Elongator is a histone H3 and H4 acetyltransferase important for normal histone acetylation levels *in vivo*

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The elongating, hyperphosphorylated form of RNA polymerase II is associated with the Elongator complex, which has the histone acetyltransferase (HAT) Elp3 as a subunit. Here we show that, in contrast to the isolated Elp3 subunit, the activity of intact Elongator complex is directed specifically toward the amino-terminal tails of histone H3 and H4, and that Elongator can acetylate both core histones and nucleosomal substrates. The predominant acetylation sites are lysine-14 of histone H3 and lysine-8 of histone H4. The three smallest Elongator subunits—Elp4, Elp5, and Elp6—are required for HAT activity, and Elongator binds to both naked and nucleosomal DNA. By using chromatin immunoprecipitation, we show that the levels of multiply acetylated histone H3 and H4 in chromatin are decreased *in vivo* in yeast cells lacking *ELP3*.

In eukaryotic cells, transcription is repressed by nucleosomes, the repeating unit of chromatin (1). Each nucleosome is composed of about 146 bp DNA wrapped around two copies of each core histone H2A, H2B, H3, and H4. Repressive chromatin structure can be altered by chromatin remodeling complexes, which can be divided into two broad groups: factors that require ATP hydrolysis to locally alter nucleosome structure, and others, such as histone acetyltransferases (HATs) and histone methylases, that facilitate transcription by posttranslational modification of the conserved amino-terminal histone tails (2–4).

Histone acetylation neutralizes the positive charge of the target lysine residue, thereby changing the overall charge distribution of the histone tails without grossly altering nucleosome structure. HAT activity is important for transcription initiation, and a number of transcriptional coactivators, including yeast Gcn5, have been shown to possess intrinsic HAT activity (5–7). Gcn5 HAT activity is required for full remodeling of, for example, the *PHO8* promoter *in vivo* (8), and can also stimulate transcription from reconstituted chromatin templates *in vitro* (9). Several HAT complexes can be recruited to promoter regions by direct interactions with DNA-binding activators, resulting in increased DNA accessibility and stimulation of transcription initiation (9–11). HATs may also assist RNA polymerase II during transcript elongation through chromatin (12). Indeed, transcript elongation is adversely affected by nucleosomes (13–15), and this inhibition can be relieved, at least in part, by histone acetylation (16).

The elongating, hyperphosphorylated form of yeast RNA polymerase II is associated with the Elongator complex (17). Holo-Elongator is an unstable six-subunit complex composed of two subcomplexes: core-Elongator, comprised of Elp1, Elp2, and Elp3 (12, 17, 18), and a smaller three-subunit module composed of Elp4, Elp5, and Elp6 (19–21). The largest subunit of Elongator (Elp1) is the homologue of the human protein encoded by *IKAP*, which is mutated in familial dysautonomia, a severe neurodegenerative disorder (22–24). Elp2 contains several WD40 repeats, and its murine homologue StIP1 was recently identified on the basis of its interaction with the transcriptional activator signal transducer and activator of transcription 3 (STAT3; ref. 25). Elp3 is a conserved member of the GNAT (Gcn5-related N-acetyltransferase) protein family, and recombinant Elp3 possesses acetyltransferase activity directed toward all four core histones in a gel-based HAT assay (12). The genes encoding Elongator proteins (*ELP* genes) are not essential in yeast, but have been shown to play a role in transcriptional activation and transcript elongation *in vivo* (17). Deletion of the different *ELP* genes results in almost identical phenotypes, which include slow adaptation to changing growth conditions, insensitivity to *Kluyveromyces lactis* killer toxin, as well as sensitivity to elevated temperature and high salt concentration (12, 17–19, 26). In addition, deletion of *ELP3* is synthetic lethal with deletion of the tail of histone H4, and confers a severe growth defect in combination with the gene encoding the HAT Gcn5 (27). The phenotypes of *elp3 gcn5* cells can be suppressed by concomitant deletion of the genes encoding the histone deacetylases *HDA1* and *HOS2*, suggesting that Elp3 functions as a HAT *in vivo* and is important for an enzyme-mediated balance between transcription-associated histone acetylation and deacetylation (27).

Here, we identify Elongator as a HAT specific for histone H3 and H4. Elongator can physically interact with DNA and nucleosomes, and lysine-14 of histone H3 and lysine-8 of histone H4 are the predominant targets for its acetyltransferase activity. Furthermore, we show that holo-Elongator, but not three-subunit core-Elongator, possesses HAT activity, thereby identifying a role for Elp4, Elp5, and Elp6. Finally, we show that Elongator HAT activity is important for the maintenance of normal histone H3 and H4 acetylation levels in chromatin *in vivo*.

Materials and Methods

Protein Purification. Holo-Elongator was purified as described (19). In most cases, highly purified proteins obtained by the anti-hemagglutinin (HA) antibody-affinity step were used. Holo-Elongator, core-Elongator, and fractions containing the trimeric complex composed of Elp4, Elp5, and Elp6 were obtained by anion-exchange chromatography as described (19). Briefly, holo-Elongator (≈3.5 ml) was applied onto a Mono Q HR5/5 column (Amersham Pharmacia-Pharmacia Biotech) and eluted with a 20-column volume gradient from 150 to 1,500 mM KOAc, collecting 0.35-ml fractions. Elongator-containing fractions were dialyzed against a buffer containing 40 mM Hepes-KOH (pH 7.6), 1 mM EDTA, 1 mM DTT, 20% glycerol, and 100 mM NaCl or KOAc.

Histone Acetyltransferase Assay. Histone acetyltransferase reactions (15 μl) were carried out in buffer containing 10 mM

Abbreviations: HAT, histone acetyltransferase; HA, hemagglutinin.

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Hepes-KOH (pH 7.6), 5 mM $MgCl_2$, 10 mM sodium-butyrate, 5 mM DTT, 5% glycerol, 0.25 mg/ml BSA, and 0.25 μCi (1 Ci = 37 GBq) [^{3}H]acetyl-CoA (0.25 mCi/ml, 2–10 Ci/mmol). As a substrate, reactions contained 1–5 μg HeLa core histones, 1 μg nucleosomes reconstituted with recombinant histones (a generous gift of S. Tan, Pennsylvania State University), or 5 μg synthetic peptide mimicking the first 32 (H2A), 33 (H2B), 28 (H3), or 27 (H4) amino acids of the histone amino-terminal tails, respectively. Reactions were carried out for 45–60 min at 30°C. To quantitate the amount of [^{3}H]acetate transferred onto the substrate, reactions were stopped by spotting onto P81 paper or GF/C glass microfiber filters (Whatman). Filters were washed three times in 50 mM sodium carbonate (pH 9.2), once in ethanol, allowed to dry, and subjected to scintillation counting. Alternatively, reactions were terminated by addition of SDS-sample buffer followed by SDS/PAGE using 18% Tris·HCl (acrylamide:bisacrylamide 120:1) gels or precast 16.5% Tris-Tricine peptide gels (Bio-Rad). After staining of the histones with Coomassie blue, the gels were subjected to fluorography.

For identifying the histone H3 and H4 amino acid residues that are targets for Elongator activity *in vitro*, three HAT reactions containing the relevant histone peptide were combined and incubated for 90 min at 30°C. Unincorporated [^{3}H[acetyl-CoA was removed by using a G-25 microspin column (Amersham Pharmacia-Pharmacia Biotech), and the desalted mixture was lyophilized and subjected to N-terminal automated microsequencing (28). The ^{3}H released in each cycle was determined by scintillation counting.

Electrophoretic Mobility-Shift Assay (EMSA). A 3′ end-labeled DNA fragment containing a 5S rDNA repeat (obtained by *Eco*RI digestion of plasmid pIC208-5S, a generous gift from J. Workman, Pennsylvania State University) was used as a probe. This radiolabeled fragment was also used for nucleosome reconstitution by octamer transfer (29). Holo-Elongator was allowed to bind to the (nucleosomal) DNA probe for 20 min at 30°C in a buffer containing 11 mM Hepes-KOH (pH 7.6), 0.6 mM Tris·HCl (pH 7.5), 1 mM EDTA, 5% glycerol, 5 mM DTT, 0.006% Nonidet P-40, 10 μg/ml BSA, 10 mM NaCl, and 20 mM KOAc. Binding reactions were directly loaded onto a 20 × 20 × 0.075-cm 4% polyacrylamide gel [acrylamide:bisacrylamide 29:1; 0.5× TBE (90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3)] and run in 0.5× TBE for 2.5 h at room temperature. For antibody supershift experiments, reactions contained 0.25 μl or 0.5 μl 12CA5 ascites (anti-HA antibody (30), recognizing the Elp1 C-terminal HA epitope-tag, or 8WG16 (anti-RPB1) control antibody (31).

Chromatin Immunoprecipitation. The congenic *Saccharomyces cerevisiae* strains W303-1A (32), JSY130 (*elp3*Δ::*LEU2*) (17) and JSY141 (*gcn5*Δ::*HIS3*) (27) were grown in yeast extract/peptone/dextrose (YPD) media to a density of 1–1.5 × 10^7 cells per ml and fixed in 1% formaldehyde for 15 min at room temperature. Cells were lysed in FA lysis buffer (50 mM Hepes, pH 7.5/140 mM NaCl/1 mM EDTA/1% Triton X-100/0.1% sodium deoxycholate/protease inhibitors), and chromatin was fragmented by rigorous sonication. Whole cell extract of 1 × 10^7 cells was used for immunoprecipitations with antibodies against tetraacetylated (K5, K8, K12, and K16) histone H4 or diacetylated (K9 and K14) histone H3 (Upstate Biotechnology, Lake Placid, NY). One thirtieth of immunoprecipitated and 1/20,000 of input DNA were used for analysis by quantitative PCR in the presence of 0.1 μCi/μl [α-^{32}P]dCTP. Reactions were done with 22 different primer pairs covering promoter and/or coding regions of the *RPS5, ADE5–7, tD(GUC)G1, ACT1, ADH1, ARG5–6, CYC1, PKG1, STE6, TDH3, VPS13, SSA4, PHO5, GAL10*, and *INO1* genes (exact sequences of primers are available on request). PCR products were separated on 6% TBE PAGE, and results were quantified by PhosphorImager (Molecular Dynamics). Results were normalized according to the amount of input DNA.

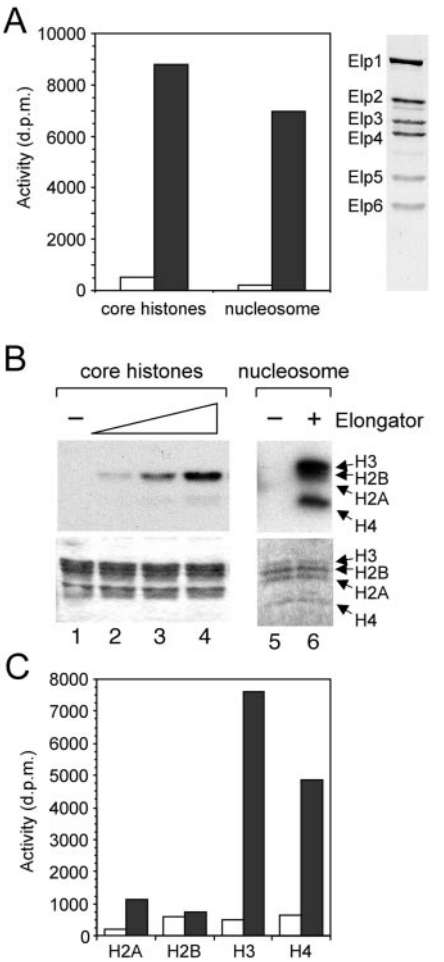

Fig. 1. Holo-Elongator is a nucleosomal histone H3 and H4 acetyltransferase. (*A Left*) HAT activity by using core histones or intact nucleosomes as substrates measured by scintillation counting. One-microgram substrate was incubated in the absence (open bars) or presence (filled bars) of holo-Elongator. (*Right*) Silver stain of the holo-Elongator fraction used in all assays. (*B Left*) HAT reactions containing increasing amounts of holo-Elongator by using core histones (5 μg) as substrates. (*Upper*) Fluorogram; (*Lower*) Coomassie-stained protein gel. (*Right*) HAT reactions using reconstituted recombinant nucleosomes (1 μg) as substrate. (*Upper*) Fluorogram of SDS/PAGE analysis; (*Lower*) Coomassie-stained protein gel. Indicated are the individual core histones. Note that reactions in the presence of core histones or nucleosomes were carried out in independent experiments by using different amounts of substrate, and different exposure times. As indicated by Fig. 1*A*, core histones and nucleosomes seem to work equally well as substrates. (*C*) HAT activity using synthetic peptides mimicking the N-terminal histone tails as substrates as measured by scintillation counting. Approximately 5 μg peptide substrate was incubated in the absence (open bars) or presence (filled bars) of holo-Elongator.

Results

Holo-Elongator Contains Nucleosomal Histone H3 and H4 Acetyltransferase Activity. Previous experiments showed that recombinant Elp3 (rElp3) has acetyltransferase activity directed toward all four core histones (12). However, because of the fact that rElp3 is insoluble, these results were obtained in an SDS gel-based assay with the histones tested individually. The availability of highly purified holo-Elongator (Fig. 1*A Right*) allowed us to investigate whether the catalytic Elp3 subunit displays HAT

activity in solution in the context of the intact Elongator complex. When holo-Elongator was mixed with core histones or recombinant nucleosomes in the presence of [^{3}H]acetyl-CoA, significant HAT activity as measured by scintillation counting was observed in both cases (Fig. 1*A Left*). Analysis of HAT reactions by SDS/PAGE and fluorography indicated that the activity of holo-Elongator was directed toward histone H3 and to a lesser extent H4, by using either histone octamers or intact recombinant nucleosomes as a substrate (Fig. 1*B*). Interestingly, when nucleosomes were used as a substrate in the HAT reactions, there was a significant increase in the relative level of histone H4 acetylation compared with core histones as a substrate (Fig. 1*B*, compare lanes 4 and 6). When synthetic peptides mimicking the N-terminal histone tails were used as substrate, significant acetylation was observed only in the case of histone H3 and H4 peptides (Fig. 1*C*), supporting the notion that holo-Elongator HAT activity is directed toward the N-terminal tails of histone H3 and H4.

We conclude that, whereas the isolated, recombinant Elp3 subunit acetylates all four core histones (12), intact holo-Elongator predominantly targets histone H3 and H4, indicating that the noncatalytic subunits of the complex are important for substrate specificity.

Acetylation Site Targets for Elongator *in Vitro*. The above results identify H3 and H4 as the major targets of acetylation by holo-Elongator. Histone H3 is also acetylated by a number of other yeast HAT complexes, such as ADA and SAGA (7), which both contain Gcn5 as their catalytic subunit, and NuA3, which contains the Sas3 catalytic subunit (7, 33). The major histone H4 acetyltransferase is the Esa1-containing NuA4 complex (34, 35). To begin to understand how these different HAT complexes might functionally interact, the preferred lysine residues acetylated by the complexes need to be determined. We determined which histone H3 and H4 tail residues are the major targets of Elongator by Edman-sequencing of the acetylated histone tails (28). Synthetic peptides mimicking the histone H3 and H4 N-terminal tails, respectively, were acetylated by holo-Elongator in the presence of [^{3}H]acetyl-CoA. After removal of nonincorporated cofactor, the peptides were subjected to amino-terminal automated sequencing, and the ^{3}H radioactivity eluting from each cycle was determined by scintillation counting (Fig. 2). A single peak corresponding to residue lysine-14 of histone H3 was observed (Fig. 2*A*), indicating that this is the predominant acetylation site for holo-Elongator *in vitro*. For the histone H4 peptide, the results were less equivocal, because of a high background count in all samples. However, the signal for lysine-8, and to a lesser extent lysine-12, was significantly above background (Fig. 2*B*).

Elp4, Elp5, and Elp6 Are Required for HAT Activity. We previously showed that three different Elongator-associated complexes are resolved by MonoQ anion-exchange chromatography: six-subunit holo-Elongator, core-Elongator composed of Elp1, Elp2, and Elp3, and a trimeric complex composed of Elp4, Elp5, and Elp6 (19). To test the HAT activity of different Elongator-related complexes, we analyzed MonoQ fractions from an Elongator purification protocol by protein silver staining and immunoblotting (Fig. 3*A*), as well as for HAT activity (Fig. 3*B*). HAT activity eluted precisely with holo-Elongator, whereas column fractions that were largely devoid of the Elp4/5/6 module showed no HAT activity, although the catalytic Elp3 subunit was present at a higher concentration in the core Elongator fractions than in the fractions containing holo-Elongator (compare fractions 42 and 48). As expected, no HAT activity was detected in fractions enriched in the Elp4/5/6 subcomplex. We have so far been unable to reconstitute holo-Elongator HAT activity by mixing core Elongator and the

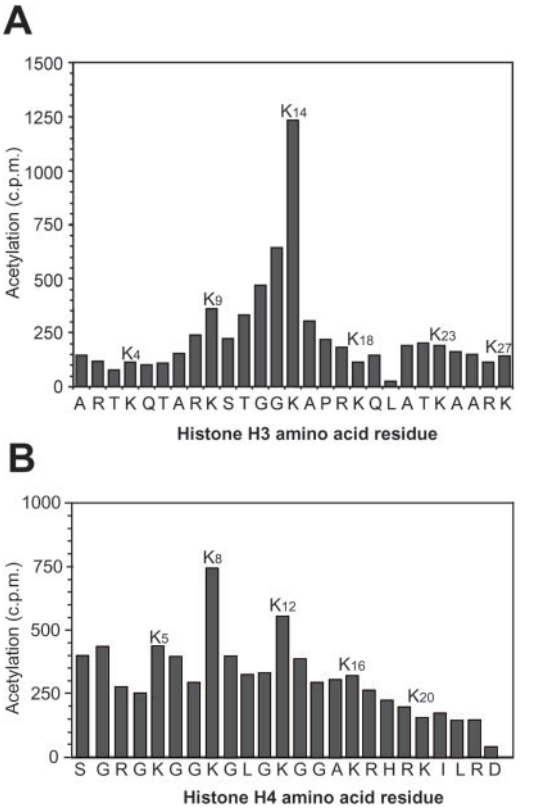

Fig. 2. Lysine-14 of histone H3 and lysine-8 of histone H4 are the predominant acetylation sites by holo-Elongator. Synthetic peptides corresponding to the N-terminal tail of histones H3 (*A*) and H4 (*B*), respectively, were acetylated by holo-Elongator in the presence of [^{3}H]acetyl-CoA. The peptides were subsequently subjected to automated N-terminal cycle sequencing and the radioactivity (^{3}H) released in each cycle was determined by scintillation counting (28).

Elp4/5/6 subcomplex, indicating that the dissociation of the complex is functionally irreversible (data not shown). Taken together, these data indicate that holo-Elongator possesses intrinsic HAT activity and that the Elp4/5/6 subcomplex is required to form an active HAT.

Holo-Elongator Physically Interacts with Naked and Nucleosomal DNA. Because holo-Elongator could acetylate the nucleosome and is tightly associated with the DNA-bound, elongating form of RNA polymerase II, we explored the possibility that Elongator might interact directly with nucleosomes and/or DNA. Increasing amounts of holo-Elongator were incubated with a labeled DNA fragment, and the reaction products were analyzed by nondenaturing PAGE. After autoradiography, several distinct mobility-shifts of the DNA fragment were observed in a manner dependent on holo-Elongator concentration, suggesting that multiple Elongator complexes could bind to the same DNA fragment (Fig. 4*A Left*). Both poly(dI-dC) and plasmid DNA could compete for binding, further indicating that the binding was not sequence specific (data not shown). To analyze nucleosome binding by holo-Elongator, histone octamer transfer was used to reconstitute a nucleosome on the labeled DNA fragment. When increasing amounts of holo-Elongator were incubated with the nucleosomal probe, concentration-dependent mobility shifts were again observed (Fig. 4*A Right*). Compared with naked DNA, a smaller fraction of probe appeared to be shifted, in all likelihood because of the large excess of unlabeled nucleosomes used for the octamer transfer reaction.

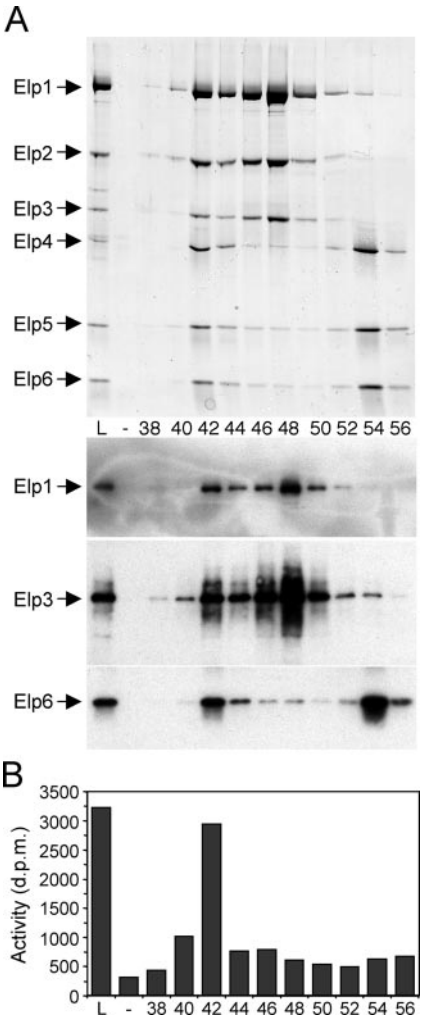

Fig. 3. Elp4, Elp5, and Elp6 are required for HAT activity. (*A Upper*) Silver stained protein gel of the MonoQ column fractions indicated (19). (*Lower*) Immunoblot analyses of MonoQ column fractions using anti-Elp1, anti-Elp3, and anti-Elp6 antibodies, respectively (19). (*B*) HAT activity of MonoQ column fractions by using core histones (5 μg) as substrates measured by scintillation counting. The MonoQ load (L, purified holo-Elongator), and the fractions used are indicated, alongside a control reaction (−) carried out in the absence of Elongator.

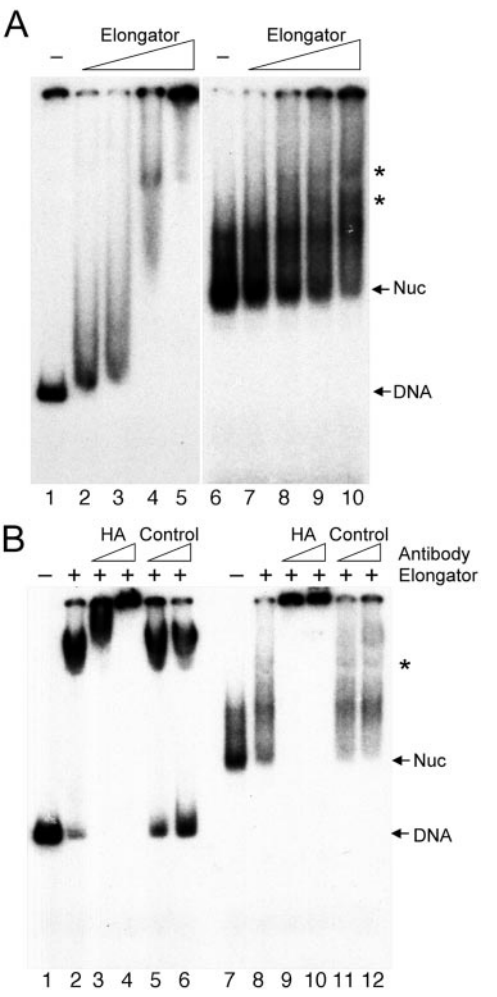

Fig. 4. Holo-Elongator can interact with both naked and nucleosomal DNA. (*A Left*) Gel-shift experiment by using a naked DNA probe. (*Right*) Gel-shift by using a reconstituted nucleosomal probe. Increasing amounts of holo-Elongator were used for binding to identical amounts of labeled naked and nucleosomal DNA. Asterisks indicate mobility shifts of the nucleosomal probe. (*B*) Antibody supershift experiment by using naked (*Left*) and nucleosomal DNA (*Right*). Increasing amounts of anti-HA antibody or a control monoclonal antibody were present during binding. The positions of the free naked DNA probe and of the unbound, labeled nucleosome probe are indicated. An asterisk indicates mobility shift of the nucleosomal probe.

To verify that Elongator and not a contaminant in the preparation was responsible for DNA and nucleosome binding, antibody-supershift experiments were carried out (Fig. 4*B*). The DNA probe was incubated with holo-Elongator in the presence of increasing amounts of either anti-HA monoclonal antibody (recognizing Elongator via the Elp1 C-terminal HA-tag), or a control monoclonal antibody (Fig. 4*B Left*). A change in mobility was observed only when anti-HA antibodies were present during binding. A similar result was obtained when a nucleosomal probe was used (Fig. 4*B Right*). These data indicate that holo-Elongator possesses intrinsic DNA and nucleosome binding activity.

Elongator Is Important for the Maintenance of Normal Acetylation Levels *in Vivo*. The above data demonstrate that holo-Elongator can interact with and acetylate nucleosomes on histone H3 and H4 *in vitro*. We next sought to investigate the relevance of these activities *in vivo* by analyzing the histone acetylation levels by chromatin immunoprecipitation, which allows an assessment of the acetylation status of histones in many different chromosomal regions. After formaldehyde crosslinking and cell lysis of wild-type, *elp3*, and *gcn5* (control) cells, histone–DNA complexes were immunoprecipitated by using antibodies specific for di-acetylated (K9 and K14) histone H3, or tetraacetylated (K5, K8, K12, and K16) histone H4. Subsequently, the amount of precipitated DNA corresponding to 20–22 different chromosomal regions (see *Materials and Methods* for a list of loci analyzed) was determined by quantitative PCR and normalized to input DNA. A histogram representing the results of the analysis using the antibodies directed against acetylated histone H3 is shown in Fig. 5*A*. In wild-type cells, a relatively broad distribution of histone H3 acetylation levels was observed. In the *elp3* mutant, both the average level and the distribution of acetylation levels were affected, in a manner indicating a significant decrease in the amount of multiply acetylated histone H3. The change in the H3 acetylation pattern in *elp3* was comparable to that observed in *gcn5* cells, albeit slightly less pronounced. Interestingly, whereas histone acetylation was more strongly affected in some genomic regions compared with others, no clear clustering of acetylation in promoter or coding regions of genes was observed in either

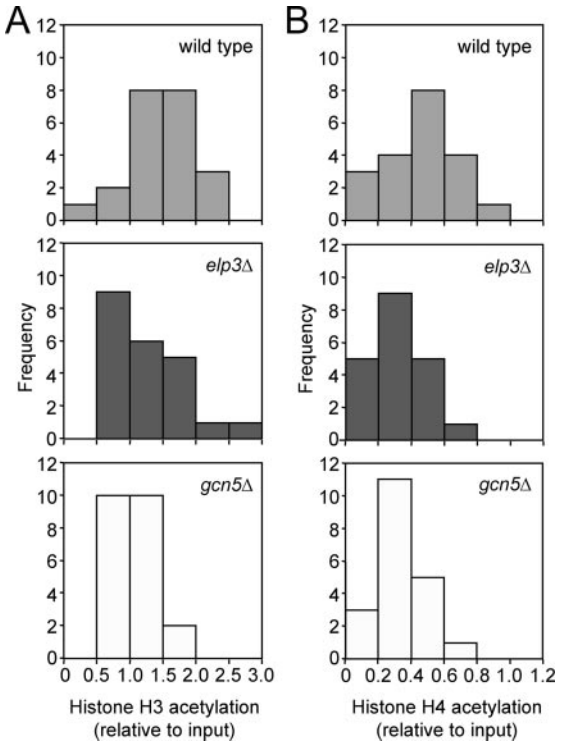

Fig. 5. Histone H3 and H4 are hypoacetylated in *elp3*Δ cells. (*A*) Chromatin immunoprecipitation analyses of the relative level of multiply acetylated histone H3 in 22 different chromosomal regions using antibodies specific for diacetylated histone H3. (*B*) Chromatin immunoprecipitation analyses of the relative level of multiply acetylated histone H4 in 20 different chromosomal regions, using antibodies specific for tetraacetylated histone H4. One thirtieth of immunoprecipitated or 1/20,000 of input DNA was used for quantitative PCR. Ratios of immunoprecipitated/input DNA were calculated, sorted in groups according to their relative value, and presented together in histograms. The *x* axis represents the value groups and the *y* axis the number of genomic regions in each group. For example, only two genomic regions had a level of multiply acetylated histone H3 that had a relative value between 0.5 and 1 in wild-type cells. By comparison, 9 regions had this characteristic in *elp3* cells, and 10 regions had this characteristic in *gcn5* cells (compare second bar in each of the histograms of *A*).

elp3 or *gcn5* cells (data not shown). This finding suggests that a global role for these enzymes in acetylation of chromatin masks what specific roles they might have during transcription. A decrease in acetylation levels was also observed when histone H4 acetylation was analyzed (Fig. 5*B*). In this case, a broad distribution of acetylation levels was again observed in wild type. In *elp3* mutant cells, the level of acetylation was decreased, but the distribution of acetylation levels was not dramatically affected. Again, the apparent decrease in histone acetylation in *elp3* was comparable to that observed in a *gcn5*Δ mutant, although again slightly weaker. The more pronounced decrease observed in histone H3 acetylation compared with histone H4 acetylation is in agreement with the *in vitro* preference of both holo-Elongator and Gcn5-containing complexes for histone H3 vs. H4 (ref. 7; this work). These results indicate that holo-Elongator HAT activity is important for the maintenance of normal levels of multiply acetylated histone H3 and, to a lesser extent, H4 in chromatin *in vivo*.

Discussion

Here, we identify Elongator as a HAT specific for the N-terminal tails of histone H3 and H4. The predominant site of acetylation *in vitro* is lysine-14 of histone H3 and lysine-8 of histone H4. Holo-Elongator can interact with and acetylate nucleosomes, and also acetylates core histones. Furthermore, we show that the recently identified Elp4, Elp5, and Elp6 subunits are required for Elongator HAT activity. Finally, we demonstrate a significant reduction in the level of multiply acetylated histone H3 and, to a lesser extent, H4 in *elp3* cells, which is comparable to that observed in the *gcn5* mutant.

Previously, we showed that the recombinant Elp3 subunit displays acetyltransferase activity toward all four core histones in a gel-based assay using individual histones as a substrate (12). In this report, we demonstrate that, by contrast, holo-Elongator specifically acetylates histone H3 and H4. In addition, we demonstrate that holo-Elongator, but not the core complex composed of Elp1, Elp2, and Elp3, possesses HAT activity. Together, these results indicate an important role for the noncatalytic subunits, including the recently identified Elp4, Elp5, and Elp6 proteins (19–21), in substrate recognition and/or the structural organization of the Elongator complex. The activity of the yeast holo-Elongator complex is similar to that of the human Elongator complex (22): both yeast and human Elongator predominantly acetylate histone H3, but also acetylate histone H4 to a lower level.

In agreement with a role during transcription of chromatin templates, we found that Elongator interacts with both naked DNA and DNA wrapped in nucleosomes. Surprisingly, we have so far been unable to chromatin immunoprecipitate significant amounts of DNA by using antibodies directed toward Elongator (A.K., unpublished data). This result might indicate that the Elongator epitopes tested so far are inaccessible in nucleoprotein complexes formed during transcription, or that the Elongator-DNA interaction is too transient *in vivo* to allow efficient formaldehyde crosslinking.

The *in vitro* specificity of the holo-Elongator HAT complex is in good agreement with the observed decrease in acetylation levels of histone H3, and to a lesser extent H4, in *elp3* cells. The finding that Elongator is important for acetylation of histone H3 in chromatin *in vivo* provides a rationale for the strong synthetic phenotype observed for strains lacking both *ELP3* and *GCN5* (27), in that the HAT activity of both SAGA and Elongator is directed toward the same histone substrate (ref. 7; this work). Indeed, holo-Elongator and SAGA both predominantly acetylate lysine-14 of histone H3 *in vitro*, suggesting that, in *elp3 gcn5* cells, histone H3 acetylation drops below a critical level required for normal growth. Interestingly, among the selected chromosomal regions used in our analysis, no clear clustering of acetylation in promoter or coding regions was observed in either *elp3* or *gcn5* cells. This result suggests a global role for the enzymes in maintaining normal levels of histone acetylation in chromatin *in vivo*.

The substrate-specificity of holo-Elongator also correlates well with the previously observed genetic interactions between Elongator and histone tails (27). Because normal histone H3 function becomes essential in the absence of the H4 tail (36), the synthetic lethality of concomitant deletion of *ELP3* and the histone H4 tail is likely caused by a decrease in H3 acetylation below a level that can support viability. Moreover, Elongator acetylates histone H4 to a lesser extent than H3, and deletion of *ELP3* in combination with histone H3 tail deletion accordingly causes only increased temperature-sensitivity (27). This result is in all likelihood because other HATs, such as NuA4, under these circumstances can maintain the histone H4 acetylation levels required for survival.

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