetics) or 15 \textmu g/ml of nocodazole (NOC) at 22°C. Alternatively, cells were incubated at 22°C in the presence of \textit{\alpha f} or NOC for 2 h and then shifted to 37°C for 1-1.5 h. Trichostatin A (TSA) in methanol (5 mg/ml) was added directly to cultures to a final concentration of 30 \textmu g/ml. Cells were released from \textit{\alpha f} or NOC arrest by centrifugation, a YPD wash and then resuspension in liquid YPD at 22°C or YPD.
prewarmed to 37°C. Cells were collected at 20 min intervals (or as described in results) for flow cytometry, Northern, and Western analyses (see Protein techniques). For flow cytometry, cells were fixed in 70% ethanol, resuspended in 50 mM Tris-HCl (pH 7.5), sonicated, treated with 1 mg/ml of RNase A at 50°C for 1 h and stained with 0.05 mg/ml propidium iodide. Approximately 2-3 x 10^4 cells were analyzed using Cell Quest software and a FACSCalibur flow cytometer (Becton-Dickinson) for each time point. For Northern analysis, 25 ml of culture were collected and RNA was extracted as described (27), separated on a 1% formaldehyde agarose gel and transferred onto a nylon membrane (Osmonics). Probes labeled with ^32P-dATP or –dCTP (ICN) were generated with PCR or Klenow and used to detect YNG2, CLN2, CLB5, CLB1, CLB2 mRNA and a PhosphorImager detection system and ImageQuant software (Molecular Dynamics) were used for analysis of blots.

Microarray analysis

Strains were grown in YPD media to mid-log phase and then harvested. RNA was collected from cells using a Qiagen Rneasy kit (Qiagen). 24µg of total RNA was used as template for double stranded cDNA synthesis using a Life Technologies Supersript Double Strand cDNA synthesis kit and protocol described by Affymetrix (Affymetrix GeneChip Expression Analysis Technical
Manual). Briefly, total RNA is synthesized into double-stranded cDNA by means of the SuperScript Choice system (Gibco BRL Life Technologies) and a T7-(dT)24 Primer (Genset Corp.). Samples were then extracted with phenol:chloroform:isoamyl alcohol (25:24:1) and precipitated with 7.5M ammonium acetate and ethanol. An Enzo Bioarray Highyield RNA transcript labeling kit (Enzo Diagonistics, Inc.) was used according to manufacturer's instructions to transcribe biotin labeled cRNA, which was purified using an RNeasy kit (Qiagen). These products were fragmented into 50-200 nt pieces with a Trizma base solution as described by Affymetrix. 5 µg of fragmented cRNA was hybridized on an Affymetrix Test 2 Array. After washing, the array was stained with streptavidin–phycoerythrin (Molecular Probes), amplified by biotinylated anti-streptavidin (Vector Laboratories, Inc.), and then scanned on an HP Gene-array scanner. The intensity for each feature of the array was captured with Affymetrix GeneChip Software, according to standard Affymetrix procedures. All 3’ to 5’ ratios fell between normal parameters. 15 µg of fragmented cRNA was assayed on Yeast Genome S98 Arrays (Affymetrix) utilizing the same protocol and equipment. Data were analyzed on the Affymetrix MicroArray Suite data analysis package. Out of a total of 9159 probe sets representing ~7000 genes, including non-annotated ORFs from SAGE analysis, 7616 (83.2%) were detected and exceeded the probe pair threshold.
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determined by Affymetrix GeneChip Software. The 54 open reading frames that decreased includes 18 transcripts scored by Affymetrix MicroArray Suite as ‘no change’. Nonetheless, these were included for completeness. Molecular function and gene names for each open reading frame was obtained from the Saccharomyces Genome Database web page at genome-www.stanford.edu/Saccharomyces.

**Protein techniques**

For protein extracts, 25-50 ml of culture at O.D. 600 nm ~0.1 was pelleted, washed, and resuspended in buffer containing protease inhibitors (50 mM Tris-HCl (pH 7.5), 10% glycerol, 0.5% Nonidet P-40, 2 mM ethylenediamine tetraacetic acid (EDTA), 150 mM NaCl, 500 µM benzamidine HCl, 10 µg/ml aprotinin, 1 µg/ml leupeptin, 1 µg/ml pepstatin A, 1 mM phenylmethanesulphonyl fluoride). An equal volume of 0.5 mm glass beads was added, the mixture vortexed at high speed for 30 min at 4°C and centrifuged at 20,000 x g at 4°C to clear the lysate. For Western analysis, 50 µg of protein, determined by Bradford assay (BioRad), was loaded per lane on a 12% SDS-polyacrylamide gel, then transferred to Hybond C nitrocellulose (Amersham). Blots were processed as described by the manufacturer for enhanced chemiluminescence (Amersham) and exposed to Hyperfilm ECL (Amersham).
The following concentrations of antibodies were used to detect Yng2p-Myc, Epl1p-Ha, Esa1p and Clb2p: 1/1000 of A14 anti-Myc polyclonal (Santa Cruz), 1/3300 of 16B12 anti-Ha polyclonal (Covance), 1/200 of anti-Esa1p (Santa Cruz) and 1/2000 anti-Clb2p (28). For immunoprecipitation reactions, 3 µg of a monoclonal anti-Myc antibody (9E10, Covance) or anti-Ha antibody (12CA5, Roche Biochemicals) was added to 400 µl of total yeast extract (0.2-1.0 mg/ml) and incubated on a rocker at 4°C for 2-3 h or overnight. Then, 50 µl of a 50% slurry of protein A-Sepharose beads (Pharmacia), washed once with lysis buffer, was added and incubated on a mixer at 4°C for 2-3 h. Immune complexes were collected by centrifugation at 800 x g for 2 min, washed three times with 1 ml of lysis buffer then resuspended in 1X SDS-PAGE sample buffer (62.5 mM Tris (pH 6.8), 10% glycerol, 2% sodium dodecylsulfate (SDS), 16 mM DTT, 0.004% bromophenol blue) or washed once with 1 ml of HAT buffer for HAT assays (see below).

**Histone acetyltransferase assays**

HAT assays were performed essentially as described (29). Immunoprecipitates were incubated at 30°C for 45 min with 50 µg of calf thymus histones (type IIA, Sigma), 0.3 µCi of [3H]-acetyl-CoA (ICN) in HAT buffer A (50 mM Tris-Cl (pH 8.0), 10% glycerol, 10 mM sodium butyrate, 1 mM...
PMSF, 1 mM DTT, and 1.5 µM acetyl-CoA in a total volume of 50-80 µl.

Reactions were terminated by addition of 4X SDS-PAGE sample buffer and separated on a 15 or 18% SDS-PAGE gel, transferred onto PVDF (Millipore), treated for fluorography using En3Hance (NEN) as described by the manufacturer, and exposed to Hyperfilm ECL (Amersham) at -80°C for one to two weeks.

**Immunofluorescence and visualizing GFP-Yng2p**

Cultures grown to O.D.600nm ~0.2-0.4 at 22°C or 37°C were fixed by addition of 1/10 volume of formaldehyde (37% w/v) directly to the media and incubation at 22°C or 30°C for 1-2 h, then washed and resuspended in phosphate buffered saline (PBS, 50 mM sodium phosphate, 150 mM NaCl (pH 7.5)). To detect acetylated H4 isoforms, fixed cells in PBS were spheroplasted for 1 h at 30°C with 0.05 mg/ml zymolyase (Seikagaku) in 1 M sorbitol, washed three times, immobilized onto poly-lysine slides (Sigma) and blocked in 2% powdered milk in PBS containing 0.2% Tween-20 (PBS/Tween-20) overnight at 4°C. Cells were then treated with the following antibody dilutions for 2 h at 22°C: 1/100 of anti-H4K8, 1/100 of anti-H4 acetylated at lysine 5, 8, 12, 16 (anti-tH4; Upstate Biotechnology Cat. #06-598), 1/1000 of anti-H4K12, 1/1000 of anti-acH3. Slides were washed three times with PBS/Tween-20
before treating slides for 1 h at 22°C with a 1/1000 dilution of goat anti-rabbit secondary antibodies conjugated to Alexa 488 (Molecular Probes) in PBS/Tween-20. Afterwards, slides were washed three times with PBS/Tween-20. Anti-fade reagent (2.5% 1,4-Diazabicyclo[2.2.2]octane in 70% glycerol containing 0.5 μg/ml 4,6-Diamidino-2-phenylindole (DAPI)) and coverslips were applied and fluorescence visualized using a Zeiss AxioSkop microscope equipped with epifluorescence optics, High Q fluorescein and DAPI filter sets (Chroma Technologies) and a Sensys CCD camera (Photometrix) controlled with IP-Lab Spectrum software (Signal Analytics).

Cells carrying GFP-YNG2 (SKY2303) were grown to O.D.600nm ~0.2-0.4 in synthetic media at 30°C and washed with water. To examine GFP-Yng2p localization through the cell cycle, cells were subjected to an αf arrest-release regimen. Samples were collected at 30 min intervals after release and imaged using a High Q fluorescein filter set (Chroma) and the microscope system described above. Cells carrying GFP-Yng2p were grown in the presence of DAPI (2.5 μg/ml) in synthetic media for 3-4 h for colocalization studies.
Results

**Yng2p is required for full NuA4 HAT activity**

Recently, Loewith et al. (2000) reported that Yng2p is associated with both Tra1, an ATM-like subunit of NuA4, and Esa1p, the catalytic subunit of NuA4 (9). Independently, by two-hybrid screening and co-immunoprecipitation (data not shown), we found that Yng2p physically interacts with Epl1p, a stable subunit of the NuA4 HAT complex (11). Moreover, by Western analysis of total histone protein, Loewith et al. (2000) observed decreased levels of acetylated forms of histone H4 in *YNG2* deletion mutants. These results suggest that Yng2p is a *bona fide* NuA4 subunit and is important for H4 acetylation *in vivo*. However, Yng2p may be important for NuA4 HAT catalytic activity, may act to target NuA4 to its chromosomal histone H4 substrate or serve some other role. Given that *YNG2* is not essential for vegetative growth at 22°C (12), we sought to assay the NuA4 HAT activity in *YNG2* deficient cells. Because Epl1p is a stable subunit of NuA4 (11) and the expression of Epl1p-Ha in *yng2* mutants was comparable to that in wildtype cells (Fig. 1A), immunoprecipitation of Epl1p-Ha provided a means to assay NuA4 HAT activity *in vitro*. Epl1p-Ha immune complexes from wildtype cells possessed ~ 5 fold greater H4 HAT
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Esa1p in wildtype cells versus \textit{yng2} mutant cells. Strikingly, we found that Esa1p coimmunoprecipitated with Epl1p-Ha to a similar degree in both wildtype and \textit{yng2} mutant cells (Fig. 1C). These data suggest that Yng2p is not necessary for NuA4 complex formation but required for wildtype levels of NuA4 H4 HAT activity.

\textbf{\textit{yng2} mutant cells exhibit slow mitotic progression}

The physiological processes which Yng2p may mediate remain unclear. Previous studies by McGee \textit{et al.} (1995) demonstrate that yeast cells harboring a non-acetylatable mutant histone H4 allele delay in G2 and mitosis. Moreover, Clarke \textit{et al.} (1999) showed that cells carrying a temperature-sensitive allele of \textit{ESA1} display a G2/M arrest. To examine whether a similar delay might underlie the slow growth at 22°C and temperature sensitivity at 37°C of \textit{yng2} mutant cells (12), we performed flow cytometry on \textit{YNG2} wildtype and mutant cells synchronized by αf-induced G1 arrest. To limit growth to a single cell cycle, αf was added back after cells entered S phase, trapping cells in the subsequent G1. Virtually all wildtype cells released at 22°C completed DNA synthesis by 60
minutes, progressed through mitosis and reentered G1 as indicated by complete return to a 1N DNA peak within 140 minutes (Fig. 2A left). Under the same conditions, yng2 mutants completed S phase with near-wildtype kinetics. However, cells with 2N DNA content persisted and only began to re-enter G1 at 160 minutes (compare Fig. 2B left to Fig. 2A left). Furthermore, 45% of yng2 mutant cells did not exit from the 2N DNA content fraction for the duration of the experiment (Fig. 2B left). At 37°C, wildtype cells completed DNA replication by 40 min, promptly entered mitosis and began to return to G1 by 80 min (Fig. 2A right). yng2 mutants exhibited a brief delay at the onset of S phase but then completed DNA replication by 60 min (Fig. 2B right). However, a long mitotic arrest ensued. While some yng2 mutant cells were able to reenter G1 by 120 min, approximately 48% of yng2 mutant cells persisted with a 2N DNA content for the duration of the experiment (compare Fig. 2B right to 3A right). These data suggest that the growth defect observed in yng2 mutant cells is largely due to slow mitotic progression.

**Loss of Yng2p-dependent NuA4 HAT activity inhibits meiosis**

Previous studies by Park and Szostak (1990) (30) showed that sporulation efficiency is dramatically reduced in N-terminal histone H4 mutants, suggesting that yng2 mutants might also be defective for meiosis. To address
this possibility, we determined sporulation efficiency for diploid wildtype or \textit{yng2} homozygous mutant cells. After a three day incubation on sporulation media, only \textasciitilde1.5\% of \textit{yng2} mutant cells formed four-spore asci compared to \textasciitilde30\% in wildtype cells (Fig. 2C). Staining the \textit{yng2} mutant cells with DAPI revealed that, except for the few cells that successfully formed four spores, \textit{yng2} mutant cells arrested unbudded and with a single nuclear mass (Fig. 2C), suggesting that Yng2p-dependent NuA4 activity is critical for early meiotic progression.

\textbf{Transcription is largely unchanged in \textit{yng2} mutants}

Histone acetylation has been shown to be important for transcriptional activation (31,32). In general, increased histone acetylation is associated with highly expressed genes while deacetylation is coupled with transcriptional repression (33). We hypothesized that hypoacetylation, leading to decreased expression of mitotic cyclins, might underlie the cell cycle delay in \textit{yng2} mutants. We performed Northern analyses using wildtype and \textit{yng2} mutants synchronously released from $\alpha$f mediated G1 arrest at 22°C. The G1 cyclin \textit{CLN2} appeared with similar kinetics to wildtype but persisted longer, the S phase cyclin \textit{CLB5} is expressed at nearly normal levels and with similar kinetics to wildtype cells, and the expression of \textit{CLB2} in \textit{yng2} mutants began later and peaked at a lower level but persisted longer than in wildtype cells (Fig. 3A).
addition, transcription of *CLB1* was not detectable in *yng2* mutants but occurred with similar kinetics and abundance as *CLB2* in wildtype cells. These changes in timing and abundance correspond to the kinetics of S phase entry and mitotic progression revealed by flow cytometry (Fig. 3B).

Towards a comprehensive and quantitative examination of transcription in *yng2* mutants, we used Affymetrix DNA oligonucleotide microarray analysis to compare genome-wide expression levels between asynchronously growing wildtype and *yng2* mutant cells at 22°C. 83% of all probe sets were detected in this experiment. Surprisingly, only 54 annotated open reading frames (ORFs) (Table 1), 9 non-annotated ORFs from SAGE analysis, the rRNAs (5S, 25S, 35S), and one snRNA (*SNR31*) were decreased 2 fold or greater. Nearly 87% of transcripts were unchanged (<2 fold difference) relative to wildtype. Suspected targets of Esa1p identified by chromatin immunoprecipitation experiments (18) all fell in the unchanged class. Of the genes whose expression significantly decreased in a *yng2* mutant, the only known mitotic regulator is *CLB1*, confirming the Northern analysis. Expression of the other cyclins and known cell cycle regulators was < 2 fold different between wildtype and *yng2* mutants. A number of genes important in biosynthetic pathways also fell within the 54 down-regulated ORFs (Table 1). However, we observe similar growth defects in *yng2* mutants grown on rich or synthetic media suggesting that down
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regulation of these genes is unlikely to contribute to the \( yng2 \) mutant phenotypes (data not shown).

The mitosis promoting cyclin, Clb2p, persists during the mitotic delay in \( yng2 \) mutant cells

Previous studies have shown that \( CLB2 \) is the major mitosis promoting cyclin (34). In the absence of \( CLB2 \), cells perform G1 and S phase functions on schedule but then delay at mitosis (34,35). To test whether the lower peak \( CLB2 \) mRNA expression in \( yng2 \) mutants led to a corresponding under-expression of Clb2p protein, we performed Western analysis on cells collected at 15 minute intervals after release from an \( \alpha f \)-induced G1 block at 37°C. To limit growth to a single cell cycle, \( \alpha f \) was added after S phase onset, trapping cells in the subsequent G1. From G1 and through onset of replication as indicated by a rightward shift in the flow profile, Clb2p is absent from both \( yng2 \) and wildtype cells (Fig. 4A and B). By 30 minutes after release, wildtype cells replicated their genome as indicated by a 2N DNA peak in the flow profile and Clb2p began to accumulate. By 45 minutes, wildtype cells displayed maximal levels of Clb2p protein and entered mitosis, by 60 minutes Clb2p protein was destroyed (Fig. 4A). Similarly, \( yng2 \) mutant cells expressed Clb2p protein upon completion of S phase at 60 minutes and displayed a peak in
Clb2p 15 minutes later. However, unlike wildtype, Clb2p protein in \textit{yng2} mutant cells was not rapidly destroyed but remained high through 105 minutes (Fig. 4B). Most \textit{yng2} mutant cells persisted with a 2N DNA content during this interval. Importantly, both 2N cells and Clb2p protein remained through 170 minutes (Fig. 4B).

\textbf{Yng2p function is required prior to mitosis}

The temperature sensitive mitotic delay in \textit{yng2} mutants suggests at least two possibilities for the time at which Yng2p performs its function(s). Yng2p activity may be necessary during anaphase for normal mitotic progression. Alternatively, advancement through mitosis may depend on pre-mitotic events mediated by Yng2p. To distinguish between these two possibilities, we analyzed the mitotic delay by applying an execution point test used by Hartwell and colleagues to characterize \textit{cdc} mutants (36). When synchronously growing CDC mutant cells are shifted to non-permissive temperature before reaching their execution point, these cells arrest homogeneously with a characteristic terminal morphology within the first cell cycle. However, if mutant cells pass the execution point prior to the temperature shift, cells will complete the first cell cycle and then arrest in the second cell cycle.
Towards determining if the temperature sensitive mitotic delay reflected a requirement for Yng2p during or prior to mitosis, we performed nocodazole arrest and release experiments at 37°C. Wildtype and \textit{yng2} mutant cells were arrested with nocodazole at 22°C and then shifted to 37°C before release from nocodazole into \textit{\alpha f} to trap cells in the subsequent G1. We found that when \textit{yng2} mutants are released from a nocodazole block at 37°C, they progress through mitosis and re-enter G1 with nearly identical kinetics to wildtype cells (Fig. 5A and B). Importantly, Clb2p protein accumulated to similar levels in both wildtype and \textit{yng2} mutant cells during the nocodazole arrest and then was destroyed with similar kinetics after release (Fig. 5C). These results suggest that Yng2p performs its critical function prior to anaphase and that delaying mitotic progression can relieve the defect.

\textbf{Yng2p is constitutively expressed and remains nuclear localized throughout the cell cycle}

Suppression of the mitotic delay in \textit{yng2} mutants after nocodazole arrest-release suggested a requirement for Yng2p-dependent NuA4 activity during or prior to mitosis. Toward examining whether expression or activity of Yng2p might be cell-cycle regulated, we examined \textit{YNG2} mRNA and protein abundance. In each of the following analyses, \textit{\alpha f} was added back at 40 minutes after cells
entered S phase, trapping cells in the subsequent G1 by 120 minutes. Northern analysis of \textit{YNG2} expression in \textit{\alpha f} synchronized cultures revealed uniform levels of \textit{YNG2} mRNA throughout the cell cycle (data not shown), confirming the results of microarray analysis (37). Western analysis performed with extracts collected from \textit{\alpha f} synchronized cells expressing a single integrated copy of \textit{YNG2}-13Myc revealed that Yng2p-Myc protein was present at relatively constant levels throughout the cell cycle (Fig. 6A). Alternatively, Yng2p-associated NuA4 HAT activity might be upregulated during or before mitosis. \textit{In vitro} NuA4 HAT activity reactions performed with Yng2p-Myc immune complexes isolated from cells synchronized in an identical manner revealed relatively similar levels of activity throughout the cell cycle (Fig. 6B).

Although Loewith \textit{et al.} (2000) previously observed that GFP-Yng2p was nuclear localized in asynchronously growing cells, it remained possible that Yng2p might shuttle between the nucleus and cytoplasm during a specific cell cycle stage. Towards testing this possibility we followed cells expressing GFP-Yng2p that were arrested with \textit{\alpha f} and then allowed to synchronously enter the cell cycle to more precisely determine if any changes occurred in GFP-Yng2p localization. Fluorescent microscopic analysis of these cells revealed that GFP-Yng2p protein remained exclusively in the nucleus throughout the cell cycle (Fig. 6C). We found that GFP-Yng2p colocalized with DAPI staining in
asynchronously growing cells demonstrating that GFP-Yng2p is nuclear localized (Fig. 6D).

**Immunofluorescence reveals cell-autonomous differences in acetylated histone H4 isoforms in yng2 mutants**

The measured decrease in total acetylated histone H4 in yng2 mutants (12) may reflect a proportional loss of acetylated histone H4 in every cell or a more dramatic loss in only a sub-population of cells. To distinguish between these two possibilities we performed indirect immunofluorescence on asynchronously growing wildtype and yng2 mutant cells with polyclonal rabbit antibodies specific to N-terminally acetylated isoforms of histone H4. In wildtype cells immunostaining for histone H4 tetra-acetylated at lysine 5, 8, 12, and 16 (tH4), or acetylated at lysine 8 (H4K8), or acetylated at lysine 12 (H4K12) was observed in each nucleus at all points in the cell cycle at 22°C (Fig. 7A-F) or 37°C (Fig. 7M-R). However, qualitatively stronger immunostaining was observed for each acetylated histone H4 isoform in G1 or S phase cells (i.e. unbudded, small and medium budded cells) compared to G2 or mitotic cells (i.e. large budded cells). When an identical set of experiments was performed with yng2 mutants, we observed dramatic differences in immunostaining for each acetylated histone H4 isoform in different cells in the population with no strict
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correlation to cell cycle position. At 22°C, ~3% of yng2 mutant cells displayed a
complete absence of H4K12 immunostaining while others displayed qualitatively
reduced or wildtype levels of immunostaining (Fig. 7H and K). When yng2
mutants were grown at 37°C, ~28% of cells displayed an absence of H4K12
immunostaining and the remainder of the cells exhibited a relative reduction in
H4K12 signal compared to wildtype cells (Fig. 7I and W). Similarly,
immunostaining for tH4 in yng2 mutant cells grown at 22°C (Fig. 7I and L) and
37°C (Fig. 7U and X) was significantly compromised. Approximately 40% of
yng2 mutants at 37°C displayed no tH4 immunostaining while the remaining
cells contained tH4 immunostaining that was reduced relative to wildtype cells
(compare Fig. 7O and R to 7U and X). Surprisingly, the distribution of H4K8
immunostaining in yng2 mutants was less heterogeneous than that of H4K12 or
tH4. Virtually all yng2 mutants grown at 22°C (Fig. 7G and J) or 37°C (Fig. 7S
and V) possessed H4K8 immunostaining, although qualitatively reduced relative
to wildtype cells.

When identical antibody immunostaining experiments were performed
with cells carrying only non-acetylatable mutants of histone H4 (6), no
immunostaining was observed, demonstrating that the apparent differences
between yng2 mutant and wildtype cells are specific (data not shown). We also
performed immunofluorescence to detect acetylated forms of histone H3 in
wildtype and \textit{yng2} mutant cells, since defects in NuA4 should not affect H3 acetylation. Indeed, identical staining patterns for acetylated H3 were observed in both wildtype and \textit{yng2} mutants (data not shown). These findings demonstrate that in the absence of Yng2p, loss of specific isoforms of acetylated histone H4 is highly cell autonomous.

\textbf{The deacetylase inhibitor trichostatin A suppresses the H4 acetylation defect and mitotic delay of \textit{yng2} mutants}

Abundance of acetylated isoforms of histone H4 \textit{in vivo} is determined by competing acetylation and deacetylation activities (38). Considering that \textit{yng2} mutants have reduced NuA4 HAT activity, we tested if inhibiting the antagonistic deacetylase activity might restore levels of acetylated isoforms of H4. Towards this end, we examined the levels of tH4 immunoreactivity in \textit{yng2} mutant cells grown in the presence of the deacetylase inhibitor trichostatin A (TSA) (39). Whereas \textit{yng2} mutant cells grown at 37°C displayed a marked decrease in tH4 immunostaining (Fig. 7U and X), TSA treatment significantly suppressed this defect (Fig. 8A, right). While 40\% of \textit{yng2} mutant cells incubated at 37°C displayed an absence of tH4, TSA treatment decreased this proportion to 4\%. To determine if inhibiting deacetylation might also relieve the mitotic delay, we performed flow cytometry on synchronized wildtype and \textit{yng2}
mutant cells collected after αf-induced G1 block and release in the presence of TSA. Wildtype cells treated with TSA progressed through all stages of the cell cycle with kinetics similar to untreated cells (compare Fig. 2A to 8B). Remarkably, TSA-treated yng2 mutant cells no longer delayed in mitosis but reentered G1 by 160 min at 22°C and 120 min at 37°C, yielding kinetics nearly identical to those of wildtype cells (compare Fig. 2B to Fig. 8B).
Discussion

The NuA4 HAT complex is comprised of at least 11 subunits (9), yet functional studies have been challenging as most subunits are individually essential for viability. We took advantage of the conditional viability of mutants lacking YNG2 to examine the role of NuA4 mediated histone H4 acetylation in cell cycle progression. We find that full NuA4 activity depends on Yng2p and is required for both mitotic and meiotic progression. In addition we discovered cell autonomous differences in histone H4 acetylation in yng2 mutant cells rather then a uniform decrease in acetylated histone H4.

We found that Yng2p physically interacts with the NuA4 subunit Epl1p, confirming work by Loewith et al. (2000) that showed Yng2p physically associates with Tra1, another NuA4 subunit. Yng2p deficient cells display decreased total acetylated histone H4 in vivo (12) and in this report we find significantly reduced NuA4 HAT activity in vitro. Furthermore, we show Epl1p-Ha and Esa1p coimmunoprecipitated in the presence or absence of Yng2p suggesting that Yng2p is a component of the NuA4 complex that is necessary for normal NuA4 activity but not for complex formation. Analysis of cells synchronously released from an αf-mediated G1 arrest revealed a mitotic delay associated with persistent Clb2p protein levels in yng2 mutants, indicating that
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full NuA4 activity is required for the onset of metaphase and/or anaphase. Towards determining the time at which Yng2p functions during the cell cycle, we applied the execution point paradigm used to characterize cdc mutants (36) to analyze the origin of the mitotic delay. Importantly, we show that a nocodazole-mediated metaphase arrest relieves both the mitotic delay and the accumulation of Clb2p protein observed in yng2 mutants when released from G1. This result suggests that the time at which Yng2p normally serves its function(s) most critical for cell cycle progression is likely at or before metaphase. We infer that the cell cycle defects may be a direct result of inadequate histone H4 acetylation rather than a NuA4-independent function of Yng2p. Perhaps NuA4-mediated H4 acetylation of histone H4 reassembled onto nascent DNA during S phase is a critical event that allows mitosis to occur in a timely manner.

The loss of both acetylated histone H4 and NuA4 HAT activity in extracts from yng2 mutant cells would predict a proportional loss of acetylated histone H4 in every yng2 mutant cell. Unexpectedly, we discovered a heterogeneous distribution of acetylated histone H4 in yng2 mutant cells. Apparently normal immunoreactivity for acetylated histone H4 was present in some cells while it was completely absent from others. Although in asynchronously growing cells we did not observe a strict correlation between cell cycle stage or
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morphologically aberrant cells and a complete lack of histone H4 acetylation, following synchronized yng2 mutant cells released at 37°C we observed that the anaphase/telophase cells at late time points are particularly deficient for immunoreactivity of tetra-acetylated histone H4 (Choy JS and Kron SJ, unpublished observation). One inference is that the heterogeneous mitotic delay and morphology of yng2 mutants may be determined on a cell-by-cell basis by the degree of histone H4 acetylation. Indeed, treatment of yng2 mutant cells with the deacetylase inhibitor TSA restores histone H4 acetylation to all cells and returns the population to nearly normal mitotic progression. Future studies to understand the basis for the cell autonomous differences in H4 acetylation may provide new insights into roles and regulation of NuA4 through the cell cycle.

We envision at least three potential roles for Yng2p-dependent NuA4 histone H4 acetylation in mitotic and meiotic progression. First, Yng2p might participate in NuA4-dependent histone acetylation to promote transcription of specific genes. Second, Yng2p-dependent NuA4 HAT activity might facilitate genome-wide acetylation that ensures proper chromatin structure during mitotic and meiotic progression. Last, Yng2p-dependent NuA4 HAT activity may mediate acetylation of non-histone substrates that regulate cell cycle progression or checkpoints.
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Numerous studies have established that histone acetylation/deacetylation modulates transcription (32,40). Indeed, NuA4 HAT activity stimulates transcription on nucleosomal array templates \textit{in vitro} (41,42). However, to date, Esa1p has been implicated in regulation of only a surprisingly small group of genes (11,18). The growth defect and other phenotypes of \textit{yng2} mutants may well derive from repression of critical genes. Suggesting that the mitotic delay might originate from a transcriptional defect, expression of the mitotic cyclin \textit{CLB1} was undetectable by Northern analysis. However, cells deficient for \textit{CLB1} demonstrate no vegetative growth phenotypes (34). We also observed a delay in onset of transcription and lowered peak expression level of the mitotic cyclin \textit{CLB2}. Despite the altered \textit{CLB2} transcription pattern, Western analysis demonstrated that Clb2p appeared on schedule after completion of S phase and accumulated to normal abundance in \textit{yng2} mutant cells. Potentially, other cell cycle regulators under similar transcriptional regulation to \textit{CLB2} (e.g. members of the Clb2 cluster (37)) are also NuA4 targets and their delayed expression may underlie the cell cycle defects. Nonetheless, Yng2p deficiency confers surprisingly little effect on transcription. Microarray analysis revealed that expression of most genes, including nearly all members of the Clb2 and Sic1 clusters, differs by less than two-fold between wildtype and \textit{yng2} mutant cells. Of the 54 genes repressed by two fold or more in the \textit{yng2} mutant, \textit{CLB1} was
the only characterized cell cycle regulator.

The meiotic arrest in yng2 mutant diploids may arise from a specific defect in Clb1p expression, a gene essential for proper meiotic progression (43,44). Yet, no obvious transcriptional defect explains the observed cell cycle delays in vegetative growth. Perhaps, the genome-wide loss in H4 acetylation per se contributes to the mitotic delay in yng2 mutants. Indeed, histone H4 acetylation in large regions of chromatin is greatly reduced in esa1 mutants, suggesting that NuA4 activity is normally responsible for acetylation over large chromatin domains (45). Our studies provide further evidence for a NuA4 role in genome-wide acetylation. Total acetylated histone H4 is reduced in yng2 mutant cell extracts (12) and immunofluorescence reveals complete loss of tetra-acetylated histone H4 in many yng2 mutant cells. Furthermore, GFP-Yng2p is localized throughout the nucleus rather than to specific loci.

Regulation of chromatin structure by histone acetylation is likely a critical process during mitosis and meiosis as each require dramatic structural rearrangements such as chromosome pairing, condensation, and decondensation. Future studies of the role of acetylation in chromosome structure and function during mitosis and meiosis may shed light on the defects seen in yng2 mutants.

Finally, although we have no evidence for non-histone substrates of
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NuA4, this remains more than a formal possibility. Studies from metazoans suggest that the function of key cell cycle regulators such as p53 is modulated by acetylation (46). As the human homolog of Yng2p, ING1, associates with p53 in vivo (47), one possibility is that ING1 is a subunit of a multi-subunit HAT complex in humans that also participates in regulation of or by p53.

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Figure Legends

**Figure 1.** Yng2p is required for full NuA4 HAT activity (A) Western analysis demonstrates that Epl1p-Ha protein abundance is similar in wildtype (YNG2) and yng2 mutant cells. (B) Immunoprecipitated Epl1p-Ha complexes from wildtype cells contain HAT activity directed towards H4 (lanes 1 and 2) while a dramatic loss in HAT activity is observed in yng2 mutant cells (lanes 3 and 4). (5X) indicates five fold more extract for immunoprecipitation reactions than in (1X) lanes. (C) Epl1p-Ha immunoprecipitated from wildtype and yng2 mutant cells is associated with the NuA4 HAT Esa1p (lanes 9-12). Control immunoprecipitation reactions (lanes 5-8) were performed with Protein A beads alone. Western analysis of whole cell extracts (WCE) demonstrates that Esa1p is expressed at similar levels in wildtype and yng2 mutant cells (lanes 1-4). Extracts were prepared from four independently derived strains expressing Epl1p-Ha in a wildtype (lanes 3,7,10 and 4,8,12) or yng2 mutant (lanes 1,5,9 and 2,6,11) background.

**Figure 2.** Yng2p deficient cells display a mitotic delay and fail to sporulate. (A and B) Wildtype and yng2 mutant cells were collected every 20 min after αf-
induced G1 arrest. αf was added back after cells entered S phase in order to arrest cells in the subsequent G1. Flow cytometry reveals that wildtype cells complete one cell cycle by 140 min at 22°C and 100 min at 37°C. In contrast, yng2 mutant cells delay with a persistent 2N DNA peak (arrows) at 22°C and this was exacerbated at 37°C. (C) Diploid wildtype or yng2 homozygous mutant cells were sporulated on solid media for 3 days. Only ~1.5% of yng2 mutant cells formed four spore asci compared to ~30% in wildtype cells. Cells stained with DAPI revealed only a single nucleus in yng2 mutant cells that failed to sporulate, suggestive of an early meiotic defect.

**Figure 3.** Transcription of the mitotic cyclins is decreased while G1, and S phase cyclins is normal in yng2 mutant cells. (A) Northern analysis was performed on RNA isolated from wildtype (YNG2) and yng2 mutant cells collected after αf-induced G1 block and release at 22°C. (B) Cells used in (A) were also analyzed by flow cytometry to determine cell cycle position. αf was added back after cells entered S phase (~60 min) to trap cells in the subsequent G1 (~120 min).

**Figure 4.** Clb2p protein is expressed on schedule and accumulates to wildtype levels in yng2 mutant cells. (A) and (B) Using a polyclonal anti-Clb2p antibody,
Clb2p protein was analyzed from wildtype and \textit{yng2} mutant cells treated identically as in Fig. 2. Expression of Clb2p in \textit{yng2} mutants occurs on schedule and to similar levels as in wildtype cells. However, unlike wildtype cells, Clb2p protein levels persist in \textit{yng2} mutant cells during the latter half of the time course and begins to decline only when cells re-enter G1. Below each Western are flow profiles which show cell cycle position of the cells at each time point. Dots below each flow profile denote DNA content- single dot is 1N and double dots are 2N. \textit{a}f was added back after cells entered S phase (~60 min) to trap cells in the subsequent G1.

\textbf{Figure 5.} A nocodazole mediated delay relieves the M/G1 delay in \textit{yng2} mutants suggesting that Yng2p functions prior to or during metaphase. (A) and (B) Cells were first arrested in nocodazole (15 µg/ml) at 22°C and released into YPD containing \textit{a}f (5 µM) at 22°C or 37°C. Both wildtype cells and \textit{yng2} mutant cells return to G1 by 120 minutes at 22°C or 60 minutes at 37°C. (C) Clb2p protein is rapidly destroyed in wildtype and \textit{yng2} mutant cells released from nocodazole arrest at 37°C.

\textbf{Figure 6.} Yng2p protein expression, associated NuA4 HAT activity, and nuclear localization are largely unchanged throughout the cell cycle. (A) Wildtype cells
(Yng2p-Myc) were collected after αf-induced G1 block and release. αf was added back after cells entered S phase (~40 min) to trap cells in the subsequent G1 (~120 min). Western analysis demonstrates that Yng2p-Myc protein abundance remains relatively similar throughout the cell cycle. (B) Yng2p-myc associated HAT activity assayed from cells treated as in (A) remains relatively unchanged throughout the cell cycle. (C) GFP-Yng2p localization was followed in synchronously (αf arrest/release) growing cells. In each case, GFP-Yng2p remained exclusively nuclear localized. (D) DAPI staining of asynchronously growing cells expressing GFP-Yng2p demonstrates that GFP-Yng2p is nuclear localized.

**Figure 7.** Cell autonomous levels of acetylated forms of histone H4 are observed in yng2 mutant cells. (A-X) Indirect immunofluorescence performed on wildtype or yng2 mutant cells grown at 22°C or 37°C using polyclonal antibodies specific for tetra-acetylated histone H4 at lysines 5, 8, 12, 16 (tH4), specific for acetylated histone H4 at lysine 8 (H4K8), and specific for acetylated histone H4 at lysine 12 (H4K12). (DIC) indicates visualization by Nomarski optics and (IF) indicates immunofluorescence. Immunostaining for all three isoforms of histone H4 was present in wildtype cells at all stages of the cell cycle when grown at 22°C (A-F) or 37°C (M-R). In contrast, cell to cell
differences in immunostaining for each H4 isoform in yng2 mutants was observed. A complete absence or severe reduction of H4K12 immunostaining was observed in a small population of yng2 mutants at 22°C (arrows) (H and K) and this increased at 37°C (arrows) (T and W). Qualitatively reduced or even wildtype levels of H4K12 immunostaining was observed in the remaining cells. H4K8 immunostaining in yng2 mutants was less heterogeneous than that of H4K12 in that virtually all cells contained H4K8 at 22°C (G and J) and 37°C (S and V), although qualitatively reduced relative to wildtype cells. A reduction in immunostaining of tH4 was also observed in yng2 mutants at both 22°C and 37°C, with a large population of cells displaying an absence of tH4 immunostaining (arrows). Exposure times for photographs of immunofluorescence were identical for wildtype and yng2 mutant cells and have not been manipulated.

**Figure 8.** Trichostatin A suppresses both the acetylation defect and mitotic delay in yng2 mutant cells. (A) Immunofluorescence performed on yng2 mutant cells grown at 37°C in the presence of the deacetylase inhibitor, TSA, displayed immunostaining for tH4 comparable to wildtype cells. (B) Flow cytometry performed on wildtype and yng2 mutant cells grown in TSA at 22°C or 37°C markedly suppresses the observed mitotic delay in yng2 mutant cells (arrows)
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(compare to Fig. 2B).
Table 1. Open reading frames down-regulated in yng2 mutants.
54 open reading frames identified by microarray analysis were down-regulated by two fold or more in yng2 mutants compared to wiltype cells.

| Fold Change | SGD Name   | Gene Name | Function                                      |
|-------------|------------|-----------|-----------------------------------------------|
| -10.9       | YJR025C    | BNA1      | Biosynthesis of nicotinic acid                 |
| -5.6        | YGR108W    | CLB1      | Mitotic cyclin                                |
| -4.9        | YCR013C    | Hypothetical ORF | Unknown                        |
| -4.5        | YPL112C    | Hypothetical ORF | Unknown                        |
| -3.9        | YAR061W    | Hypothetical ORF | Unknown                        |
| -3.6        | YMR316C    | Hypothetical ORF | Unknown                        |
| -3.5        | YNL204C    | SPS18     | Sporulation-specific protein                  |
| -2.9        | YOR146W    | Hypothetical ORF | Unknown                        |
| -2.8        | YGL177W    | Hypothetical ORF | Unknown                        |
| -2.7        | YOR315W    | Hypothetical ORF | Unknown                        |
| -2.7        | YBR197C    | Hypothetical ORF | Unknown                        |
| -2.7        | YKL083W    | Hypothetical ORF | Unknown                        |
| -2.6        | YOL140W    | ARG8      | Ornithine biosynthesis                        |
| -2.6        | YMR042W    | ARG80     | Arginine transcription factor                 |
| -2.6        | YJR147W    | HMS2      | Heat shock transcription factor homolog       |
| -2.5        | YPL197C    | Hypothetical ORF | Unknown                        |
| -2.5        | YOR302W    | Hypothetical ORF | Unknown                        |
| -2.4        | YLL044W    | Hypothetical ORF | Unknown                        |
| -2.3        | YGR143W    | SKN1      | (1→6)-beta-glucan biosynthesis               |
| -2.3        | YAL005C    | SSA1      | Heat shock protein of HSP70 family           |
| -2.3        | YLL038C    | ENT4      | Cytoskeletal adaptor                         |
| -2.3        | YPR142C    | Hypothetical ORF | Unknown                        |
| -2.3        | YMR161W    | HLJ1      | Homologous to dnaJ protein                   |
| -2.3        | YPR116W    | Hypothetical ORF | Unknown                        |
| -2.3        | YOL141W    | PPM2      | Carboxy methyl transferase                   |
| -2.2        | YLR022C    | Hypothetical ORF | Unknown                        |
| -2.2        | YOL058W    | ARG1      | Arginine biosynthesis                        |
| -2.2        | YJL088W    | ARG3      | Arginine biosynthesis                        |
| -2.2        | YER069W    | ARG5,6    | Ornithine biosynthesis                       |
| -2.2        | YEL021W    | URA3      | Orotidine-5'-phosphate decarboxylase         |
| -2.2        | YKL153W    | Hypothetical ORF | Unknown                        |
| -2.2        | YNL171C    | Hypothetical ORF | Unknown                        |
| -2.2        | YNR025C    | Hypothetical ORF | Unknown                        |
| -2.1        | YLL168W    | SDL1      | L-serine dehydratase                         |
| -2.1        | YML019W    | OST6      | Subunit of N-oligosaccharyltransferase complex|
| -2.1        | YGR078C    | PAC10     | Non-native actin binding complex polypeptide 3|
| -2.1        | YDR413C    | Hypothetical ORF | Unknown                        |
| -2.1        | YLR339C    | Hypothetical ORF | Unknown                        |
| -2.1        | YDR187C    | Hypothetical ORF | Unknown                        |
| -2          | YNR003C    | RPC34     | Subunit of RNA polymerase III                |
| -2          | YML113W    | DAT1      | Oligo(dA).oligo(dT)-binding protein          |
| -2          | YOL028C    | YAP7      | (bZIP) transcription factor                  |
| -2          | YNL132W    | KRE33     | Killer toxin resistant                       |
| -2          | YNR060W    | FRE4      | Similar to FRE2                              |
| -2          | YDL038C    | Hypothetical ORF | Unknown                        |
| -2          | YJR012C    | Hypothetical ORF | Unknown                        |
| -2          | YLR393W    | ATP10     | Mitochondrial ATPase complex assembly        |
| -2          | YBL062W    | Hypothetical ORF | Unknown                        |
| -2          | YPR087W    | Hypothetical ORF | Unknown                        |
| -2          | YPR136C    | FYV15     | K1 killer toxin hypersensitivity             |
| -2          | YPR044C    | Hypothetical ORF | Unknown                        |
Figure 1

A.  

B. IP: α-HA  

YNG2  

EPL1-HA  

Epl1-HA  

H2A  

H4  

C.  

| Yng2: | - | - | + | + | - | - | + | + | - | + | - | +  
|-------|---|---|---|---|---|---|---|---|---|---|---|---  
| Epl1-HA | + | + | + | + | + | + | + | + | + | + | + | +  

| Blot: α-Esa1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-------------|---|---|---|---|---|---|---|---|---|---|---|---|
Figure 2

A. 

B. 

C. DIC DAPI

\[ \frac{YNG2}{YNG2} \] 

\[ \frac{yng2}{yng2} \]
Figure 3

A. YNG2 @ 22°C

B. YNG2 @ 22°C

yng2 @ 22°C
Figure 4

A.  

YNG2 @ 37°C

| 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 130 |
|---|---|---|---|---|---|---|-----|-----|
| Clb2p |

B.  

yng2 @ 37°C

| 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 130 | 170 |
|---|---|---|---|---|---|---|-----|-----|-----|
| Clb2p |
Figure 5

A. YNG2 @ 22°C  yng2 @ 22°C

B. YNG2 @ 37°C  yng2 @ 37°C

C. 0 30 60 min 0 30 60 min

YNG2 @ 37°C yng2 @ 37°C
Figure 6

A. Yng2p-Myc

B. H4

C. G1 S/G2 G2/M M/G1 αf arrest/release

D. DIC GFP-Yng2p DAPI
Figure 7

DIC

YNG2@37°C

H4

H4K8

H4K12

tH4

DIC

yng2@37°C

H4

H4K8

H4K12

tH4
Yng2-dependent NuA4 histone H4 acetylation activity is required in mitosis and meiosis
John S. Choy, Brian T.D. Tobe, Joon H. Huh and Stephen J. Kron

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