The Yeast NuA4 and Drosophila MSL Complexes Contain Homologous Subunits Important for Transcription Regulation*

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RAC, and ACF in Drosophila; and RSP and WCRF in mammals) and those that are recruited to alter chromatin conformation via the acetylation of histones (e.g. NuA4, ADA, ADA, Elongator, and SAGA in yeast and TAFII250, human GCN5, p/CAF, SRC-1/ACTR, and CBP/p300 complexes in mammals) and their deacetylation (reviewed in Refs. 2 and 3; see Refs. 4 and 5). The mechanisms whereby the enzymatic functions identified with these complexes are translated into the modulation of transcription still remain largely unresolved. Significant insights into the functional aspects of chromatin remodeling can be garnered by comparing complexes that share homologous components in different model systems where specific biochemical and genetic tools are available. We have begun such an analysis by studying two complexes with homologous protein subunits, the dosage compensation or MSL complex of Drosophila melanogaster and the NuA4 complex of Saccharomyces cerevisiae.

In Drosophila, the transcription of most genes on the single male X chromosome is increased 2-fold relative to the transcription of the same genes on each of the two female X chromosomes (6, 7). This compensation for the difference in dosage of X-linked genes between males and females requires a group of gene products: MLE (maleless); MSL1, MSL2, and MSL3 (male-specific lethal 1, 2, and 3, respectively), and MOF (males absent on the first), known collectively as the MSLs for their male-specific lethal loss-of-function phenotype. In males, the MSLs form a complex with roX RNA that binds the X chromosome along numerous sites and that results in the presence of a particular isofrom of histone H4 acetylated at lysine 16 (8–13). The enzymatic activity of two members of the complex has been established: MLE is an RNA helicase (14, 15) that exhibits 49% identity and 85% similarity to mammalian RNA helicase A (16), and MOF is a histone acetyltransferase from the MYST family responsible for the X-specific acetylation of H4 at lysine 16 (11, 17–19). The yeast homolog of MOF, Esa1p (essential Sas2-related acetyltransferase-1 protein), expressed as a fusion protein in bacteria, acetylates free histone H4 in vitro (20, 21). Surprises

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†The abbreviations used are: NuA4, nucleosome acetyltransferase of histone H4; ADA, Ada acetyltransferase; SAGA, Spt-Ada-Gen5 acetyltransferase; MSL, male-specific lethal; MOF, males absent on the first; MYST, MOZ/TIF2/SAS2/Tip60; Esa1p, essential Sas2-related acetyltransferase-1 protein; HAT, histone acetyltransferase; Tra1p, transfor-

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2 Domain-containing region; EMM, Esa1p/MSL3/MRG15; HPLC, high pressure liquid chromatography; Epl1p, enhancer of Polycomb-like-1 protein; Arp4p, actin-related protein-4 protein; Alp13, altered polarity mutant-13; MRG15, MORF-related gene on chromosome 15; MORF4, mortality factor on chromosome 4.
using, although a replacement of the ESA1 gene is lethal, a mutation in a conserved glycine in the acetyl-CoA-binding site of Esa1p, which results in male-specific lethality in the Drosophila MOF protein and eliminates the acetyltransferase activity of the recombinant protein in vitro, has no observable phenotypic effect on yeast viability (20). In contrast, temperature-sensitive point mutations in Esa1p were recently shown to arrest cells at the G2/M border in a RAD9-dependent manner, suggesting that Esa1p activity is essential for cell cycle progression (21). We have recently shown that Esa1p is a component of the 1.3-MDa NuA4 chromatin-remodeling complex and is responsible for its HAT activity (22). This complex stimulates transcription mediated by a variety of activation domains in vitro in a chromatin- and acetyl-CoA-dependent manner, i.e. through histone acetylation (23–26). Recruitment of the complex by physical interaction with activation domains has also been observed (23, 26). Another component of the NuA4 complex is the ATM-related cofactor Tra1p (22). Tra1p is also essential for growth; and its human ortholog, TPRAP, interacts directly with c-Myc and E2F activation domains and is required for their cell-transforming activities (27, 28). These observations suggest that NuA4 is required to modulate expression of genes important for cell cycle control.

In this study, we report that a second yeast protein, homologous to a component of the Drosophila dosage compensation complex, is also a subunit of the NuA4 complex. This protein, which we call Eaf3p (Esa1p-associated factor-3 protein), has extensive similarity to Drosophila MSL3. To elucidate the role of Eaf3p and the other common component of the NuA4 and MSL complexes (Esa1p) and their functional interaction within the Drosophila and yeast complexes, we performed a genetic and biochemical analysis of NuA4 complexes isolated from various mutant strains. Our results demonstrate that Esa1p and Eaf3p are integral components of the NuA4 complex. In contrast to the role of MSL3 in the Drosophila complex, Eaf3p is not essential for growth; this is consistent with our finding that a NuA4 complex lacking this component exhibits normal levels of histone acetyltransferase activity in vitro. Consistent with the important but subtle 2-fold effect on transcription of the dosage compensation complex, we show that a strain lacking Eaf3p has an ~2-fold decrease in the transcription of specific target genes.

EXPERIMENTAL PROCEDURES

Media and Strains—Strains were grown at 30 °C in rich (yeast extract/Bacto-peptone/dextrose (YPD)) or minimal medium supplemented with the appropriate nutrients. Medium containing 6-azauracil (Sigma) was made by addition of the appropriate concentration of a sterile solution of the drug (dissolved in 1 mM ammonium hydroxide) to autoclaved and cooled minimal medium. Media for potential mutant phenotypes were prepared as follows. A sterile stock solution of sorbitol was added to YPD to a concentration of 8 mM. Benomyl or to minimal medium lacking glucose. A sterile stock solution of caffeine or ethanol medium was prepared by adding a sterile stock solution from nickel-agarose resin at 160 mM imidazole. Partially purified and affinity-purified anti-Esa1p sera have been described (22), as has anti-Tra1p serum (30).

Purification of the NuA4 HAT Complex—Preparation of whole cell extract from yeast strains and fractionation over Ni2+-nitrotriacetic acid-agarose (Qiagen Inc.) and Mono Q HR 5/5 columns (Amersham Pharmacia Biotech) were performed essentially as described (22, 31, 32). Typically, a 1-ml fraction was tested for HAT activity on 0.5 μg of oligonucleosomes for 30 min at 30 °C. When indicated, peak nuclease H4/H2A-specific acetyltransferase activity (NuA4) from the Mono Q salt gradient elution was pooled and concentrated to 0.6 ml with 50 μg/ml insulin, frozen in liquid nitrogen, and stored at ~80 °C. Peak Superox 6 fractions were used in analytical and preparative immunoprecipitation experiments.

NuA4 assays were performed essentially as described (31) using 0.5 μg of oligonucleosomes, protein fractions, and 0.125 μCi of [3H]acetylated CoA (4.7 Ci/mmol) in HAT buffer (50 mM Tris-HCl (pH 8.0), 50 mM KCl, 0.1 mM EDTA, 5% glycerol, 1 mM phenylmethylsulfonyl fluoride, and 10 mM sodium butyrate) for 30 min at 30 °C. Quantification was performed by spotting the reactions on Whatman P-81 filters and liquid scintillation counting as described (33). Visualization of histone specificity was obtained by loading the reactions on an 18% SDS-polyacrylamide gel, followed by Coomassie Blue staining to ensure equivalent loading of histones in each lane, destaining, and fluorography with Enhance (PerkinElmer Life Sciences).

Ion Trap Mass Spectrometry and Peptide Sequencing—The immunopurified 47-kDa NuA4 SDS-PAGE excised band was subjected to gel digestion, carbamylation, and tryptic digestion (Promega). Multiple peptide sequences were determined in a single run by a microcapillary reverse-phase chromatography directly coupled to an LCQ quadrupole ion trap mass spectrometer (Finnigan MAT) equipped with a custom nanoelectrospray source. The column was packed in-house with 5 μm of C18 support into a one-piece 75-μm inner diameter column terminating in a 15-μm tip (New Objective). The flow rate was 190 nl/min. The peptide samples and column were heated to a temperature of 35 °C. Multiple scan modes consisting of full-scan mass spectrometry over alternating ranges of 395–800 or 1300–1800 m/z, followed by two data-dependent scans on the most abundant ions in those full scans. These dependent scans allowed the automatic acquisition of a high resolution (znom) scan to determine charge state and exact mass and tandem mass spectra for peptide sequence information at low femtomole quantities. Tandem
mass spectra were acquired with a relative collision energy of 30%, an isolation width of 2.5 Da, and recurring ions dynamically excluded. Interpretation of the resulting tandem mass spectra of the peptides was facilitated by programs developed in the Harvard Microchemistry Faculty (34) and by data base correlation with the algorithm SEQUEST (35).

Western Blotting, Immunoprecipitations, and Pull-down Assays—10-μl fractions were typically used for Western blotting after electrophoresis on a 10% SDS-polyacrylamide gel. Immunoblotting with anti-Esa1p, anti-Tra1p, and anti-Eaf3p sera was done as 1:3000, 1:2000, and 1:100 dilutions, respectively, in 1% nonfat dry milk in phosphate-buffered saline and 0.1% Tween 20 for 4 h at room temperature or overnight at 4 °C. For immunoprecipitation studies, affinity-purified anti-Esa1p and anti-Eaf3p sera were cross-linked to protein A-Sepharose resin (Amersham Pharmacia Biotech) with dimethyl pimelimidate (Pierce) following standard protocols (36). Superox 6 NuA4 fractions were diluted in buffer B (40 mM Hepes (pH 7.5), 0.1% Tween 20, 0.5 mM dithiothreitol, and 10% glycerol plus protease inhibitors) to 150 mM NaCl, and precleared with protein A-Sepharose. Precleared supernatants were incubated with preimmune or immune cross-linked beads for at least 3–4 h at 4 °C as a 30% slurry. After incubation, the beads were washed twice with buffer C containing 350 mM NaCl and twice with 150 mM NaCl. HAT assays were performed directly on equivalent fractions (obtained from Research Genetics) without cross-linking. Signals were quantified using a PhosphorImager (Molecular Dynamics, Inc.).

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Superox 6 columns (22). The Mono Q fractionation allowed separation of the four previously identified native HAT complexes (Fig. 2A) (31). The fractions containing the NuA4 complex displayed nucleosomal H4 HAT activity (fractions 24–26). Western blot analysis showed strict coelution of Eaf3p with this activity and with two known components of NuA4, Esa1p and the ATM-related cofactor Tra1p (Fig. 2A). Furthermore, Eaf3p was specifically coeluted with NuA4 HAT activity and components from the subgradient gel filtration column (Fig. 2B), suggesting that Eaf3p is associated with the large-MDA complex.

To further demonstrate a physical association of Esa1p and Eaf3p, we used the anti-Eaf3p antibody in an immunoprecipitation assay with the peak NuA4 fraction from the Superox 6 column. Whereas incubation with preimmune serum left NuA4 activity in the supernatant, anti-Eaf3p beads brought down a

RESULTS

Eaf3p Is a Component of the S. cerevisiae NuA4 Acetyltransferase Complex—Data base comparisons have already revealed that MOF, a histone acetyltransferase of the MYST family involved in dosage compensation, shows strong sequence similarity to the essential yeast protein Esa1p (17). This similarity extends beyond the 250-amino acid core of the protein family to include a region that contains a chromodomain (Fig. 1A). Data base comparisons also revealed that MSL3, another gene product involved in Drosophila dosage compensation, shares strong similarity with a protein encoded by the yeast open reading frame YPR025C that, because of the interactions described in this study, we have named Eaf3p. As shown in Fig. 1B, these two gene products belong to a family of related proteins characterized by two chromodomain-containing regions (CRI and CRII) and three additional domains of similarity that have been designated as the EMM1, EMM2, and EMM3 boxes. The chromodomain-containing region CRI is highly related to the chromodomain found in the histone acetyltransferases MOF and Esa1p. Since Esa1p is a component of the NuA4 transcription adaptor/histone H4 acetyltransferase complex (22), we wished to investigate whether Eaf3p is also part of this complex. We partially purified NuA4 from whole cell extracts over a nickel-agarose resin, followed by Mono Q and

NuA4 Subunits Homologous to Dosage Compensation Proteins

FIG. 1. Yeast proteins homologous to Drosophila dosage compensation proteins. A, shown is a schematic representation of the S. cerevisiae Esa1p (ScEsa1p) and D. melanogaster MOF (DmMOF) proteins. The shaded areas represent the region of similarity shared by all MYST histone acetyltransferase family members (amino acids 539–792 in MOF and amino acids 163–413 in Esa1p), and the hatched boxes designate the chromodomain-containing region (CHROMO); amino acids 41–78 in Esa1p. The segment containing the conserved acetyl-CoA-binding site is enlarged (amino acids 401–438 in MOF and amino acids 41–78 in Esa1p). The segment containing the conserved acetyl-CoA-binding site is enlarged (amino acids 401–438 in MOF and amino acids 41–78 in Esa1p). The segment containing the conserved acetyl-CoA-binding site is enlarged (amino acids 401–438 in MOF and amino acids 41–78 in Esa1p). The segment containing the conserved acetyl-CoA-binding site is enlarged (amino acids 401–438 in MOF and amino acids 41–78 in Esa1p). The segment containing the conserved acetyl-CoA-binding site is enlarged (amino acids 401–438 in MOF and amino acids 41–78 in Esa1p). The segment containing the conserved acetyl-CoA-binding site is enlarged (amino acids 401–438 in MOF and amino acids 41–78 in Esa1p). The segment containing the conserved acetyl-CoA-binding site is enlarged (amino acids 401–438 in MOF and amino acids 41–78 in Esa1p).

This similarity extends beyond the 250-amino acid core of the protein family to include a region that contains a chromodomain (Fig. 1A). Data base comparisons also revealed that MSL3, another gene product involved in dosage compensation, shows strong sequence similarity to the essential yeast protein Esa1p (17). Data base comparisons have already revealed that MOF, a histone acetyltransferase of the MYST family involved in dosage compensation, shows strong sequence similarity to the essential yeast protein Esa1p (17).
significant amount of activity with a concomitant depletion from the supernatant (Fig. 3A). In the reciprocal experiment, Eaf3p was completely depleted with anti-Esa1p antibodies and was fully recovered on the beads (Fig. 3B). This clearly demonstrates the stable association of Eaf3p with Esa1p in the NuA4 complex. Preparative immunoprecipitation using anti-Esa1p antibodies was then performed to visualize NuA4 subunits as described previously (22). A silver-stained gel of the purified complex is shown in Fig. 3C. The NuA4 stoichiometric subunits are marked on the left, and two are close to the molecular mass predicted for Eaf3p, i.e. p44 and p47. In a separate report, p44 was identified as Act1p, cellular actin (38). The p47 band was excised, digested with trypsin, and analyzed by microcapillary reverse-phase HPLC/nanoelectrospray tandem mass spectrometry. The 15 peptides obtained correspond to sequences from the EAF3 gene product and cover 45% of the 401-amino acid protein (Fig. 3D, boldface). Together, these data establish Eaf3p as a stable stoichiometric subunit of the yeast NuA4 complex.

The esa1-G315E Mutation Has no Effect on Growth, yet Reduces HAT Activity—A deletion of the ESA1 gene is lethal (20, 21). In Drosophila, MOF was identified on the basis of the male-specific lethality of a Gly-to-Glu substitution at position 691 (17). In contrast, the analogous esa1-G315E mutation (Fig. 1A), representing an amino acid substitution that eliminates all HAT activity of the recombinant fusion protein (20), has no effect on viability or growth of the mutant strain. This conclusion was reached by raising the strains under haploid or homozygous diploid conditions at 18, 30, or 37 °C in complete or synthetic media containing 50 and 25% increases in cell doubling time were observed for Eaf3p and Esa1p, respectively (data not shown).

A possible explanation for the above results is that, in the context of the NuA4 complex, the mutant protein exhibits residual HAT activity at a level sufficient for normal growth. To test this possibility, we determined the effect of the mutation on the structure and HAT activity of the NuA4 complex at the Mono Q chromatographic step. The relative activities of the other HAT complexes and the presence of Tra1p in the SAGA complex (30) are useful internal controls to analyze mutation effect on the NuA4 complex. In contrast with the mutant recombinant protein, the esa1-G315E mutation allowed a significant level (10% compared with the wild type) of residual HAT activity (Fig. 4, lower versus upper HAT assay). Western analysis showed that this decrease in acetyltransferase activity was not due to a lower amount of NuA4 complex, but rather to a reduction in specific activity. The finding of residual HAT activity in the native complex, but not in the recombinant protein, is reminiscent of certain mutations in another HAT, Gcn5p (39, 40).

eaf3::KAN Has No Effect on Growth or HAT Activity—Given the lethality associated with the absence of Esa1p and the close association of Esa1p and Eaf3p homologs in Drosophila (41), we wished to determine whether a replacement of EAF3 would be lethal or at least have a severe growth phenotype. To this end, we generated a gene replacement and tested the mutant strain using the same assays as described above. No difference in growth characteristics was detected, nor were any differences detected when this mutation was combined with the esa1-G315E mutation (data not shown). Thus, the Eaf3p subunit differs from the other five subunits of NuA4 (Esa1p, Tra1p, Epl1p, Act3p/Arp4p, and Act1p) in that it is not required for cell growth (22, 38). Mention should be made that, when expressed from a strong glyceraldehyde-3-phosphate dehydrogenase promoter on multicopy plasmids, 50 and 25% increases in cell doubling time were observed for Eaf3p and Esa1p, respectively (data not shown).

The esa1-G315E mutation reduced HAT activity without affecting growth. To determine whether the eaf3::KAN (where KAN is kanamycin) mutant strain exhibits similar functional characteristics, we tested the mutant NuA4 complex from the Mono Q column for HAT activity. We found no difference in NuA4 acetyltransferase activity compared with the wild type (Fig. 4, compare middle and upper panels). We purified further the mutant complex over a Superose 6 gel filtration column (Fig. 5A) and observed that it has a smaller apparent molecular mass (peaking at fraction 22 instead of fraction 21). The de-
crease in apparent size of the complex led us to analyze its
subunit composition. A silver-stained gel of the affinity-puri-
fied wild-type and mutant complexes is presented in Fig. 5
B. From the subunits visualized on the gel, only Eaf3p is missing
in the mutant complex. Loss of only a 47-kDa protein does not
seem to justify the chromatographic difference between wild-
type and mutant NuA4 complexes. This could suggest an ar-
chitectural role of Eaf3p within NuA4 (its absence creating a

Fig. 3. Eaf3p is a stable stoichiometric component of the NuA4 complex. A, immunoprecipitation of NuA4 activity with anti-Eaf3p serum. The peak Superose 6 NuA4 fraction was incubated with preimmune serum or anti-Eaf3p beads, and equivalent amounts of input material (Initial, lane 1), supernatant (flow-through (FT), lanes 2 and 4), and beads (lanes 3 and 5) were used in nucleosomal HAT assays. B, Esa1p and Eaf3p are communoprecipitated by anti-Esa1p serum. Equivalent amounts of input material (lane 1), preimmune serum (lane 2), and anti-Esa1p beads (lane 3), as described for A, were probed with anti-Esa1p or anti-Eaf3p serum by Western blotting. C, silver-stained gel of the purified NuA4 complex. CY396 protein extract was purified using Ni²⁺-nitrilotriacetic acid-agarose and Mono Q and Superose 6 columns, followed by preparative immunoprecipitation (IP) with anti-Esa1p beads. The complex was eluted with glycine HCl and analyzed by SDS-PAGE. Specific NuA4 bands are indicated by their respective names, and asterisks mark nonspecific bands also present in the protein A control fraction (not shown). Ion trap mass spectrometry of tryptic peptides obtained from the 47-kDa/Eaf3p band identified it as the protein encoded by the yeast open reading frame YPR023C. Molecular mass standards are indicated. D, amino acid sequence of Eaf3p. The 15 peptide sequences obtained by tandem mass spectrometry analysis are shown in boldface. The two chromodomain regions are shown in italics.

Fig. 4. Eaf3p is dispensable for NuA4 histone acetyltransferase activity, whereas a point mutation in the Esa1p acetyl-CoA-binding site decreases it. Nickel-agarose eluates from wild-type (WT), eaf3Δ, and esa1-G315E yeast extracts were fractionated on a Mono Q column, and the even-numbered fractions were analyzed by nucleosomal HAT assay and Western blotting with anti-Esa1p and anti-Tra1p sera. The SAGA HAT activity and its associated Tra1p signal serve as internal controls for sample comparison. Note the low specific activity of the NuA4 complex from the esa1-G315E strain.
beads (lanes 5–7) were then analyzed by Western blotting using anti-Esa1p serum. Interacted directly with Esa1p in GST pull-down assays. 0.8 mC from the mutant complex. NuA4 subunits are indicated on the left, and molecular mass standards are on the right. From wild-type and C complexes (like in Fig. 3 type). Again, ADA HAT activity and an unrelated Tra1p-containing complex serve as effective internal controls. Normal level of HAT activity, but the downshift in the apparent molecular mass (peak in NuA4 at fraction 22 compared with fraction 21 in the wild type). Again, ADA HAT activity and an unrelated Tra1p-containing complex serve as effective internal controls. B, immunoaffinity-purified NuA4 complexes (like in Fig. 3C) from wild-type and eaf3A strains were analyzed by 10% SDS-PAGE and silver staining. Note that only Eaf3p is missing from the mutant complex. NuA4 subunits are indicated on the left, and molecular mass standards are on the right. C, the Eaf3p C terminus interacted directly with Esa1p in GST pull-down assays. 0.8 µg of recombinant Esa1p were incubated with GST beads alone or harboring the N-terminal (N-term) or C-terminal (C-term) half of Eaf3p (4 µg). Equivalent amounts of the initial sample (lane 1), supernatant (lanes 2–4), and beads (lanes 5–7) were then analyzed by Western blotting using anti-Esa1p serum.

Eaf3p Interacts Directly with Esa1p within the NuA4 Complex—In Drosophila, MOF and MSL3 appear to interact directly in that the inclusion of MSL3 in the complex is dependent on the presence of MOF (41). The fact that Eaf3p is the only component lost in the mutant complex (Fig. 5B) argues for a conserved direct physical interaction between Eaf3p and Esa1p within NuA4. To test whether Eaf3p and Esa1p physically interact, we performed GST pull-down assays using fusion proteins consisting of the N- or C-terminal halves of Eaf3p. When recombinant Esa1p was incubated in the presence of the two types of GST beads, an efficient interaction was detected with the C-terminal half of Eaf3p (Fig. 5C). These data strongly suggest that Eaf3p interacts directly with Esa1p in the NuA4 complex. This interaction may be mediated by the chromodomains in CRII and/or by one or both of the EMM boxes in the C-terminal half of the protein. Chromodomains have been proposed to be protein interaction modules, and some were shown to self-associate (reviewed in Ref. 42). However, Eaf3p interaction was dispensable for NuA4 assembly and HAT activity (Fig. 5, A and B).

The eaf3::KAN Mutation Identifies Specific Gene Targets of the NuA4 Complex—The absence of a measurable effect of the eaf3 deletion on complex assembly or HAT activity does not preclude in vivo effects on gene expression. To search for such effects, we performed Northern analysis with wild-type and mutant cells grown in rich medium and measured the mRNA levels of specific genes. The results presented in Fig. 6 indicate that PHO5, HIS4, and TRP4 mRNA levels were decreased by 50% or more in the eaf3 mutant cells, whereas GAL1 and ACT1 were unaffected. The pattern of effects on gene expression of esa1-G315E was different from that of the eaf3 deletion. The esa1-G315E cells showed reduced PHO5 gene expression, whereas the HIS4, TRP4, GAL1, and ACT1 genes remained unaffected (Fig. 6). Haploid cells harboring both esa1-G315E and eaf3::KAN mutations did not show any increased transcription defects compared with the single mutant cells (data not shown). In a separate report, we have shown that temperature-sensitive mutations in Esa1p and in the Act3p/Arp4p subunit of NuA4 affect specifically PHO5 and HIS4 gene expression (38); our experiments with the eaf3 deletion strain have identified TRP4 as an additional target of the complex. The fact that more genes are affected by the eaf3 mutation compared with the HAT domain point mutation argues for an important change in three-dimensional conformation).

Eaf3p is required for normal expression of NuA4-dependent genes. RNA was isolated from wild-type (wt; lane 1), esa1-G315E (lane 2), and eaf3A (lane 3) yeast strains that were grown to A = 1 in YPD. RNA was blotted, and the membrane was hybridized with the probes indicated on the left. Both eaf3 and esa1-G315E mutations affected PHO5 gene expression, but only the EAF3-deleted strain had decreased HIS4 and TRP4 mRNA levels. The GAL1 and ACT1 genes were not affected.
role of Eaf3p in NuA4 function in gene-specific transcription regulation. The results with the esa1-G315E mutation also underscore the importance of NuA4 in PHO5 transcription regulation and demonstrate that the effect detected with the esa1 temperature-sensitive mutants was not due to indirect cell cycle/growth effects at the nonpermissive temperature, since the esa1 mutant analyzed here has no growth phenotype.

**DISCUSSION**

The ubiquitous occurrence of numerous chromatin-remodeling complexes in eukaryotes has highlighted the existence of a new level of regulation of gene function. These specialized multiprotein regulatory complexes interact with chromatin components to control the level of transcription. It appears that these complexes are assembled using some common basic components as well as several unique components that are thought to tailor individual complexes to specific targets for particular functions (43–45). The dosage compensation or MSL complex of *Drosophila* and the NuA4 complex of *Saccharomyces cerevisiae* are distinct from other HAT complexes in their substrate specificity since they acetylate histone H4 in chromatin (11, 22). In contrast to NuA4, which can acetylate all four conserved lysines of the histone H4 tail in a nucleosome (22), the MSL complex acetylates H4 at lysine 16 specifically (11), a modification that has been correlated with an enhancement in the rate of transcription of most X-linked genes in males (9, 10). This enhancement is precisely regulated so that, on average, the steady-state level of X-linked gene messages is similar in males and females. A recent report demonstrated that the Esa1p homolog in the MSL complex (MOF) stimulates in vitro transcription through histone H4 acetylation and activates transcription in yeast when fused to the Gal4 DNA-binding domain (19). In the case of NuA4, whereas general histone H4 acetylation appears to stimulate transcription, NuA4 preferentially stimulates transcription via interaction with specific activation domains (23, 25, 26). It would therefore appear that the activity of both complexes can be modulated in response to specific transcription level requirements.

The presence of Eaf3p in the NuA4 complex, already known to include Esa1p, was suggested by the association of the homologs of these proteins in the MSL complex of *Drosophila*. A further indication was that both proteins possess a highly related chromodomain. The only other gene encoding a chromodomain-hearing protein reported in the *Saccharomyces* genome is *CHD1*, and its chromodomain is only distantly related to those of Esa1p and Eaf3p. Our data indicate that Esa1p and Eaf3p are in direct contact within the NuA4 complex and that the interaction is through the Eaf3p C-terminal region (Fig. 5C). Concurring data in *Drosophila* argues for the direct in vivo association of MSL3 with MOF and for MSL3-MOF binding together in the MSL complex through the MSL1 C terminus (41, 46). Such an association could be mediated by the interaction of chromodomains of each protein with each other. The Esa1p chromodomain could also interact with a specific region of the C-terminal portion of Eaf3p, most likely within the conserved EMM2 or EMM3 box. Alternatively, the Eaf3p CRII chromodomain could bind a specific region within the Esa1p MYST domain.

The disparate effects of the lethal esa1-KAN mutation and the esa1-G315E mutation, which is fully viable, were unexpected in light of the total lack of HAT activity in the recombiant Esa1-G315E protein produced in *Escherichia coli* (29). We found that yeast strains carrying the esa1-G315E mutation are still able to assemble normal levels of NuA4 complex and that this complex has some residual nucleosomal HAT activity. Clearly, the native interaction of the mutant protein with the other members of the complex suppresses to some degree the effect of the single amino acid substitution, as is the case for mutant Gcn5p in Ada complexes (39). The normal viability of the esa1-G315E-bearing strain is concordant with the observation that yeast can tolerate decreases in total mRNA of 20–50% without exhibiting mutant growth phenotypes (47). It is also important to note that another point mutation in the Esa1p HAT domain (L327S) destroys the HAT activity of the recombinant protein in vitro and that cells harboring that mutation are arrested at restrictive temperatures (21), a result confirming the essential role of Esa1p HAT activity in cell cycle progression.

The precise role of Eaf3p in the NuA4 complex remains unclear. An attractive current model for chromodomain-containing proteins implicates them in propagating memory of epigenetically determined transcription states (42). If such were the role of the NuA4 complex or of some of its components, the absence of a growth phenotype in the mutants analyzed in the present study could be explained. One member of the NuA4 complex, Act3p/Arp4p, is involved in epigenetic transcriptional control of a δ-element-inactivated *HIS4* gene in yeast (38). Another member, EPL1, is a homolog of *Drosophila* E(Pc), a modifier of position-effect variegation, an epigenetic regulatory phenomenon (48–50). Furthermore, it was suggested that chromodomains could play a role similar to bromodomains, another protein module found in many transcription regulators (51). Bromodomains have been deleted from proteins without apparent phenotype, but recent studies have shown that these domains bind specifically to acetylated isoforms of histone tails (52, 53). Eaf3p chromodomains may recognize acetylated H4 tails and play a similar role in stabilizing NuA4-induced transcriptionally active states. The fact that the eaf3Δ strain shows transcription defects of more genes than the HAT-weakened esa1-G315E strain suggests a similar role in stabilizing the transcription/acetylation state of chromatin at these promoters through mitosis. A 2-fold effect on transcription levels is strikingly reminiscent of the X chromosome dosage compensation in *Drosophila* males.

Other clues about Eaf3p function were obtained by identifying its homologs in other species (Fig. 1B). A strain carrying a mutation of the fission yeast homolog Alp13 is temperature-sensitive, has cell polarity defect, and is sterile (54). The closest Eaf3p homolog found in human cells is MRG15 (55). Overexpression of MRG15 results in abnormal nuclear morphologies and cell death. Furthermore, MORF4, an expressed pseudogene derived from MRG15, induces senescence in a subset of immortal cell lines, including HeLa cells. One report referred to it as the “immortality gene” (56). Strikingly, MORF4 is a truncated version lacking the N-terminal chromodomain. Based on our data, one could suggest that MORF4 still binds to a human Esa1-like protein (e.g. Tip60 (57)) through the C-terminal region. This is in complete agreement with a model arguing that MORF4 may act in a dominant-negative manner in transcription complexes involving MRG15. MORF4 could block the chromatin-modifying/binding activity of the N-terminal chromodomain of MRG15, causing major changes in gene expression and cell proliferation (55).

Our results validate the comparative approach in the study of chromatin-remodeling complexes. Further understanding of the role played by specific components such as Eaf3p in NuA4 will be obtained by additional experimental work on the MSL complex of *Drosophila* as well as by the characterization of its homologs in additional species such as Alp13 in fission yeast and MRG15 in humans.

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