

## Esa1p Is an Essential Histone Acetyltransferase Required for Cell Cycle Progression

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**Histones are dynamically modified during chromatin assembly, as specific transcriptional patterns are established, and during mitosis and development. Modifications include acetylation, phosphorylation, ubiquitination, methylation, and ADP-ribosylation, but the biological significance of each of these is not well understood. For example, distinct acetylation patterns correlate with nucleosome formation and with transcriptionally activated or silenced chromatin, yet mutations in genes encoding several yeast histone acetyltransferase (HAT) activities result in either no cellular phenotype or only modest growth defects. Here we report characterization of *ESA1*, an essential gene that is a member of the MYST family that includes two yeast silencing genes, human genes associated with leukemia and with the human immunodeficiency virus type 1 Tat protein, and *Drosophila mof*, a gene essential for male dosage compensation. *Esa1p* acetylates histones in a pattern distinct from those of other yeast enzymes, and temperature-sensitive mutant alleles abolish enzymatic activity *in vitro* and result in partial loss of an acetylated isoform of histone H4 *in vivo*. Strains carrying these mutations are also blocked in the cell cycle such that at restrictive temperatures, *esa1* mutants succeed in replicating their DNA but fail to proceed normally through mitosis and cytokinesis. Recent studies show that *Esa1p* enhances transcription *in vitro* and thus may modulate expression of genes important for cell cycle control. These observations therefore link an essential HAT activity to cell cycle progression, potentially through discrete transcriptional regulatory events.**

Cytogenetic and biochemical differences between transcriptionally active and inactive chromatin have long been recognized (for reviews, see references 21, 60, 66, and 71). Particular focus in defining these distinctions has been on the core histones, which in the context of the nucleosome serve as the scaffold for packaging DNA into chromatin. Ultimately, it is chromatin that serves as the *in vivo* template from which RNA transcription and DNA replication occur, and it has been of central importance to define how histones function in the regulation of these processes.

Previous biochemical and genetic studies and the recent high-resolution structure of the nucleosome (37) point to the amino-terminal tails of the conserved core histones as important effectors for regulation. Extending from the nucleosomal particle, these tails participate in internucleosomal interactions and may be posttranslationally modified to result in local differences in chromatin structure and function by influencing accessibility and activity of polymerases and other regulatory proteins. Understanding these modifications may be crucial for understanding cell- and gene-specific functions. For example, phosphorylation of histone H3 on serine 10 occurs in late G<sub>2</sub> and has been proposed to promote binding of factors that may drive chromatin condensation as cells enter mitosis (24).

Among the observed histone modifications, acetylation has been perhaps most thoroughly studied, and it may contribute

both to assembly of chromatin in general and to setting transcriptional states for specific loci or entire chromosomes (21, 40, 60). Progress in defining these roles for acetylation came first from mapping and mutating sites on the core histones that were modified and most recently through identification of the enzymes, histone acetyltransferases (HATs) and deacetylases, that catalyze the modifications (for reviews see references 40, 49, and 60). The yeast gene *HAT1* was found to encode an enzyme that acetylates primarily newly synthesized histone H4, potentially as part of its assembly into nucleosomes (29, 48). *Hat1p* associates with *Hat2p*, an Rba48-like protein that contributes to substrate specificity of the enzyme (48). Although no phenotypes have been observed for yeast cells harboring mutations in these genes, a similar HAT complex exists in human cells (62), suggesting that such conserved activities are functionally important.

The first transcription-linked HAT was identified in *Tetrahymena* as the homologue of yeast Gcn5p (9). *GCN5* is not essential in yeast, but it is necessary for full transcriptional activation of some genes (17), and mutational analyses demonstrate that HAT activity correlates with transcriptional function (33, 65). Although Gcn5p provides the catalytic activity, it is only one component of two large multisubunit HAT complexes that exist in the cell (19). Interestingly, the noncatalytic subunits are also important for *in vivo* function of the complexes, suggesting that they may be finely regulated for both catalysis and specificity.

Many other proteins with HAT activity have also been identified; these include p300 and the CREB binding protein CBP (4, 43), SRC-1 family proteins (10, 59), and the basal transcription factor TAF<sub>II</sub>250 (41). The biological significance of the activities of these proteins has not been defined fully, and it is likely that HAT activity is a common feature of both gene-specific and basal regulation.

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Sequence analysis identifies a superfamily of other proteins that are either known or predicted acetyltransferases (reviewed in reference 42). For some of these there are no known functions, whereas others have provocative connections to chromatin. We have been especially interested in the *SAS* (something about silencing) genes of the MYST family (6), which were first identified for their roles in transcriptional silencing in the yeast *Saccharomyces cerevisiae* (15, 53). Although *SAS2* and *SAS3* exhibit significant sequence similarity, they have distinct mutant phenotypes suggesting that they can either promote or silence transcription, depending on the locus. Both proteins contain the A motif, a relatively short sequence that may contribute to acetyl coenzyme A binding (42), although neither has been demonstrated to have catalytic activity. We have identified a third yeast *SAS* family member, *ESAI*, that encodes an essential HAT. Further, *Esa1p* functions as the catalytic subunit in the 1.3-MDa NuA4 complex that acetylates both free histones and chromatin and, notably, can promote transcription *in vitro* (14). Our analysis of recessive conditional alleles reveals that *ESAI* is important for progression through the cell cycle, since cells grown under restrictive conditions arrest with a G<sub>2</sub>/M DNA content and partial depletion of an acetylated form of histone H4. The cell cycle arrest observed upon loss of *ESAI* function is dependent on the checkpoint gene *RAD9*. These observations therefore link specific histone modifications to cell cycle control, potentially mediated through transcriptional regulation.

#### MATERIALS AND METHODS

**Yeast methods.** An *esa1Δ::HIS3* null mutation was generated in the S288c diploid strain LPY2553 (*MATa/MATα his3Δ200/his3Δ200 leu2-3,112/leu2-3,112 trp1Δ1/trp1Δ1 ura3-52/ura3-52*) to create LPY2639 (*MATa/MATα his3Δ200/his3Δ200 leu2-3,112/leu2-3,112 trp1Δ1/trp1Δ1 ura3-52/ura3-52 esa1Δ::HIS3/ESAI*) according to standard procedures (5). Temperature-sensitive alleles were generated by amplifying the *ESAI* gene by using *Taq* polymerase in conditions recommended by the manufacturer (Promega), except that deoxynucleoside triphosphates were used at a final concentration of 250 mM. Products were subcloned into a *TRP1*-CEN vector and tested for complementation of the *esa1* null strain LPY2641 (*MATa his3Δ200 leu2-3,112 trp1Δ1 ura3-52 esa1Δ::HIS3* bearing an *ESAI-URA3*-CEN plasmid) by plasmid shuffle (23). Temperature-sensitive alleles were identified as having complementation defects at 34°C and above. Alleles were sequenced by using an Applied Biosystems automated facility.

Three alleles, *esa1-L327S*, *esa1-L254P*, and *esa1-414*, were integrated according to standard procedures (23) in LPY2639 at the *esa1Δ::HIS3* locus with *URA3*-marked integrating vectors pLP951, pLP949, and pLP952, creating LPY3425, LPY3454, and LPY3443, respectively. Integration events were confirmed by standard methods, and diploids LPY3425 and LPY3454 were sporulated to create the haploids carrying the following alleles for further experiments: *esa1-L327S* (LPY3430 *MATa his3Δ200 leu2-3,112 trp1Δ1 ura3-52 esa1Δ::HIS3 esa1-L327S::URA3*), *ESAI* (LPY3431 *MATa his3Δ200 leu2-3,112 trp1Δ1 ura3-52 ESAI*), *esa1-L254P* (LPY3500 *MATa his3Δ200 leu2-3,112 trp1Δ1 ura3-52 esa1Δ::HIS3 esa1-L254P::URA3*), and *ESAI* (LPY3498 *MATa his3Δ200 leu2-3,112 trp1Δ1 ura3-52 ESAI*). Strains for *rad9Δ esa1-L254P* mutant analysis were generated by crossing LPY3500 and LPY3719 (*MATα ade2-101 his3Δ200 lys2-801 trp1Δ1 ura3-52 rad9Δ::HIS3*) to create the diploid LPY3779 (*MATa/MATα ADE2/ade2-101 his3Δ200/his3Δ200 leu2-3,112/LEU2 LYS2/lys2-801 trp1Δ1/trp1Δ1 ura3-52/ura3-52 esa1Δ::HIS3 esa1-L254P::URA3/ESAI RAD9/rad9Δ::HIS3*); after sporulation and dissection, the haploids with the following mutations were recovered from tetrads with either a 2 *HIS*:2 *his* or 4 *HIS*:0 *his* segregation pattern: *rad9Δ esa1-L254P* (LPY3780 *MATα ade2-101 his3Δ200 LEU2 lys2-801 trp1Δ1 ura3-52 rad9Δ::HIS3 esa1-L254P::URA3*), *rad9Δ* (LPY3784 *MATa ade2-101 his3Δ200 leu2-3,112 LYS2 trp1Δ1 ura3-52 rad9Δ::HIS3*), and *esa1-L254P* (LPY3785 *MATa ADE2 his3Δ200 LEU2 lys2-801 trp1Δ1 ura3-52 esa1Δ::HIS3 esa1-L254P::URA3*). Additional strains used included LPY2991, a *MATa esa1Δ::HIS3* isolate from LPY2639, with a CEN-*ESAI* plasmid (pLP795); LPY3291, the same as LPY2991 except that plasmid (pLP863)-borne *esa1-414* replaces the *ESAI* plasmid; LPY3068, the same as LPY2991 except that *ESAI* on a *TRP1*-2 $\mu$ m plasmid, pLP798, replaces pLP795; LPY4184, the same as LPY2991 but a *MATα* isolate in which *esa1-414* on a *TRP1*-2 $\mu$ m plasmid, pLP1128, replaces pLP795; a *mad3Δ* strain (LPY4222 *MATα leu2-3,112 his3Δ200 trp1Δ1 ura3-52 mad3Δ::URA3*); and a *mad3Δ esa1-L254P* strain (LPY4223 *MATα leu2-3,112 his3Δ200 trp1Δ1 ura3-52 mad3Δ::URA3 esa1Δ::HIS3 esa1-L254P::URA3*).

**Protein expression.** Exponentially growing *Escherichia coli* BL21(DE3)pLysS cells transformed with either pLP820 (pRSETc vector [Invitrogen]), pLP831 (pRSETc-*ESAI*), pLP1138 (pRSETc-*esa1-L254P*), pLP942 (pRSETc-*esa1-414*), or pLP832 (pRSETc-*esa1-L327S*) were induced by addition of isopropyl- $\beta$ -D-thiogalactopyranoside to a final concentration of 0.5 mM. Cells were harvested after 3 h at 25°C, and lysate was prepared by a freeze-thaw cycle followed by sonication in buffer (20% sucrose, 30 mM Tris [pH 8.0], 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 100  $\mu$ l per optical density at 600 nm of 0.25). Supernatants from total cell equivalent lysates that had been clarified by centrifugation were used in HAT assays.

**Acetyltransferase activity assays.** Fifty micrograms of calf thymus histones (Sigma), 20  $\mu$ g of yeast histones (a gift of J. Pilon and P. Laybourn), or 500  $\mu$ g of unacetylated bovine serum albumin (BSA; Amresco) was combined in reaction buffer (32) with 15  $\mu$ g of recombinant *Esa1p*, *Esa1-L327Sp*, or pRSETc total soluble cell lysate and 0.1  $\mu$ Ci of <sup>3</sup>H-acetyl coenzyme A (2.3 Ci/mmol; ICN). Following incubation at 30°C for 10 min, samples were processed as described elsewhere (7) prior to scintillation counting. Approximately equivalent as well as sevenfold more *Esa1-L327Sp* than *Esa1p* was used in HAT assays based on quantitation of Coomassie blue-stained gels, using the Alpha Digital Imaging System 2000 program. For fluorography, aliquots of the HAT assay reactions were separated on a sodium dodecyl sulfate (SDS)-18% polyacrylamide gel and stained with Coomassie brilliant blue. The gel was then treated for fluorography with Intensity enhancer (NEN) and processed according to the manufacturer's instructions.

**Microsequencing.** HAT assays were performed essentially as described above except that 3  $\mu$ g of synthetic peptide corresponding to the N terminus of histone H4, H3, or H2A was combined with 3  $\mu$ g of recombinant *Esa1p* total soluble cell lysate and 1.0  $\mu$ Ci of <sup>3</sup>H-acetyl coenzyme A (2.58 Ci/mmol; NEN). Reaction mixtures were incubated for 30 min at 22°C (H4 peptide) or for 3 h at 15°C (H3 and H2A peptides). Individual reactions were microsequenced as described elsewhere (32, 58) at the Protein Chemistry Core Facility at the Baylor School of Medicine. H4 and H2A peptides were gifts of M. Parthun and D. Gottschling. The H3 peptide was synthesized on an Applied Biosystems instrument, using standard chemistry and manufacturer's recommendations.

**Flow cytometry and microscopic analyses.** Flow cytometry of 10,000 cells/sample was performed as described elsewhere (69). For 4',6-diamidino-2-phenylindole (DAPI) staining, cells were fixed in 70% acetone-30% methanol on dry ice for at least 1 h, washed twice in phosphate-buffered saline (PBS), and then stained with DAPI (Boehringer Mannheim) at a final concentration of 0.25  $\mu$ g/ml. For indirect immunofluorescence, 37% formaldehyde was added to cultures to a final concentration of 5%. Samples were agitated for 5 min at their relevant experimental temperatures and then fixed for an additional 45 min at 22°C. Cells were washed once in PBS, washed twice in 1.2 M sorbitol in PBS (SPBS), and then resuspended in SPBS with 24 mM  $\beta$ -mercaptoethanol and Zymolyase 70000 (ICN) at a final concentration of 29  $\mu$ g/ml. After 30 min at 30°C, samples were washed once in SPBS and twice in PBS. Staining was performed on poly-L-lysine-coated multiwell slides. Samples were preblocked (PBS with 10 mg of BSA per ml and 0.5% Nonidet P-40). Rabbit anti-Kar2p serum was used at a dilution of 1:1,000, and rat antitubulin serum (YOL1/34; Serotec, Inc.) at was used 1:2,000. Primary incubations for 1 h at 37°C were followed by six PBS washes. Secondary antibodies, fluorescein isothiocyanate-conjugated goat anti-rabbit (Jackson Laboratories) at 1:300 and Texas red-conjugated donkey anti-rat (Jackson Laboratories) at 1:50, were incubated at 22°C for 30 min. After six PBS washes, DAPI was added as described above. After washing, slides were mounted in Citifluor (Ted Pella, Inc.), staining was visualized with a Zeiss Axiophot fluorescence microscope, and images were collected with a COHU charge-coupled device camera using the Scion Image 1.57 program. For electron microscopy in collaboration with Tom Giddings at the University of Colorado, samples were prepared as described elsewhere (44). Sections of 80 nm were cut and examined on a Philips CM10 microscope at 80 kV with a magnification of either  $\times 10,500$  or  $\times 25,000$ . Images were collected by using a Gatan BioScan camera and Gatan Digital Micrograph 2.5 (PPC) software.

**Immunoblotting analysis.** Exponentially growing cells were split, pelleted, and resuspended into 28 or 37°C medium and incubated at the appropriate temperature for 4 h (except LPY3430, which was incubated for 8 h). Protein from  $5 \times 10^8$  cells was recovered by glass bead lysis. Briefly, cells were washed in lysis buffer (0.3 M sorbitol, 0.1 M NaCl, 5 mM MgCl<sub>2</sub>, 10 mM Tris [pH 7.5]), and the pellets were frozen at -20°C and then resuspended in 0.4 to 0.8 ml of lysis buffer containing 1  $\mu$ g each of tosylsulfonyl phenylalanyl chloromethyl ketone (TPCK), leupeptin, pepstatin A, aprotinin per ml and 0.2 mM phenylmethylsulfonyl fluoride. Acid-washed glass beads were added, and the cells were vortexed six times in 1-min bursts. Extracted protein was quantitated by the Bio-Rad protein assay, diluted into sample buffer, boiled for 5 min, and loaded onto an SDS-18% polyacrylamide gel. The separated proteins were then transferred to nylon membrane, blocked in TBSTM (10 mM Tris [pH 8.0], 150 mM NaCl, 0.05% Tween 20, 2% dry milk) for 1 h at room temperature, and then incubated with antiserum directed against the histone H4-acetylated Lys5 residue (1:500 in TBSTM; Serotec) or antiserum directed against total histones (0.5  $\mu$ g/ml in TBSTM; Boehringer-Mannheim) for 8 h at 4°C. Blots were washed, incubated with horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse antiserum (1:10,000 or 1:2,500, respectively; Promega) for 1 h at room temperature, washed, and processed for chemiluminescence as described elsewhere (56).

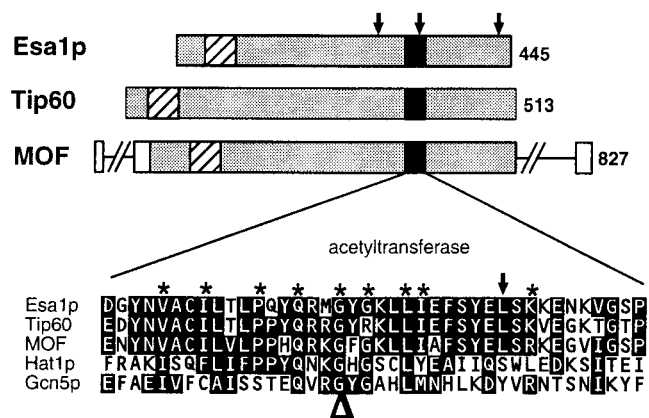


FIG. 1. Esa1p has sequence similarity (gray boxes) with human Tip60 and *D. melanogaster* MOF. A region of strong sequence similarity to acetyltransferases including yeast Hat1p and Gcn5p is indicated (black boxes). Similar and identical residues in this region are highlighted. Residues most conserved among known and predicted acetyltransferases in the A motif as defined by Neuwald and Landsman (42) are noted with asterisks. Positions of temperature-sensitive *esa1* alleles (see text) are noted by arrows above the Esa1p cartoon; the position of the *esa1-L327S* allele is also shown by an arrow in the sequence expansion. The conserved G→E mutation in *mof* (25) is marked with an arrowhead below the sequence. An N-terminal chromo domain (1, 25, 31, 47) is found in these three SAS family members (diagonal stripes). The number of amino acids in each protein is indicated at the right.

## RESULTS

***ESAI* is an essential gene in the MYST family with similarity to acetyltransferases.** During analysis of the yeast *SAS2* and *SAS3* silencing genes (53), we discovered that another, very similar but previously uncharacterized open reading frame (ORF) (YOR244w) existed on yeast chromosome XV. This ORF is predicted to encode a 445-amino-acid protein (Fig. 1) with sequence features that include an acetyltransferase motif and an amino-terminal chromo domain, a motif found in many proteins thought to regulate transcription via their chromatin association (1, 31, 47). YOR244w is most similar [BLASTP (3) scores of  $P = 10^{-100}$  or better] to the human MYST family proteins Tip60 and MOZ and the *Drosophila melanogaster* MOF protein (6, 25, 26). To investigate the function of YOR244w, we constructed a null allele by deleting one copy of the ORF (5) in a diploid yeast strain. This diploid was sporulated, and of the four spores from each tetrad, only two formed colonies. Microscopic analysis demonstrated that all four spores germinated with approximately the same kinetics, but cells from two of the spores in every tetrad stopped growing after three to four rounds of division. Genotyping revealed that the surviving spores were all wild type for YOR244w, and a plasmid-borne copy of the wild-type gene rescued the lethality associated with the deletion. Thus, the YOR244w gene product is essential for cellular viability, and we have named it *ESAI*, for essential SAS family acetyltransferase.

**Esa1p acetylates histones with a distinct specificity pattern.** A clue for the cellular role of *ESAI* came from the motif that it shares with other yeast proteins demonstrated to have in vitro catalytic acetyltransferase activity, such as Hat1p, thought to function in chromatin assembly, and Gcn5p, which functions in transcription (9, 29, 48). To test if Esa1p had HAT activity in vitro, we cloned the gene into a vector allowing its inducible expression in *E. coli*. Extracts prepared from bacteria expressing Esa1p or Gcn5p were tested for HAT activity in a standard assay (7, 32) by mixing the extracts with  $^3\text{H}$ -acetyl coenzyme A and histones from either yeast or calf thymus. These reaction

mixtures were resolved by using SDS–18% polyacrylamide gels that were stained (Fig. 2A) and subjected to fluorography (Fig. 2B). Recombinant Esa1p acetylated histone substrates, but the pattern of incorporation was distinct from that of Gcn5p. Esa1p acetylated primarily histone H4 and to a lesser extent H3 and H2A. In contrast, Gcn5p was most active on H3, although upon longer exposures, as in published reports (32), weak H4 acetylation was observed. In parallel liquid HAT assays performed with extracts prepared from bacteria transformed with vector alone, or without histones or with BSA substituted for histones, no acetylation was observed (Fig. 2C). Therefore, Esa1p's activity is specific for histones under these reaction conditions. To identify the lysines acetylated by recombinant Esa1p, we used synthetic peptides corresponding to the lysine-rich amino-terminal tails of yeast H4, H3, and H2A as substrates in standard reactions. The labeled peptides were then subjected to microsequencing coupled with the direct determination of radioactivity incorporated into each position of the peptide (Fig. 2D to F). The results show clearly that Lys5 was the major site of Esa1p acetylation on the yeast H4 peptide. Lysines at positions 8, 12, and 16 were also acetylated but less efficiently, demonstrating that each of the acetylatability lysines in the H4 tail is a potential target for Esa1p. For H3, Lys14 was the major target, with modest acetylation of Lys4. The yeast H2A peptide was acetylated predominantly at Lys4 and moderately at Lys7. This pattern of substrate specificity is distinct from those previously defined for yeast HATs and agrees with independent results for Esa1p obtained in assays using purified histones, including nonyeast proteins, as substrates (57). For example, the deposition-related Hat1p acetylates H4, predominantly on Lys12, although under conditions of reduced specificity Lys5 is also acetylated as is H2A (29, 48). The transcription-linked HAT, Gcn5p, can acetylate H4 on Lys16 but has significant preference for Lys14 of histone H3 in vitro (32). H2A is not a substrate for Gcn5p. However, Lys4 of H2A was identified previously as the sole in vivo site of acetylation in murine leukemia L1210 cells (46), although to date the relevant L1210 HAT has not been reported. Thus, it is clear that *ESAI* encodes a HAT with distinct substrate specificities, which may indicate novel or perhaps multiple cellular functions. To begin to understand these functions, we identified conditional alleles so that loss-of-function phenotypes could be examined.

**Temperature-sensitive *esa1* mutants lack HAT activity in vitro and deplete cellular pools of histone H4 acetylated on Lys5 in vivo.** We screened a library of plasmids encoding *ESAI* that had been amplified by using standard procedures. We recovered those plasmids that fully complemented lethality of strains with the *esa1Δ* allele at permissive temperatures (28°C or below) but that failed to complement at restrictive temperatures (34°C or above). Twenty-two *esa1* alleles met these criteria, and growth phenotypes of strains harboring three recessive *esa1* mutants are shown in Fig. 3. These three mutant alleles have been integrated into the normal chromosomal locus of *ESAI*. The *esa1-414* phenotype is most severe in that haploid strains with this allele are viable only when the mutated gene is plasmid borne and presumably present at slightly increased gene dosage. In *esa1-414*, deletion of a single nucleotide at position 1887 leads to a frameshift mutation in codon 414, altering 10 amino acids before terminating the ORF 22 amino acids prematurely. The *esa1-L254P* and *esa1-L327S* mutations substitute proline or serine for conserved leucines, and notably the *esa1-L327S* substitution lies within the acetyltransferase domain itself (Fig. 1). All three mutant strains grew well at permissive temperatures but poorly at elevated temperatures (Fig. 3), suffering 60 to 80% lethality within

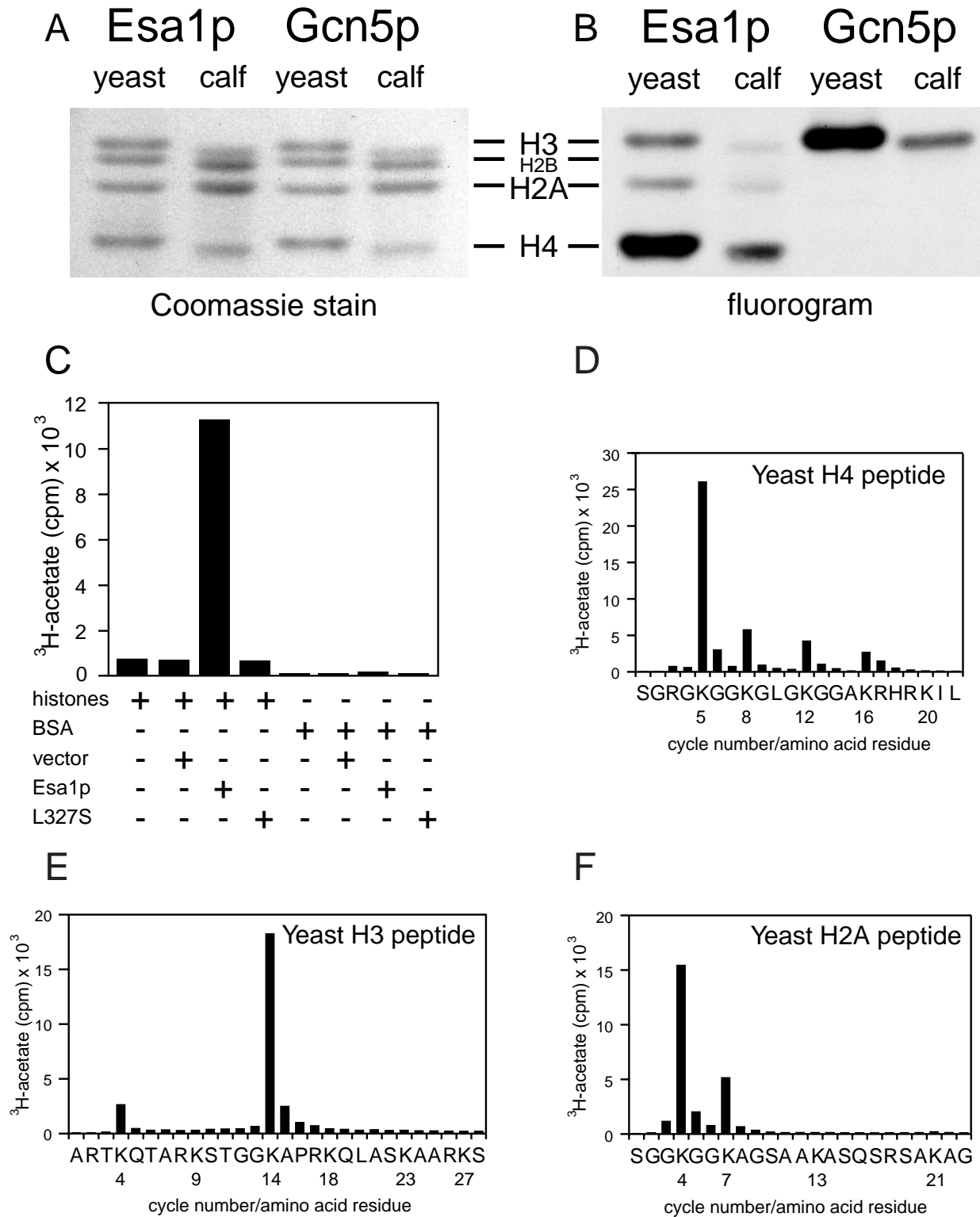


FIG. 2. Recombinant Esa1p acetylates histones. Products of HAT assays using either yeast or calf histones as substrates were resolved on an SDS-18% polyacrylamide gel that was stained with Coomassie brilliant blue (A) and treated for fluorography (B). The activities of recombinant Esa1p were compared with that of recombinant Gcn5p; whereas Esa1p preferentially acetylates H4, Gcn5p preferentially acetylates H3 (32). (C) Bacterial extracts containing equivalent amounts of recombinant Esa1p, vector control, or recombinant Esa1-L327Sp were incubated with  $^3\text{H-acetyl coenzyme A}$  and either calf histones or BSA. Esa1p acetylates calf histones but not BSA. No activity was observed with vector control or Esa1-L327Sp lysates or with two other mutant proteins tested (data not shown). The preferred targets of Esa1p are Lys5 of histone H4 (D), Lys14 of histone H3 (E), and Lys4 and Lys7 of histone H2A (F). Note that lysines 8, 12, and 16 in H4 were also acetylated. When repetitive yields for the cycles of Edman degradation were calculated, the extent of acetylation at each of these sites was equivalent and significantly less than that of Lys5.

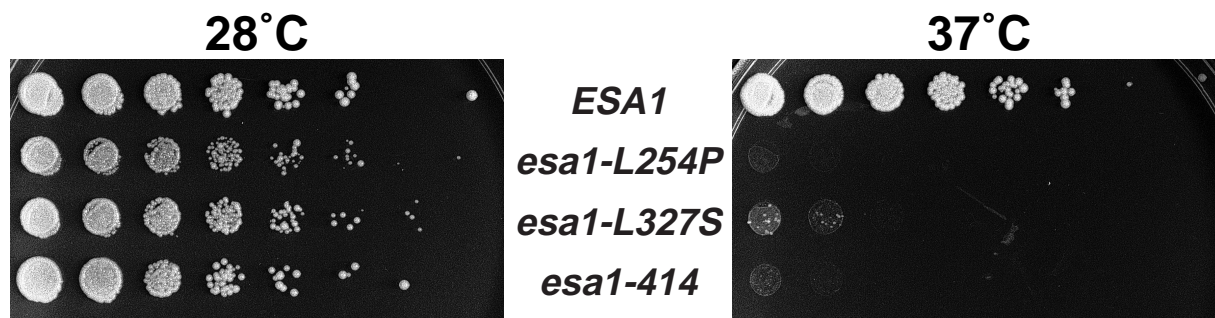


FIG. 3. *esa1* mutants are temperature sensitive. Temperature sensitivity was observed by plating wild-type *ESA1* (LPY3498) and *esa1* mutant (*esa1-L254P* [LPY3500], *esa1-L327S* [LPY3430], and *esa1-414* [LPY3291]) strains in fivefold serial dilutions at 28°C (left) and 37°C (right). All three alleles are recessive mutations.

the first 4 h of incubation. When recombinant protein encoded by *esa1* mutant alleles was tested in standard liquid HAT assays using either equivalent or excess amounts of mutant compared to wild-type protein, no activity was detected (Fig. 2C and data not shown) at any temperature tested from 1 to 37°C.

To determine whether the loss of HAT activity *in vitro* correlated with changes in histone acetylation *in vivo*, we assayed protein extracts prepared from mutant and control strains by immunoblotting. In particular, we used an antiserum specific for the acetylated Lys5 residue of histone H4, the preferred site for recombinant Esa1p activity, to compare relative levels of acetylation of wild-type and mutant strains at permissive and restrictive temperatures (Fig. 4). Each of the three *esa1* mutants had detectable levels of the acetylated isoform at the permissive temperature, although there were modest reductions relative to the wild-type control. Under restrictive conditions, each of the mutant strains had decreased levels of acetylation, with the most severely affected mutant, the *esa1-414* strain, showing the greatest reduction. This reduction was not simply due to loss of viability, because extracts prepared from a *dam1* temperature-sensitive mutant that also suffers significant loss of viability under these conditions (25a) had wild-type levels of Lys5 acetylation (data not shown). The change in Lys5-acetylated H4 does not simply reflect a decrease in cellular pools of H4. When the same samples of wild-type and mutant proteins prepared at 37°C were probed with an antibody to evaluate total histones, levels of H4-immuno-

reactive material were comparable. Although this loss of Lys5 acetylation is correlated with loss of viability of the *esa1* mutant strains, it is likely that other sites on H4, other histone targets, or the cumulative effects of loss of acetylation on all of Esa1p's targets together contribute to the essential function of Esa1p. These possibilities may be distinguished in future studies, but it is clear from Fig. 2 and 4 that the *esa1* temperature-sensitive alleles encode proteins that are defective for HAT activity both *in vitro* and *in vivo*.

***esa1* mutant strains are defective in cell cycle control.** To explore further the nature of the striking loss of viability that correlates with loss of HAT activity, we used a variety of cell biological techniques to compare wild-type *ESA1* and *esa1* temperature-sensitive strains. Mutant and wild-type cells were first grown at the permissive temperature and then split so that a portion of each was shifted to the restrictive temperature. At multiple time points after the shift to 37°C, we compared the cells for morphology and DNA content. The mutant cells became distinctly blocked in the cell cycle at the restrictive temperature. As shown in Fig. 5, 4 h after the temperature shift, *ESA1* cells were dividing normally and two peaks, indicative of G<sub>1</sub> and G<sub>2</sub>/M positions in the cycle, were easily distinguished by flow cytometric analysis. In contrast, the mutant cells stopped dividing after being shifted to 37°C and accumulated at G<sub>2</sub>/M. This terminal arrest phenotype was observed for every *esa1* temperature-sensitive strain examined, although the time required to achieve the arrest varies between alleles (data not shown). Because the mutant cells arrest with G<sub>2</sub>/M DNA

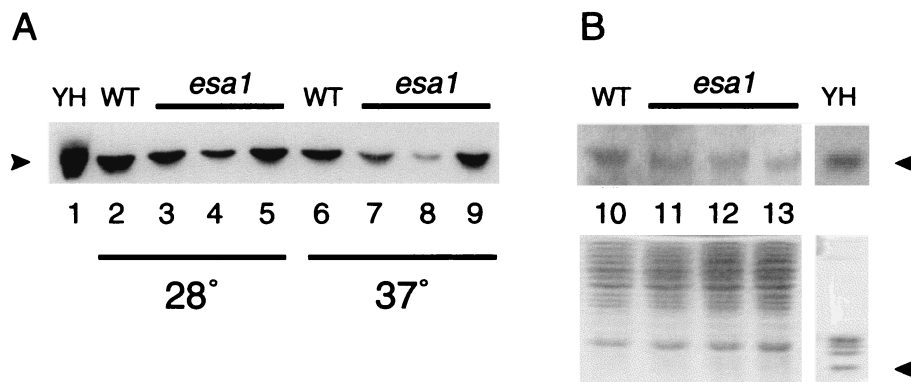


FIG. 4. *esa1* mutants display conditional decreases in the level of histone H4-Lys5 acetylation *in vivo*. Whole-cell lysates from wild-type (WT; lanes 2, 6, and 10) or *esa1* temperature-sensitive (lanes 3 to 5, 7 to 9, and 11 to 13) strains grown at 28°C (lanes 2 to 5) or 37°C (lanes 6 to 13) were separated on an SDS-18% polyacrylamide gel and either transferred to nylon and probed with an antiserum directed against Lys5-acetylated H4 (A) or probed with a control antiserum directed against histones (B; upper panel shows H4 immunoreactive band) or stained with Coomassie brilliant Blue (B, lower panel). Purified yeast histones (YH; approximately 6  $\mu$ g; lane 1) were run in parallel. *esa1-L254P* (LPY3500; lanes 3, 7, and 11), *esa1-414* (LPY3291; lanes 4, 8, and 12), and *esa1-L327S* (LPY3430; lanes 5, 9, and 13) strain displayed decreased Lys5-acetylated immunoreactivity at the nonpermissive temperature of 37°C. Arrowheads denote migration of histone H4.

content, loss of *ESAI* function apparently does not interfere with DNA synthesis, but the *esa1* mutants are unable to proceed through mitosis and cytokinesis.

To evaluate the morphology of the mutant arrest, cells from the wild-type strain and the three mutants shown in Fig. 3 were fixed and stained with DAPI to visualize DNA (Fig. 6). The wild-type *ESAI* cells were observed at all positions in the cell cycle, with normal populations of 37% unbudded, 27% small-budded, and 36% large-budded cells. Apparently normal mitotic cells, in which DAPI-staining material extended between mother and daughter buds, were seen in only 8% of the mutant cells examined. More than 70% of the mutant cells were arrested with a large bud. Of these, 75% contained a single region of DAPI-stained chromatin; the remaining 25% contained what appeared to be fragmented chromatin. Interestingly, the chromatin could be in either the mother or the daughter bud (data not shown) as determined by Calcofluor staining (51), indicating that normal nuclear migration occurring early in mitosis (45, 74) was not inhibited. Variants of the large-bud arrested mutant cells, in which an additional bud has formed, were also observed as <10% of the population (Fig. 6, right panels for mutants). In these cases, DAPI staining was sometimes seen in more than one of the buds, but one bud usually remained unstained. Together, the large-budded and variant large-budded forms comprised approximately 80% of the *esa1* cells at the restrictive temperature. For all of the mutants, increased incubation time under restrictive conditions led to increased cell volume. We interpret the continued growth of the arrested mutant cells to indicate that *Esa1p* is not required for cell growth but instead is necessary for cell cycle progression. The observation that the mutant cells undergo cell cycle arrest with replicated DNA after the shift to the restrictive temperature reveals that *Esa1p* function may be required during  $G_2$  or mitosis.

To establish whether the failure was in mitosis, we examined cells by indirect immunofluorescence microscopy using anti-tubulin (27, 28) and anti-Kar2p (54) staining as markers for mitotic and cytoplasmic microtubules and the nuclear envelope-endoplasmic reticulum (ER) membranes (Fig. 7). In wild-type cells at both temperatures and in *esa1* cells at the permissive temperature, the staining appeared normal and consistent with the cell's position in the cell cycle as inferred by DAPI staining. At the restrictive temperature, among the more than 80% abnormal cells in the *esa1* population shown here, the anti-Kar2p staining encircled the DAPI-stained chromatin (Fig. 7) and occasionally extended in fingers throughout the cytoplasm and even into the bud of the cell pair lacking DAPI staining. This staining pattern is consistent with that expected for the nuclear envelope and ER (54). The microtubular structures in the mutant cells were generally oriented with the long axis of the cell and usually had one region of more intense staining within the DAPI-stained nucleus, along with somewhat lighter, occasionally forked structures extended into the other bud of the cell. We interpret these to be spindle and cytoplasmic microtubules, respectively, that are comparable to microtubule structures in other mutants with  $G_2/M$  arrests (18). For wild-type large-budded cells, the nucleus had migrated between mother and daughter buds, with long mitotic spindles stretched between them, and occasionally short bundles of cytoplasmic microtubules were visible extended from the cytoplasmic faces of the spindle pole bodies. The mutant cells were never observed to have long spindles, consistent with the failure of the nucleus to migrate normally between mother and daughter buds.

To evaluate the morphology of arrested *esa1* mutant cells with greater resolution, samples from a temperature shift ex-

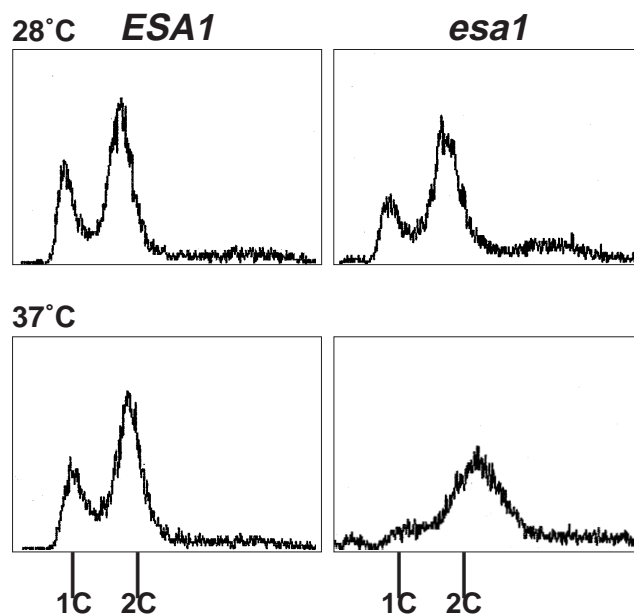
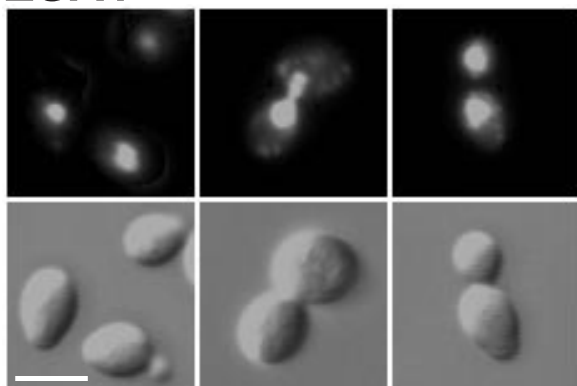


FIG. 5. *esa1* temperature-sensitive mutants arrest in the  $G_2/M$  phase of the cell cycle after DNA replication. Wild-type *ESAI* (LPY3498) (left) and mutant *esa1-L254P* (LPY3500) (right) cells were incubated at 28 and 37°C for 4 h and analyzed for DNA content by flow cytometry. The x and y axes represent relative fluorescence intensity and number of cells, respectively. *esa1* mutants arrested with  $G_2/M$  DNA content after DNA replication at the restrictive temperature of 37°C. This mutant phenotype was also observed for *esa1-L327S*, *esa1-414*, and four other temperature-sensitive alleles tested (data not shown). The time to reach the arrest varied somewhat depending on the allele of *ESAI*.

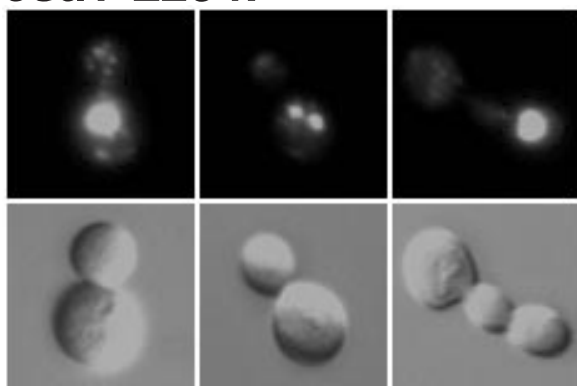
periment were prepared for electron microscopy using a rapid-freeze protocol (44). Clear differences were observed in thin sections of the wild-type and mutant cells (Fig. 8). Images of large-budded cells are shown here for comparison, although they represent only about 36% of the *ESAI* population. The cytoplasm of the *esa1* mutant cells was consistently observed to be more highly vesiculated than that of wild-type cells (Fig. 8C), but striking differences were also observed in the substructure of the nuclei. In wild-type cells, the nucleus is of relatively uniform density, with increased density found restricted to the fairly compact heterochromatic nucleolus (Fig. 8A and B). In the dividing cell, this more electron-dense material was seen at both poles of the lobed nucleus extending between mother and daughter cells. However, in the *esa1* cells, the nucleus was generally restricted to one side of the mother-daughter pair (Fig. 8C and D), confirming our results with light microscopy. The mitotic spindles appeared structurally normal, including short bipolar spindles with connecting microtubules and normally nucleated cytoplasmic microtubules. However, in some instances, the spindle was oriented aberrantly and was even observed to lie horizontally across the bud neck rather than extending through it. The nuclei themselves were also distinct in that the nucleolus was often exaggerated, and electron-dense material was consistently scattered throughout the nucleus (Fig. 8D) rather than remaining restricted to the nucleolar compartment. This dispersal was detected with various degrees of severity in 96% of the 65 mutant nuclei examined, but it was observed in only <1% of wild-type nuclei and has not been seen in other ultrastructural analyses of comparably prepared *cdc* mutants that arrest with large buds (43, 70a).

Considered together, the enzymatic and cell biological analyses of the *esa1* mutants reveal important consequences for loss of *ESAI* function. We were especially interested in the

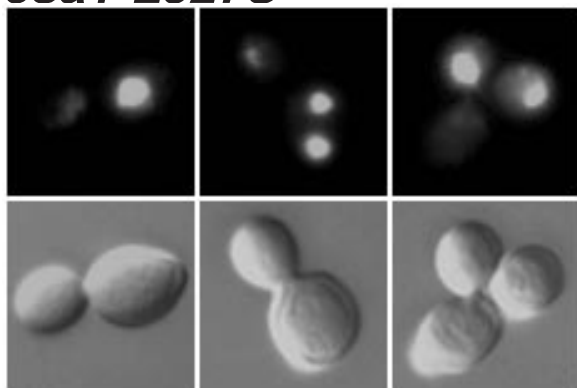
### **ESA1**



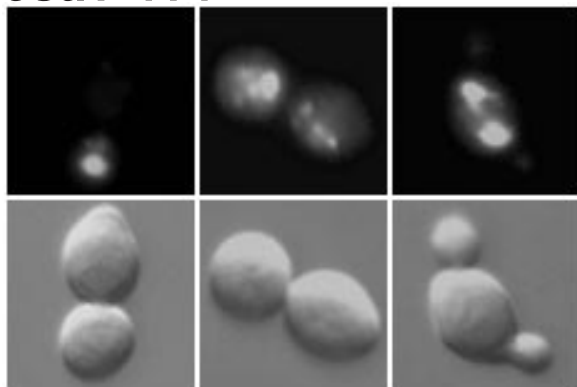
### **esa1-L254P**



### **esa1-L327S**



### **esa1-414**



defect in cell cycle progression because previous research demonstrated that the acetyltable amino-terminal H3 and H4 tails can be essential for cell cycle control. In particular, if cells are depleted of normal H3 and H4 histones and are left only with H3 and H4 lacking amino-terminal tails (36), they rapidly assume an arrest comparable to that which we observed in *esa1* cells. In considering the mechanisms leading to the arrest, it was relevant to consider the role of checkpoint functions (reviewed in references 16 and 68).

**The *esa1* cell cycle arrest is dependent on the *RAD9* checkpoint gene.** Cell cycle checkpoint functions are ordinarily defined as those functions that perceive either damaged structures or failure to complete processes necessary for normal fidelity of cell duplication (16, 68). Frequently, cell cycle arrests are triggered by checkpoint functions allowing time for repair or completion of the lagging step. We wanted to determine whether the cell cycle arrest observed in the *esa1* mutants was dependent on checkpoint functions. It seemed possible that, depending on the precise role(s) of the Esa1p HAT activity, the cells could trigger checkpoints registering failure to replicate chromatin or failure in cell cycle transcriptional control. It should be noted that DNA checkpoints defined to date, such as that controlled by the *RAD9* gene (39, 67), appear to register DNA damage, not necessarily chromatin damage. Further, the *esa1* cell cycle arrest observed is distinct from that ordinarily seen at checkpoints in that there is a substantial loss of cell viability for the *esa1* mutants, whereas in classically defined checkpoint arrests, viability remains high upon return to permissive conditions.

To assess the role of checkpoint control in the *esa1* cell cycle arrest, we constructed isogenic *rad9Δ esa1* double- and single-mutant strains for temperature shift experiments as described above. At 4 h after the shift to the restrictive temperature, the *rad9Δ esa1* cells had severe losses in viability comparable to the *esa1* single mutants, whereas the *rad9Δ* cells remained fully viable (data not shown). Flow cytometry also revealed that the *rad9Δ* single-mutant strain was unaffected by growth at 37°C (Fig. 9A), whereas the *esa1* strain arrested with 2C DNA content (Fig. 9B). The *rad9Δ esa1* double-mutant strain, however, no longer arrested with a simple 2C DNA content (Fig. 9C). Instead, both 1C and 2C peaks were apparent, indicative of cells in both G<sub>1</sub> and G<sub>2</sub>/M phases of the cell cycle. Budding indices confirmed and extended the flow cytometry data. Rather than arresting with >80% large-budded and abnormal cells, the *rad9Δ esa1* strain was 46% unbudded, 25% small budded, and 26% large budded. In this population, 64% of the cells were clearly abnormal, often appearing exaggerated in size.

To determine if the cell cycle arrest was dependent on the mitotic checkpoint, we constructed an *esa1* strain also deleted for the *MAD3* gene (34, 70). No change was observed in cell cycle arrest at the restrictive temperature assayed by flow cytometry compared to the *esa1* mutant alone (Fig. 9D and E). Therefore, the cell cycle arrest observed in the *esa1* mutant strain is dependent on the checkpoint function of *RAD9* but not on the *MAD3* mitotic checkpoint.

FIG. 6. *esa1* mutants display improper DNA segregation. *ESA1* (LPY3498), *esa1-L254P* (LPY3500), and *esa1-414* (LPY3291) cells were incubated at 37°C for 4 h, and *esa1-L327S* (LPY3430) cells were incubated at 37°C for 8 h, fixed, and stained with DAPI. *esa1* mutant cells were predominantly large budded as visualized by Nomarski optics (left and center columns, bottom), with some triple-budded cells (right column, bottom). DAPI staining indicates that *esa1* mutant cells either failed to segregate or abnormally segregated their chromatin in comparison to wild-type cells. Bar, 5 μm.

## DISCUSSION

Histone acetylation is a posttranslational modification that historically has been correlated with both assembly and distinct transcriptional states of chromatin (8, 21). Here we report identification of the yeast *ESAI* gene that is essential for cell viability and encodes a protein with HAT activity. The enzymatic activity of Esa1p has a unique pattern of substrate specificity among yeast HATs, and intriguingly, the essential role appears tied to progression through the cell cycle after DNA replication is complete. *ESAI* is one of a family of highly conserved genes which were first identified for their transcriptional regulatory functions in yeast, *Drosophila*, and humans (6, 15, 25, 26, 53). It is likely that at least part of Esa1p's essential functions are transcriptional since it is the catalytic component of NuA4, a large, multisubunit complex that can activate transcription (14, 61). These results therefore connect a specific HAT to cell cycle progression and raise the possibility that targets of HAT activity feed into checkpoint controls or contribute to their regulation through modulation of downstream transcriptional events.

**Esa1p acetylates histones with a distinct pattern of substrate specificity.** Both recombinant Esa1p and native protein purified in the NuA4 complex (14) have significant HAT activity directed toward histone H4. With synthetic peptides or purified histones as substrates, Esa1p can acetylate each of the four lysines in the N-terminal tail that are most significant for H4 function (this report and references 14 and 57). The pronounced preference toward Lys5 of H4 is consistent with a role in the deposition of newly synthesized histones (8), although the comparable site is acetylated in the transcriptionally active macronuclei of rapidly growing *Tetrahymena* (12). Esa1p's pattern of acetylation observed on H3 is also seen for HAT activity of Gcn5p and TAF<sub>II</sub>250 (32, 41), both transcriptional activators. Although H2A's acetylation has been less thoroughly studied, it can be acetylated *in vivo* in animal cells (46) at the same position as we observe for Esa1p's activity *in vitro*. These same specificities have also been observed independently and recently reported, including using purified *Drosophila* H4 and chicken H2A histones as substrates (57). Further, a catalytically active recombinant fragment of the human protein Tip60 acetylates H4, H3, and H2A free histones (72), revealing that not only are the SAs family proteins highly related, but the specificities of their activities may be as well. Although *in vitro* specificities of HATs should be interpreted cautiously (see, e.g., references 48 and 75), our data along with those of others (57, 72) do identify Esa1p as a candidate H4 HAT, distinguished comparatively by its targets, of which Lys5 of H4 appears relevant *in vivo* (Fig. 4). Because the Lys5-acetylated isoform of H4 is also depleted in *gcn5* $\Delta$  mutants (75), there likely must be other sites and other substrates that, in concert, contribute to the essential nature of *ESAI*'s function. Like *gcn5* strains which display impaired HAT activity *in vivo* when residues either within the A box or outside this region are mutated (33, 65), we recovered *esa1* mutants with lesions within the A box (*esa1-L327S*) as well as outside it (*esa1-L254P* and *esa1-414*) that exhibit decreased H4 Lys5 acetylation *in vivo*. Interestingly, the *esa1-414* mutant shows the strongest decrease, suggesting a role for the carboxy terminus in maintaining a functional complex (14).

The Esa1p-containing NuA4 complex, identified for its acetylation of nucleosomal histones H2A and H4, is both physically and genetically distinct from the other large HAT complexes such as Ada and SAGA (14, 19) that appear most active toward H3. The NuA4 complex in *esa1-414* strains retains HAT activity at permissive temperature, but this activity is lost upon

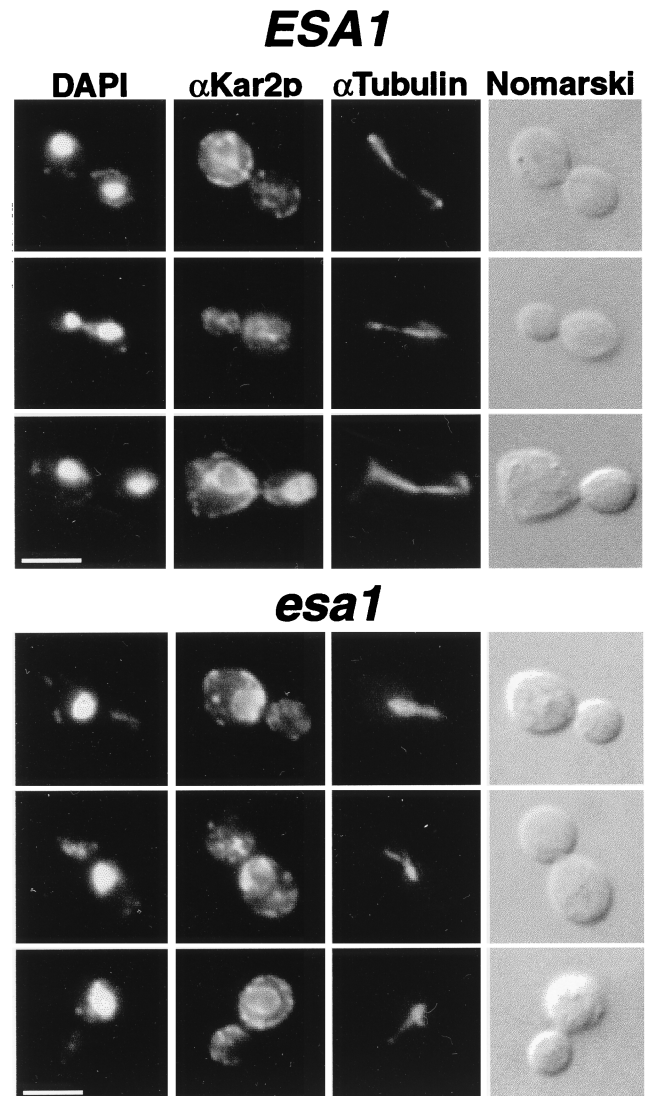


FIG. 7. *esa1* mutants disrupt mitotic cell cycle progression. *ESAI* (LPY3498) and *esa1-L254P* (LPY3500) cells were incubated at 37°C for 4 h and prepared for indirect immunofluorescence microscopy. *esa1* mutant cells exhibited aberrant nuclear and short spindle morphologies at the restrictive temperature. As visualized by double-label immunofluorescence microscopy, antisera directed against Kar2p detected the nuclear envelope and ER and  $\alpha$ -tubulin detected microtubules. Both the nuclear envelope and the microtubules extended only partially through the bud neck in comparison to the wild type but coincided with the DAPI-stained region. Bars represent 5  $\mu$ m.

a 4-h shift to the restrictive temperature. It is possible that H2A and H4 are relevant substrates *in vivo* of Esa1p and that the H3 acetylation results from relaxed stringency *in vitro* with the recombinant protein. Reduced site specificity was observed for recombinant Hat1p in the absence of its binding partner, the Rba48-like protein Hat2, which appears to function as a specificity factor (48). In contrast, broader activity *in vivo* than originally predicted has recently been suggested for Gcn5p (75). Thus, Esa1p may acetylate H2A and H4 in the context of the NuA4 complex but may act on H3 as the catalytic subunit of a different complex, influenced by different specificity factors. Determining whether Esa1p is catalytically active in more than one complex and whether it has distinct substrate specificities (including nonhistone proteins) depending on its cellular localization or its targeted genomic loci will be critical to



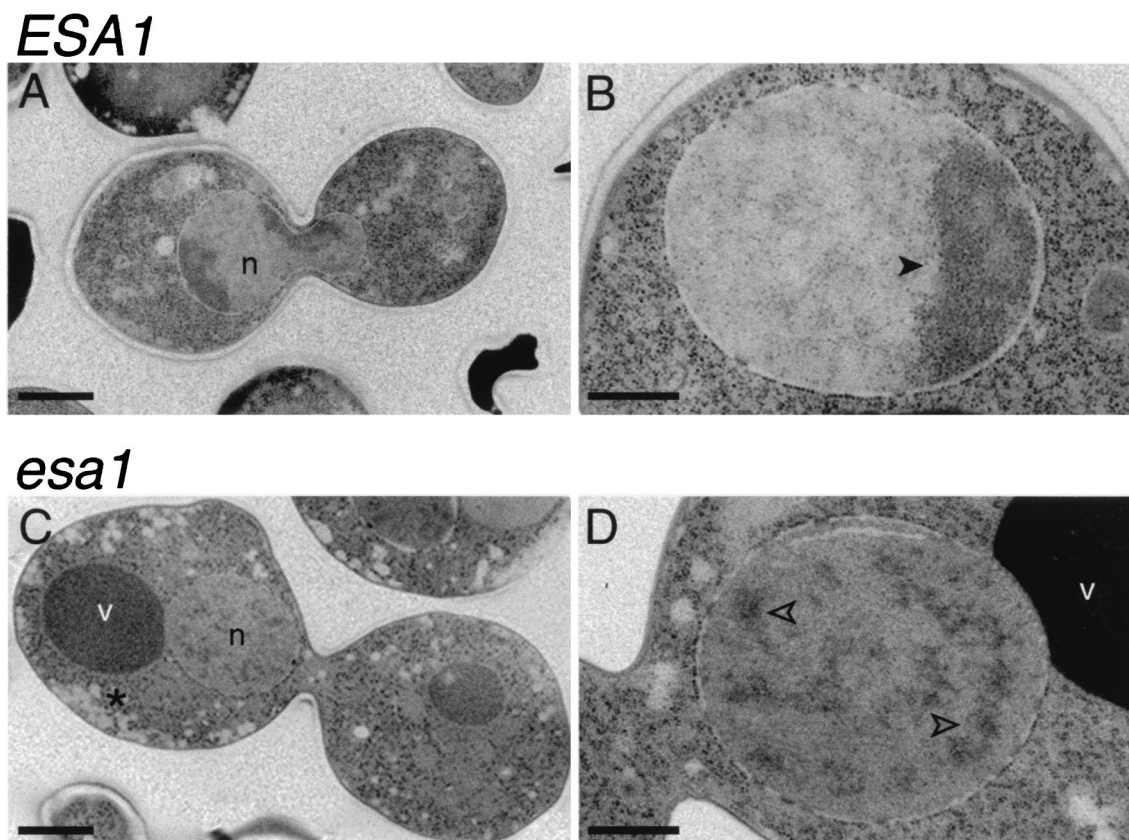


FIG. 8. *esa1* mutants have aberrant ultrastructural morphology. Wild-type *ESA1* (LPY3498) (A and B) and *esa1-L254P* (LPY3500) (C and D) cells were incubated at 37°C for 4 h and prepared by rapid freezing for electron microscopy. In wild-type cells, the nucleolar electron-dense material was at both poles of the dividing nucleus (n) (A) or in a crescent shape in a nondividing cell (B, closed arrowhead). However, in the *esa1* mutant (C and D, open arrowheads), the electron-dense material was dispersed throughout the nucleus. The majority of the mutant large-budded cells showed the nucleus on one side of the bud neck, in contrast to wild-type cells, where the nucleus extended equally through the bud neck. The cytoplasm of the *esa1* mutant (C, asterisk) was also more highly vesiculated than in wild-type cells. Each panel shows a different cell, and scale bars shown represent 1  $\mu\text{m}$  (A and C) and 0.5  $\mu\text{m}$  (B and D). v, vacuoles.

understanding its function. Previously proposed models predict that HAT complexes may be regulated through subunit exchange (55). Indeed, in early studies, a single *Tetrahymena* HAT activity was shown to have both deposition- and transcription-related activities (11). In light of the current molecular identification of HATs, it would be revealing to know the detailed molecular composition of that potentially bifunctional activity.

**Connecting HATs and checkpoint control through *ESA1*'s role in cell cycle progression.** The cell cycle arrest of temperature-sensitive *esa1* mutant cells is rapid and is accompanied by loss of viability. These effects suggest that appropriate histone acetylation is critical for cell cycle progression. Interestingly, recent results indicate that *gcn5* mutants, which ordinarily have only modest growth phenotypes, die in combination with subsets of H3 and H4 mutants and also suffer  $G_2/M$  effects (75). Previous studies revealed that mutations of H4 N-terminal lysines can lead to  $G_2/M$  delays that are *RAD9* dependent (39). Similarly, deletion of N-terminal tails of either H3 or H4 also delays the cell cycle, and deletion of both kills cells in  $G_2/M$  (36). Our results provide an explanation for these observations by demonstrating that modification of N-terminal histone tails may be essential and may couple acetylation events to cell cycle control, independent of structural changes resulting from mutation of the histones. Because the N-terminal tail of either H3 or H4 alone can support viability (36), it is possible that Esa1p may acetylate both histones in vivo and that these particular

acetylation patterns are important for chromatin structure. It is also possible that Esa1p may modify some key nonhistone target(s). Alternatively, Esa1p's HAT activity may function to regulate transcription of a gene or group of genes, which are themselves required for proper cell cycle control, although *ESA1* itself is not transcriptionally regulated through the cell cycle (13). The closely related genes *SAS2* and *SAS3* can influence transcriptional regulation of the silent mating-type loci and telomeric reporter genes (15, 53). Preliminary studies indicate that *ESA1* does not contribute to regulation of these loci, furthering the likelihood that its functions are distinct.

Insight into the nature of the *esa1* cell cycle arrest comes from flow cytometric and microscopic analyses. These studies reveal that the arrest occurs after the cells have completed DNA synthesis but before the successful completion of mitosis. Compared to overall bud and cell size, the migration of the nucleus and extension of the mitotic spindle appear abnormal. Ultrastructural analysis suggests that there may be irregularities in the electron-dense chromatin itself. The significance of this observation is not yet understood. It remains possible that Esa1p functions in multiple capacities, some directly related to chromatin structure and assembly in mitosis and others transcriptionally focused.

The observation that the *esa1* temperature-sensitive cell cycle arrest is dependent on the *RAD9* checkpoint function but not on the *MAD3* checkpoint function deepens the potential significance of understanding *ESA1*'s role in the cell cycle.

*RAD9* participates in sensing DNA damage and the execution of functions necessary for repair. Our results raise the possibility that incompletely or inappropriately acetylated chromatin may be recognized as damage, although the precise mechanisms for monitoring such damage remain to be defined. It is formally possible that the very end stages of DNA replication are not completed in the *esa1* mutants, to degrees not detectable at the limits of flow cytometric resolution, but we prefer the interpretation that the arrest occurs after S phase because of the nuclear positioning and mitotic spindle phenotypes. The high loss of viability observed at the arrest distinguishes it from normal checkpoint-regulated processes. Perhaps the damage in the *esa1* mutant cells is so severe as to be irreparable. Alternatively, *Esa1p* may itself contribute to checkpoint functions akin to the essential *RAD53* gene (2, 68), potentially by activating transcriptional targets required at cell cycle transition points, thereby regulating the synthesis of factors required for return to normal cell cycle progression.

**Essential roles for essential HATs.** *GCN5* and *HAT1* were the first yeast genes determined to encode transcription- and deposition-related HAT activities (9, 29, 48). Surprisingly, strains in which only these genes are deleted have modest growth phenotypes under special conditions or no detectable cellular phenotypes. The TFIID component  $TAF_{II}250$ , an essential factor of the basal transcriptional machinery (50, 52), has intrinsic HAT activity (41), although whether this is its essential function is not known. It is interesting that mutants with temperature-sensitive alleles of the yeast  $TAF_{II}250$  gene homologue, *TAF145* also arrest the cell cycle at their restrictive temperature (63). This arrest appears to be due to loss of transcriptional activation of  $G_1$  cyclins and not loss of global RNA polymerase II-directed transcription (64), although whether these mutant alleles are specifically defective in HAT activity remains to be determined. These results, considered together with those for the *esa1* cell cycle arrest, raise the possibility that gene-specific or global chromatin modification provides an additional control for transcriptional pathways such as those activated by DNA damage or other cell cycle perturbations.

Essential roles for other HATs are revealed by the male-specific lethality resulting from mutation of the closely related *Drosophila mof* gene. In this case, the increased transcription of the X chromosome that is correlated with H4 Lys16 acetylation fails, and death results because dosage compensation is not achieved (25). Female flies with *mof* mutations appear completely normal (25), suggesting that *mof* may encode a sex-specific HAT or that there are sex-specific requirements for histone acetylation.

Whether the vertebrate MYST family genes are essential is not yet known. Among the human homologues (6), there may be genes encoding both essential and nonessential functions, as do the yeast genes. By comparison, it has been recently reported that the murine p300 and CBP genes are both essential, and loss of either gene results in multiple developmental defects and early embryonic lethality (73). Although it has not been possible to separate p300's transcriptional activation properties from its HAT activity (38), CBP and p300's essential roles are likely to be transcriptional ones, mechanistically dependent on HAT activity. CBP is known to interact with p53 to potentiate the transcriptional activation functions of p53 in inhibiting entry into  $G_1$  (22, 35). Furthermore, p53 is also activated in response to DNA damage (for a review see reference 30). Thus, regulated transcriptional activities of HAT complexes may participate at several levels in coordinating cell cycle regulation and responses to DNA or chromatin damage.

In considering the contribution of chromatin modification to biological regulation, it is clear that histone acetylation can fa-

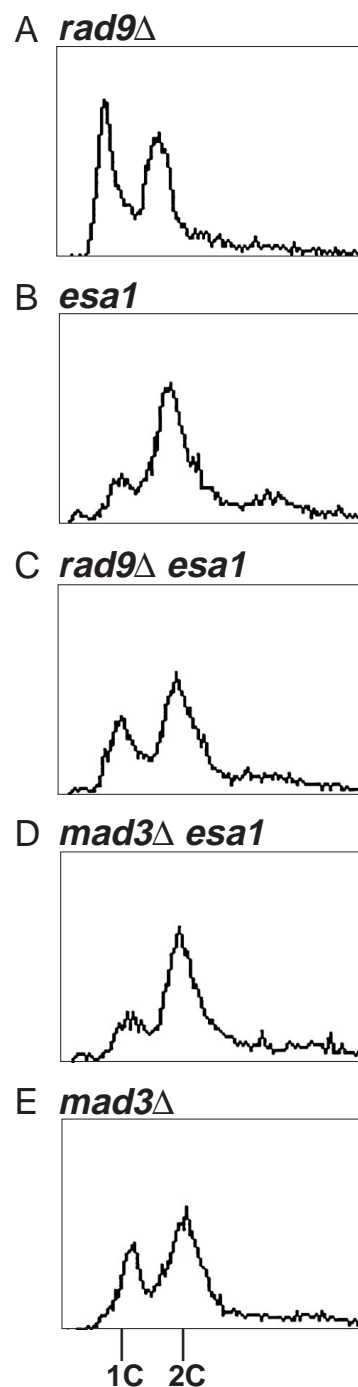


FIG. 9. The *esa1* mutant  $G_2/M$  arrest is dependent on the *RAD9* checkpoint gene. (A) *rad9Δ* (LPY3784), (B) *esa1-L254P* (LPY3785), (C) *rad9Δ esa1-L254P* (LPY3780), (D) *mad3Δ esa1-L254P* (LPY4223), and (E) *mad3Δ* (LPY4222) mutant cells were incubated at 37°C and then analyzed for DNA content by flow cytometry. The x and y axes represent relative fluorescence intensity and number of cells, respectively. The *esa1-L254P* and *mad3Δ esa1-L254P* mutants arrested after DNA replication with approximately 80% of the cells containing 2C DNA content, whereas the *rad9Δ* and *mad3Δ* cells have normal DNA content for dividing cells. The *rad9Δ esa1-L254P* double mutant had both 1C and 2C peaks, indicating that *esa1-L254P* cell cycle arrest is dependent on *RAD9* but not on *MAD3*.

cilitate fine-tuning of transcription as well as more profound changes in chromatin structure. By analogy to Gcn5p (20), *Esa1p* may participate catalytically in more than one complex. Whether its catalytic activity is strictly transcription related (14, 61),

or whether it has other roles, even in chromatin assembly, will be an important issue to address. The fact that the three closely related genes *SAS2*, *SAS3*, and *ES1* appear to have distinct, nonredundant functions in unicellular yeast implies that the homologous genes in vertebrates may also have distinct functions. For example, it is not known how loss or misregulation of Tip60 may contribute to human immunodeficiency virus type 1 Tat transcription (26) or how MOZ, when fused to CBP, may lead to acute myeloid leukemia (6). Understanding the specific functions of these proteins, and how they promote changes in chromatin structure and transcriptional control, will ultimately depend on identifying their *in vivo* targets and the complexes in which they act.

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A.S.C. and J.E.L. contributed equally to this work.

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