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An Rtt109-Independent Role for Vps75 in Transcription-Associated Nucleosome Dynamics▽†

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The histone chaperone Vps75 forms a complex with, and stimulates the activity of, the histone acetyltransferase Rtt109. However, Vps75 can also be isolated on its own and might therefore possess Rtt109-independent functions. Analysis of epistatic miniarray profiles showed that *VPS75* genetically interacts with factors involved in transcription regulation whereas *RTT109* clusters with genes linked to DNA replication/repair. Additional genetic and biochemical experiments revealed a close relationship between Vps75 and RNA polymerase II. Furthermore, Vps75 is recruited to activated genes in an Rtt109-independent manner, and its genome-wide association with genes correlates with transcription rate. Expression microarray analysis identified a number of genes whose normal expression depends on *VPS75*. Interestingly, histone H2B dynamics at some of these genes are consistent with a role for Vps75 in histone H2A/H2B eviction/deposition during transcription. Indeed, reconstitution of nucleosome disassembly using the ATP-dependent chromatin remodeler Rsc and Vps75 revealed that these proteins can cooperate to remove H2A/H2B dimers from nucleosomes. These results indicate a role for Vps75 in nucleosome dynamics during transcription, and importantly, this function appears to be largely independent of Rtt109.

Eukaryotic DNA is packaged into chromatin, comprised of repeating units of nucleosomes each containing two histone H2A-H2B dimers and one histone H3-H4 tetramer around which 147 bp of DNA are wrapped (29). The compact nature of chromatin severely impinges on processes occurring on DNA, such as replication, repair, and transcription. For transcription, several important mechanisms for relieving this inhibition exist, including covalent modification of histones, incorporation of histone variants, and the action of ATP-dependent chromatin remodelers (34). In recent years, a more drastic method to enable efficient transcriptional initiation and elongation has emerged: nucleosomes can be completely disassembled at promoters and, to a lesser extent, within the coding region of genes (70, 73). Nucleosome eviction occurs at most active genes and is often proportional to the transcription rate (5, 33, 76). In contrast to the case with promoters, loss of chromatin structure in the body of genes is highly transient, and in the wake of RNA polymerase II passage, nucleosomes are rapidly reassembled. This process is required to prevent the transcription machinery from having inappropriate access to cryptic sites of initiation within genes, which can result in spurious transcriptional events (36).

Originally thought to be simple histone binding proteins involved in intracellular histone movement and storage and in replication-associated chromatin assembly, histone chaperones are now known to also be involved in all aspects of transcription-related chromatin dynamics (47). This includes nucleosome disassembly at promoters (1, 2, 68) and within coding regions (57), as well as nucleosome reassembly in the wake of RNA polymerase II (RNAPII) passage to prevent internal transcription initiation (22, 25, 39, 57). Furthermore, histone chaperones are likely to promote enrichment of certain histone variants involved in transcription: for example, Chz1 preferentially binds H2A.Z-H2B over H2A-H2B dimers and may be involved in deposition of this histone complex into nucleosomes flanking nucleosome-free promoters throughout the yeast genome (38). In addition, it has been suggested that mammalian HIRA has a preference for the major histone variant H3.3, which is a key substrate for replication-independent chromatin assembly (65). Recent evidence also implicates histone chaperones in covalent modification of histones. For example, nucleosome assembly protein (NAP)-domain containing factors are able to stimulate the histone acetyltransferase (HAT) activity of the p300 coactivator complex (61). Conversely, TEF1/SET, another NAP family member, is a member of the INHAT complex, which inhibits the HAT activity of p300 and PCAF (60). Finally, histone chaperones appear to function in combination with ATP-dependent chromatin-remodeling factors, for example, to promote nucleosome disassembly (37, 68). The importance of these factors is

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TABLE 1. *Saccharoymyces cerevisiae* strains used in this study

Name	Genotype	Source or reference
W303 1A	*MAT***a** *leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-1*	
JSY1099	W303 1A *VPS75-8His-2TEV-9Myc*::*URA3*	59
JSY1082	W303 1A *8His-2TEV-9Myc-VPS75*::*URA3*	This study
JSY1083	W303 1A *8His-2TEV-9Myc-VPS75*::*URA3 rtt109*Δ::*HIS3*	This study
JSY1076	W303 1A *vps75*Δ::*HIS3*	59
PHY2193	*MAT***a** *his3-*Δ*200 leu2-3,112 rpb1*Δ*187*::*HIS3 ura3-52* (*pC6; rpb1-104 and pRS416*)	21
JSY1101	*MAT***a** *his3-*Δ*200 leu2-3,112 rpb1*Δ*187*::*HIS3 ura3-52* (*pC6; rpb1-104 and pRS416*) *vps75*Δ::*TRP1*	This study
JSY110	W303 1A *dst1*Δ::*HIS3*	J. Fellows
JSY1086	W303 1A *dst1*Δ::*HIS3 vps75*Δ::*TRP*	This study
JSY1102	W303 1A *rtt109*Δ::*HIS3*	This study
BY4741	*MATa his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0*	71
BY4741 *vps75*Δ	BY4741 *vps75*Δ::*KANMX4*	71
BY4741 *haa1*Δ	BY4741 *haa1*Δ::*KANMX4*	71
BY4741 *vps75*Δ*haa1*Δ	BY4741 *haa1*Δ::*KANMX4 vps75*Δ::*URA3*	This study
FY2452	*MAT***a** *ura3-52 his3 RPB3-HA1*::*LEU2 KANMX6-PGAL1-FLO8-HIS3*	44
FY2445	*MAT***a** *ura3-52 his3 RPB3-HA1*::*LEU2 spt2*::*KANMX6 KANMX6-PGAL1-FLO8-HIS3 SPT6-FLAG*	44
JSY1100	*MAT***a** *ura3-52 his3 RPB3-HA1*::*LEU2 KANMX6-PGAL1-FLO8-HIS3 vps75*Δ::*URA3*	This study
JSY1103	*MAT***a** *ura3-52 his3 RPB3-HA1*::*LEU2 KANMX6-PGAL1-FLO8-HIS3 rtt109*Δ::*URA3*	This study

highlighted by their apparent redundancy: in yeast, many nonessential histone chaperones have multiple, overlapping roles in transcription (for a review, see reference 47).

The histone chaperone Vps75 was originally identified in global genetic screens to identify factors involved in vacuolar sorting (6) and telomere maintenance (3). Sequence comparisons revealed that this gene belonged to the NAP domain family of histone chaperones, and its product was shown to bind histones and assemble nucleosomes in vitro (59). The cellular functions of Vps75 have also begun to be elucidated. Of particular note, this protein forms a complex with, and stimulates the activity of, Rtt109, the HAT that was identified as the elusive modifier of lysine 56 on histone H3 (11, 17, 67). This modification occurs on newly synthesized histone H3 molecules and is required for chromatin assembly during DNA replication and repair (8, 35). In addition, acetylation of histone 3 lysine 56 (H3K56ac) plays important roles in telomeric silencing (40, 75) and transcriptional activation (52, 69, 74). However, despite strongly stimulating H3K56ac in vitro, deletion of *VPS75* does not significantly affect bulk H3K56ac in vivo or result in sensitivity to drugs affecting replication, phenotypes that are typical of cells lacking Rtt109 (11, 59). These data suggest that Vps75 may have alternative cellular roles, an idea that was supported by two recent reports describing a catalytic function for the Rtt109/Vps75 complex in acetylation of lysine 9 on histone H3 (4, 14).

In this study, we used an unbiased genetic approach as a basis to more thoroughly investigate the function of the Vps75 histone chaperone. This strategy revealed numerous interactions between *VPS75* and genes encoding factors involved in transcription. Genomic and biochemical assays were then used to show that Vps75 mediates transcription-associated histone exchange, a function that appears to be independent of Rtt109-mediated histone acetylation. Mechanistically, Vps75-mediated histone exchange might occur in combination with ATP-dependent chromatin remodeling, since Vps75 and the well-known chromatin remodeler RSC can induce partial disassembly of nucleosomes in vitro. These data highlight a new role for Vps75 in transcription, distinct from its function as a cofactor for the Rtt109 HAT.

MATERIALS AND METHODS

Yeast manipulation. Genotypes of *Saccharomyces cerevisiae* strains used in this study are listed in Table 1. Tagging and deletion of genes was done using standard yeast genetic methods (details are available on request). Epistatic miniarray profile (E-MAP) analysis was performed as described previously (55). To test for sensitivity to acetic acid, yeast strains based on BY4741 were used. To analyze intragenic transcription of the *GAL*::*FLO8-HIS3* gene, deletions of *VPS75* and *RTT109* were made in strain FY2452 (44). 5′:3′ RNA ratios were measured in *vps75*Δ and *rtt109*Δ strains based on W303-1A. G_1 arrest was achieved by addition of α-factor (1 μg/ml) for 2 h.

ChIP. Chromatin immunoprecipitation (ChIP) analyses were performed as described previously (59). Chromatin was immunoprecipitated with 9E10 (monoclonal antibody against the Myc tag of Vps75), 12CA5 (monoclonal antibody against the hemagglutinin (HA) tag of histones H2B and H3), and polyclonal antibodies specific for the carboxy terminus of histone H3 (a kind gift of Alain Verreault) or for histone H3 acetylated at lysine 56 (Upstate). To measure recruitment of Vps75 to induced genes, the amount of precipitated DNA was divided by the amount in the input sample and corrected to a nontranscribed region (telomeric DNA on chromosome 6) and, in most cases, the equivalent signal from an untagged strain. Primer sequences are available on request.

To determine the genome-wide distribution of Vps75 (ChIP-chip), chromatin immunoprecipitates were prepared and hybridized to Affymetrix *S. cerevisiae* whole-genome forward tiling arrays (Tiling 1.0F array; P/N 520286) as described previously (45). Two replicates were hybridized to separate whole-genome tiling arrays and normalized to the signals obtained from their own respective supernatant (unbound fraction) controls (also hybridized on separate tiling arrays).

Microarray and RNA analyses. Cells were grown in yeast extract-peptone-dextrose (YPD) medium to 1×10^7 cells/ml at 30°C. RNA was prepared using a Qiagen RNeasy minikit, labeled with One Cycle target labeling (Affymetrix), and hybridized to oligonucleotide arrays (GeneChip Yeast Genome 2.0 arrays; Affymetrix) using standard techniques. Three independent experiments were performed and an average change in expression calculated.

For determination of 5′:3′ RNA ratios at various genes in mutant and wild-type yeast strains, RNA was extracted as described above and quantitated by reverse transcriptase PCR using the ABsolute QPCR SYBR green reagent (ABgene) with Multiscribe reverse transcriptase (Applied Biosystems) and a Bio-Rad MyIQ iCycler. Primer sequences are available on request.

Nucleosome disassembly assays. Nucleosome disassembly assays were carried out as described previously (37). His-tagged Vps75 and Nap1 proteins were

prepared as described previously (67, 72). The Vps75-His expression vector was a kind gift from P. Kaufman.

Microarray data accession number. Microarray data (MIAME compliant) are available at http://bioinformatics.picr.man.ac.uk/vice/ExternalReview.vice?k=kIa2QDT0u51tX4IShdNgz8ZB8rPdQErmOq91xD1ROtFjULbx4Q8rgHVoBngxfus4yWcTKjqbgOjm%0D%0A4aujRwrhww%3D%3D. ChIP-chip data were deposited in NCBI's Gene Expression Omnibus (12) and are accessible through GEO Series accession number GSE15607 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE15607).

RESULTS

VPS75 genetically interacts with RNAPII and other factors involved in transcription and chromatin function. Almost all research on Vps75 to date has focused on the mechanism by which it regulates Rtt109-mediated histone acetylation (14, 18, 19, 67), but little is known about whether it plays other roles in the cell. To gain a more complete understanding of the function of *VPS75*, we analyzed a high-density E-MAP generated using synthetic genetic array technology (9). Each gene on the E-MAP possesses a genetic interaction profile that describes its interactions with all other genes on the map. The E-MAP profile thus provides a high-resolution phenotype, and functionally related genes often have similar interaction profiles. Interestingly, even though Rtt109 and Vps75 are physically associated, the corresponding mutants gave very different genetic interaction profiles that were in fact weakly anticorrelated (Pearson correlation coefficient [CC] = −0.38) (Fig. 1A). It has previously been shown (9) that deletion of *RTT109* gives a profile that is similar to those seen with mutants known to function in DNA repair/replication (e.g., for *ASF1*, CC = 0.838) (Fig. 1A). In contrast, *VPS75* generally interacts with factors linked to transcriptional regulation, as shown by clustering of the most strongly interacting genes by their gene ontology term (Table 2). For example, deletion of *VPS75* was associated with significant growth defects when combined with mutations in components of the Mediator complex, the SAGA and NuA4 HATs and SET3 histone deacetylase, the RSC and ISW1B ATP-dependent chromatin remodeling complexes, and forkhead transcription factors, which influence the elongation phase of transcription (Fig. 1B) (41). Strong negative interactions were also observed with a DAmP allele of *RPO21* (54), encoding the largest subunit of RNAPII, and with *DST1*, which encodes the archetypal transcription elongation factor TFIIS. Conversely, the most striking positive interaction (e.g., suppression) was observed with mutants of the Elongator HAT complex, which functions in such diverse processes as transcription and tRNA modification (64). Other transcription-related genes displaying positive genetic interactions with *VPS75* included *UBP3* and *BRE5*, which encode factors involved in RNAPII deubiquitination (32), members of the Rpd3C(L) histone deacetylase complex, a component of the INO80 chromatin remodeling complex (*ARP8*), and *IWR1*, a factor of unknown function which interacts with RNAPII (31) (Fig. 1B). Collectively, these genetic data suggest that Vps75 directly functions in pathways contributing to transcriptional control.

To verify the E-MAP data and to further investigate the putative function of Vps75 in transcription, we examined two of the interactions in more detail by performing growth assays. In the first experiment, a deletion of *VPS75* was constructed in the slow-growing *rpb1-104* strain, which expresses a modified Rpb1 protein containing just 11 repeats of the C-terminal domain heptad repeat (YSPTSPS) (43). Even though deletion of *VPS75* itself had little or no consequence for growth, it negatively affected the growth of *rpb1-104* such that the *vps75*Δ *rpb1-104* double mutant grew more slowly than either single mutant alone (Fig. 1C). We next tested the functional relationship between *VPS75* and *DST1*. Deletion of *DST1* (encoding TFIIS) causes sensitivity to 6-azauracil (6-AU), a drug that depletes intracellular nucleotide pools and thereby inhibits transcript elongation (42). Interestingly, even though the *vps75*Δ single mutant grew normally on 6-AU, a *vps75*Δ *dst1*Δ double mutant exhibited significantly enhanced sensitivity to 6-AU compared to that of the *dst1*Δ single mutant (Fig. 1D). These results confirm and extend the E-MAP data and further indicate that *VPS75* influences transcription by RNAPII.

Vps75 is recruited to genes following their activation. Previous work showed that Vps75 is associated with chromatin at both active and inactive genes and at telomeric DNA (59), potentially pointing to an involvement in multiple chromatin-related processes, including replication, telomere silencing, and transcription. To more specifically examine its putative role in transcription, the presence of Vps75 at *GAL10*, *PHO5*, and *HSP104* before and after gene induction was determined by ChIP using a strain expressing Myc-tagged Vps75 from its native chromosomal location. Vps75 was detected at each of these genes even under repressive conditions (59) (data not shown). Upon induction of *GAL10* by addition of galactose, however, Vps75 density in both the promoter and open reading frame (ORF) increased markedly (Fig. 2A). A similar increase in density was also consistently observed at *HSP104* after heat shock (Fig. 2B) and at *PHO5* in response to phosphate starvation (Fig. 2C), suggesting that Vps75 recruitment to genes following transcriptional activation is a general phenomenon. Furthermore, the rapid rate of Vps75 recruitment to *GAL10* and *HSP104* was comparable to that previously reported for RNAPII (57, 58), suggesting that Vps75 is directly involved in the transcription process. This idea was reinforced by the observation that rerepression of *GAL10* (by glucose addition) and *HSP104* (by reverting to growth at room temperature) caused a loss of Vps75 from these genes to background levels (Fig. 2A and B, respectively).

The finding that Vps75 is associated with sites of active transcription and can be rapidly recruited to a gene following induction prompted us to examine whether this occurs in the context of a Vps75/Rtt109 complex. Initially, we attempted to analyze the occupancy of TAP-tagged Rtt109 at the induced *GAL10* and *HSP104* genes by ChIP, but despite repeated attempts, a signal for Rtt109 above the background was never observed (data not shown). This is consistent with a previous study which reported no binding of Rtt109 to the promoter of the activated *PHO5* gene (69) and also appears to be compatible with the finding that Rtt109 acetylates free, but not nucleosomal, histones (19, 67). As an alternative approach, *RTT109* was therefore deleted in the strain expressing tagged Vps75 to investigate whether Vps75 recruitment requires Rtt109. Interestingly, Vps75 was recruited to *HSP104* and *PHO5* in the absence of Rtt109 (Fig. 2B and C), suggesting that the function of Vps75 at active genes may be unrelated to histone H3 acetylation and certainly that it does not require

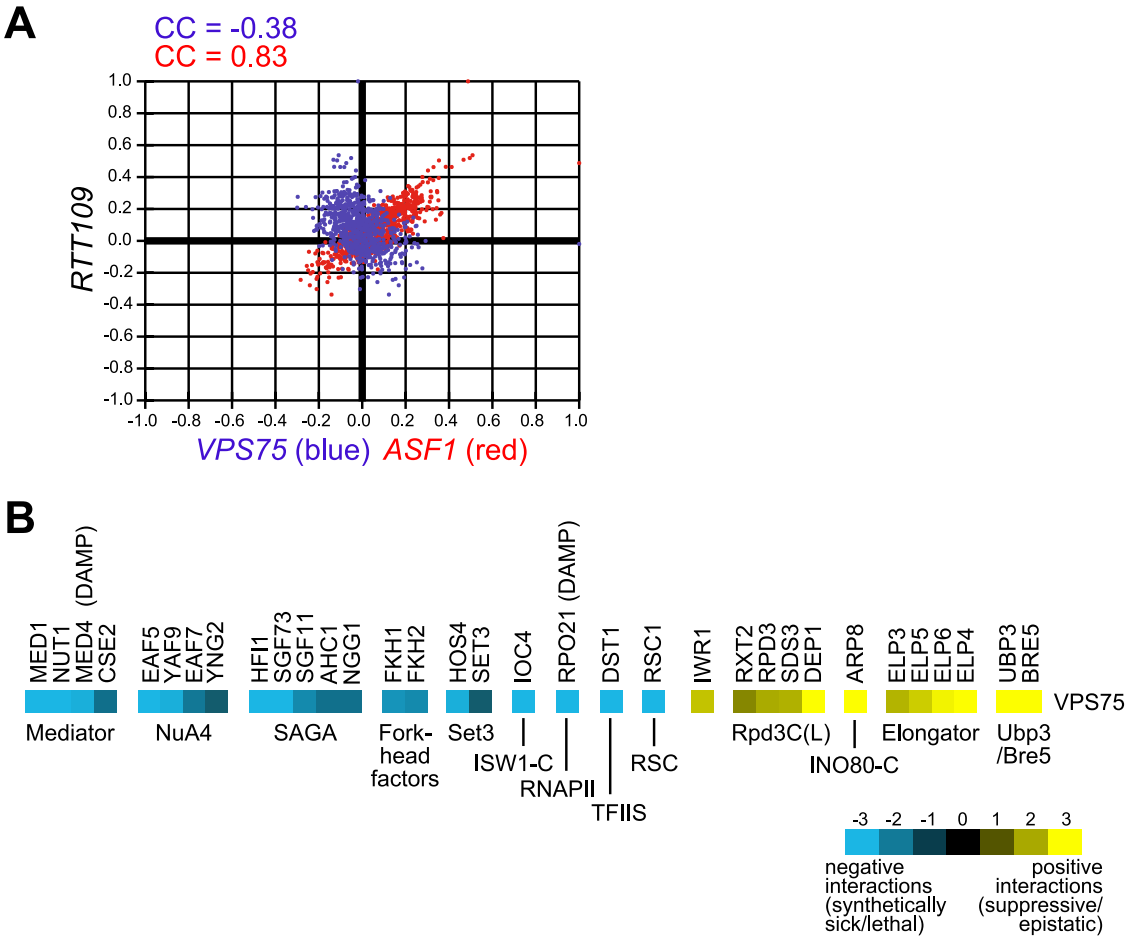

FIG. 1. *VPS75* interacts genetically with genes involved in transcription. (A) Scatter plot of the CCs of *vps75*Δ, *asf1*Δ, and *rtt109*Δ with all genetic profiles from the chromosome function E-MAP (9). The profile of *RTT109* correlates with that of *ASF1* (red) but not that of *VPS75* (blue). (B) A subset of the genetic interaction profile for *VPS75* as determined by E-MAP analysis, showing strongly interacting genes involved in transcription. Blue and yellow represent negative and positive genetic interactions, respectively. The DAmP (decreased abundance by mRNA perturbation) technique was used to generate hypomorphic alleles of essential genes (54). (C) *VPS75* interacts genetically with a conditional allele of the gene encoding Rpb1, *rpb1-104*. Serial dilutions of the indicated strains were grown on YPD at 30°C for 2 days. (D) Deletion of *VPS75* enhances the 6-AU sensitivity of a *dst1*Δ strain. Serial dilutions of the indicated strains were grown on medium lacking uracil (−U) or −U containing 50 μg/ml 6-AU (−U + 6-AU) at 30°C for 2 days.

concomitant recruitment of Rtt109. There did appear to be slightly less Vps75 at these activated genes in the absence of Rtt109, but this was within the error of the experiment. However, we cannot completely rule out the possibility that recruitment of Vps75 following gene induction may be somewhat affected by Rtt109.

An alternative possibility is that Vps75 in a complex with Rtt109 is more stable than Vps75 alone, since others have shown that Rtt109 is degraded more quickly in the absence of Vps75 (14). To test this idea, we examined whether the expression of Vps75 changes during cell cycle progression. The rationale behind this experiment relates to the observation that the production of Rtt109 is tightly coordinated with the cell cycle so that it peaks just before S phase, ensuring that newly

TABLE 2. Five most statistically overrepresented gene ontology (GO) terms scored by GOstat analysis of top 100 genes (top 50 positive and top 50 negative) interacting with *VPS75* in an epistatic miniarray profile

GO term	GO category	No. of genes[a]	*P* value
Chromosome organization	GO:0051276	41	1.45e−26
Transcription, DNA dependent	GO:0006351	38	3.29e−25
RNA biosynthetic process	GO:0032774	38	3.29e−25
Transcription from RNA polymerase II promoter	GO:0006366	30	3.32e−25
Transcription	GO:0006350	38	1.41e−22

[a] Number of genes (out of 100) present in gene ontology group.

synthesized H3 molecules are maximally acetylated at K56 prior to deposition on replicated DNA (11). Thus, if Vps75 stability is linked to the presence of Rtt109, we might expect that Vps75 levels would increase coordinately with those of Rtt109. Cells were arrested in G_1 with α-factor, and samples were then taken at different time points after release. Since an antibody to Rtt109 is currently unavailable, we used the cyclin Clb2 as a marker of cell cycle progression: this protein exhibits a peak of expression during G_2-M, approximately 20 min after that of Rtt109 (11). Unlike Clb2 and Rtt109, the level of Vps75 remained stable throughout the cell cycle (Fig. 2D), suggesting that its stability is unaffected by complex formation with Rtt109 and showing that it must exist without Rtt109 much of the time. This is in agreement with the finding that the majority of Vps75 is purified without a partner (59) and further supports the hypothesis that Vps75 possesses functions distinct from its involvement in Rtt109-mediated histone acetylation.

Given its apparent involvement in transcription, we tested whether Vps75 interacts with RNAPII. After immunoprecipitating Vps75, we were unable to detect copurifying RNAPII (Rpb1) either before or after treatments that result in significant changes in overall cellular transcription (for example, galactose addition or heat shock) (data not shown). This suggests that these proteins do not directly interact. A recent study

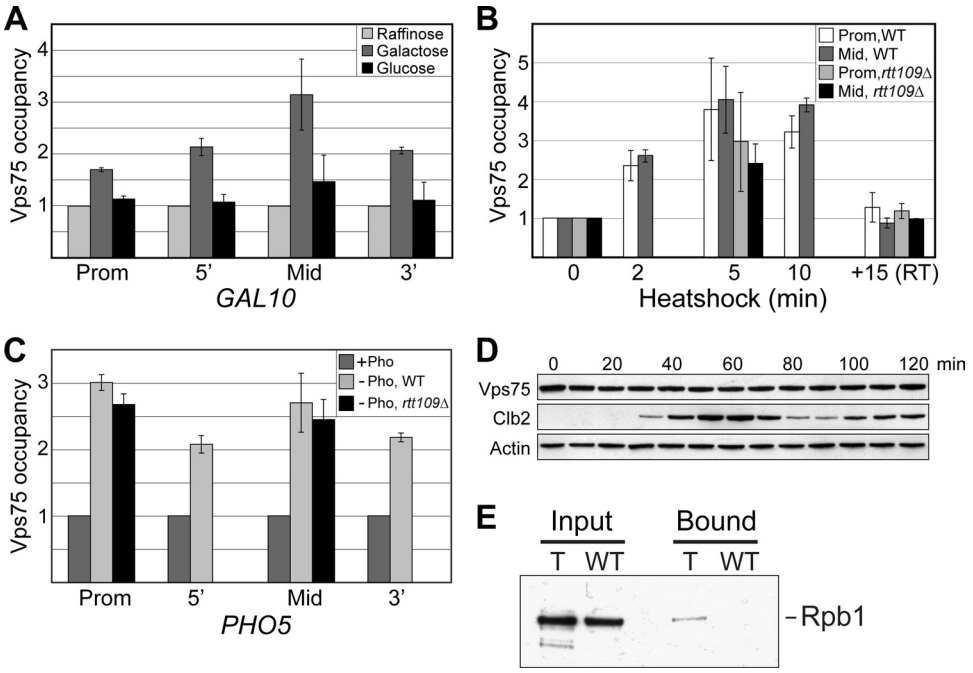

FIG. 2. Vps75 is physically associated with sites of active transcription, and its expression remains constant throughout the cell cycle. (A) Vps75 is recruited to *GAL10* following galactose induction. Cells expressing a tagged form of Vps75 were grown in medium containing raffinose at 30°C, and *GAL10* was induced by the addition of galactose for 1 h. Glucose was then added and the cells grown for a further 20 min to repress *GAL10*. The occupancy of Vps75 at the promoter (prom), 5′ end (5′), middle (mid), and 3′ end (3′) of *GAL10* in raffinose, galactose, or glucose was measured by ChIP followed by quantitative PCR. Occupancy in raffinose was set to 1. Values shown are the averages, with standard errors, of four independent experiments. (B) Vps75 is recruited to *HSP104* following heat shock. Wild-type (WT) or *rtt109*Δ cells expressing a tagged form of Vps75 were grown in YPD at 30°C, switched to 39°C for 10 min to induce *HSP104*, and then returned to room temperature for 15 min [+15 (RT)] to repress the gene. At the indicated time points, cells were cross-linked and the level of Vps75 at the *HSP104* promoter (Prom) or ORF (Mid) was measured by ChIP followed by quantitative PCR. Occupancy prior to heat shock (0 min) was set to 1. Values shown are the averages, with standard errors, of three independent experiments. (C) Vps75 is recruited to *PHO5* following induction by phosphate starvation. Wild-type (WT) or *rtt109*Δ cells expressing a tagged form of Vps75 were grown in YPD, spun down, and resuspended in either YPD (+Pho) or medium lacking phosphate (−Pho). After 2 h of induction, the occupancy of Vps75 at the promoter (Prom), 5′ end (5′), middle (Mid), and 3′ end (3′) of *PHO5* was measured as described above. Occupancy in YPD was set to 1. Values shown are the averages, with standard errors, of four independent experiments. (D) Expression of Vps75 does not change during the cell cycle. A strain expressing Vps75-Myc was arrested in G_1 with α-factor and then released. Samples were taken at the indicated time points for Western analysis. Clb2 is shown as a cell cycle-regulated G_2-M marker, and actin as a loading control. (E) Vps75 can be cross-linked to Rpb1. Cells from a Vps75-Myc (T) or untagged wild-type (WT) strain were cross-linked with formaldehyde and chromatin prepared as for ChIPs. Extracts were immunoprecipitated with 9E11 (anti-Myc) and coprecipitating Rpb1 identified by Western analysis following reversal of cross-links.

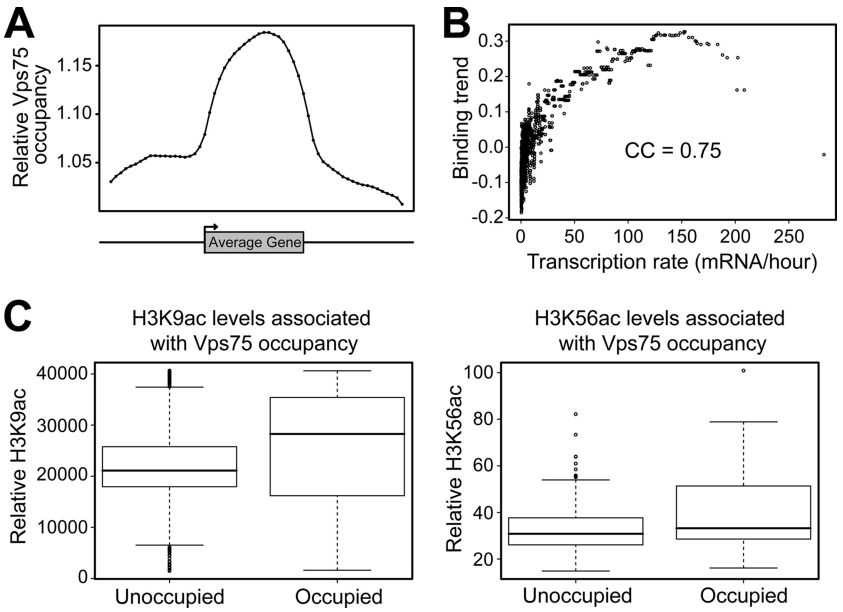

FIG. 3. Genome-wide analysis of Vps75 binding sites reveals an association with transcription and histone acetylation. (A) Composite profile of Vps75 occupancy (detected by ChIP) across the average gene. The ends of genes were defined at fixed points according to the positions of transcriptional start and termination sites. The gene and the flanking (upstream and downstream) regions were then subdivided into 20 regions each. For each of the 60 intervals, a mean Vps75 occupancy was calculated and plotted. (B) Vps75 occupancy correlates with the transcription rate genome-wide. The Vps75 binding trend was determined as described previously (44) and plotted against the transcription rate for all yeast genes (20). The Pearson CC is shown. (C) H3K9ac and H3K56ac levels are higher at sites of Vps75 occupancy. Box plots show histone H3K9 (left) and H3K56 (right) acetylation levels at Vps75-occupied and -unoccupied regions, generated by comparing peaks of Vps75 occupancy with published maps of histone acetylation (26, 50). The line in the center of each box represents the median value of the distribution, and the upper and lower ends of the box are the upper (25th) and lower (75th) quartiles, respectively.

identified new factors involved in transcription by characterizing proteins copurifying with RNAPII after formaldehyde cross-linking (66). To determine if Vps75 could be similarly cross-linked to RNAPII, cells were treated with formaldehyde, and Vps75 was immunoprecipitated after extensive DNA sonication. Rpb1 was detected in the immunoprecipitates from cells expressing tagged Vps75 but not in a control extract from untagged cells (Fig. 2E). This indicates that Vps75 is in close proximity to RNAPII, such that it can be cross-linked to it either directly or via DNA, RNA, or other proteins. These data further support the idea that Vps75 is involved in RNAPII transcription.

Vps75 localizes primarily to sites of active transcription and is associated with higher levels of histone H3 lysine 9 (H3K9)/H3K56 acetylation. The apparent link between Vps75 and active transcription prompted us to examine its global distribution on chromatin in relation to genes. To achieve this, genome-wide localization studies by ChIP-chip analysis were performed. We found 895 different chromosomal loci enriched for Vps75 (see data set S1 in the supplemental material). Approximately 65% of these loci were in the coding region of protein-coding genes, supporting the idea that Vps75 is targeted to sites of transcription. A composite profile of Vps75 occupancy aligned according to the location of transcriptional start and termination sites at all protein-coding genes containing at least one Vps75 binding site confirmed that it is tightly associated with RNAPII transcription (Fig. 3A). Of the remaining peaks of Vps75 occupancy, we found an unexpectedly high number at tRNA genes (62, compared to 5.7 expected by chance alone) and at snRNA and snoRNA genes (12, compared to 2.4 expected), suggesting that Vps75 might participate in transcription by RNA polymerase III as well (see data set S1 in the supplemental material). In addition, peaks of Vps75 were underrepresented at telomeres (0, compared to 17.1 expected) and transposable elements (2, compared to 29.0 expected) (see data set S1 in the supplemental material).

Given that Vps75 is enriched at sites of active transcription, we hypothesized that its distribution across the genome might relate more closely to gene activity than to gene function. To test this idea, we analyzed the relationship between its genome-wide occupancy and the transcription rate for all RNAPII-transcribed yeast genes (20). Indeed, we calculated a strong correlation (Pearson CC = 0.75) between the transcription rate and Vps75 occupancy (Fig. 3B), providing further support for a general role in transcription by RNAPII.

Since others have shown that Vps75 facilitates H3K9ac in vivo and H3K56ac in vitro (4, 11, 14, 67), we compared genome-wide maps of these histone modifications (26, 50) to Vps75 occupancy. A Wilcoxon rank sum test was used to assess whether Vps75-occupied and -unoccupied regions showed differences in the levels of H3K9 and H3K56 acetylation. This analysis revealed significantly greater H3K9ac at sites of Vps75 occupancy (Fig. 3C) ($P = 2.521 \times 10^{-11}$). Given that Rtt109/Vps75 acetylates only nonnucleosomal histones (19), this may simply reflect the fact that Vps75 is found at highly transcribed genes, the chromatin of which is often hyperacetylated. However, it is also possible that this overlap exists because Vps75 facilitates acetylation of H3 immediately prior to its deposition

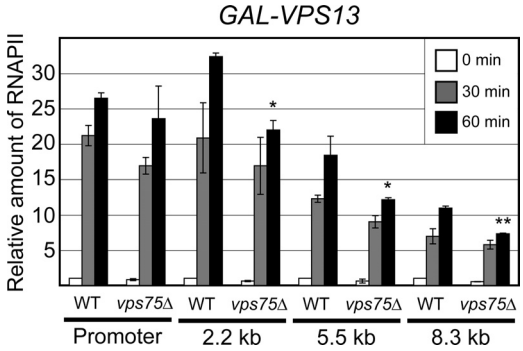

FIG. 4. Induction of *GAL-VPS13* is affected by Vps75. Recruitment of RNAPII to the promoter and through the coding region (2.2 kb, 5.5 kb, and 8.3 kb) of *GAL-VPS13* was measured by ChIP assays followed by quantitative PCR in wild-type (WT) and *vps75*Δ cells after 0, 30, and 60 min of induction. Values shown are the averages, with standard errors, for three independent experiments and are expressed as the amount of immunoprecipitated DNA corrected to input DNA. The value for the wild-type strain at 0 min was set to 1 and other values expressed relative to that. An unpaired *t* test was used to determine the significance of the difference between mutant and WT cells at each location after 60 min, with one asterisk denoting a *P* value of <0.05 and two asterisks denoting a *P* value of <0.01.

onto chromatin. A minor but still statistically significant ($P = 0.044$) increase in H3K56ac was observed at peaks of Vps75 (Fig. 3C), suggesting a connection between Vps75 and this histone mark in vivo.

Transcriptional defects in the absence of Vps75. If Vps75 functions in transcription, we might expect that the absence of this factor would affect the expression level of a subset of genes. To test the potential effect of deleting *VPS75* on transcription, we first analyzed RNAPII density in a long, galactose-inducible gene, *GAL-VPS13* (30). As described previously, RNAPII is rapidly recruited to the promoter and is detected throughout the coding region of this transcription unit as the gene is activated (Fig. 4). In the absence of Vps75, a slight reduction in RNAPII levels was observed at the *GAL-VPS13* promoter (Fig. 4). However, the decrease was more pronounced in the coding region of the gene. For example, after 60 min of activation, the density of RNAPII at the promoter in *vps75* was approximately 89% of that for the wild type. However, in the coding region (at 2.2, 5.5, and 8.8 kb), it was only 68, 66, and 66%, respectively. This suggests that Vps75 can affect the movement of RNAPII along the chromatin of the *GAL-VPS13* gene.

The transcription of other inducible, shorter genes, including *GAL10*, *PHO5*, and *HSP104*, appears to be largely unaffected in cells lacking Vps75 (see below; also data not shown), possibly due to the high level of redundancy in the yeast histone chaperone system (47). The observation that *VPS75* deletion has a very modest phenotype may also reflect the functional redundancy of these factors. To identify genes which require Vps75 for normal expression, the transcription profiles of wild-type and *vps75*Δ cells were compared using Affymetrix microarrays. The expression of 57 genes (approximately 1% of the genome) was altered by a factor of 2 or more (data not shown), while 30 genes were changed by at least 2.5-fold in *vps75*Δ (Table 3). Of these genes, the products of eight are yet to be experimentally verified and the function of most of the remainder is largely unknown. Interestingly, however, many of the differentially expressed genes are predicted to encode membrane proteins, including the most significantly upregulated gene, *HSP30*, which encodes the integral plasma membrane heat shock protein in yeast.

To verify the microarray results, we measured the expression of five of the affected genes by reverse transcription followed by quantitative PCR. This independent analysis confirmed the data obtained in the microarray study (Table 4). To determine whether the expression of these same genes was regulated by Rtt109, we also quantitated RNA isolated from *rtt109*Δ cells by reverse transcription followed by quantitative PCR. Of the five genes tested, four were unaffected while the expression of *BSC1* (significantly upregulated in the absence of Vps75) was repressed in *rtt109*Δ cells (Table 4). This further highlights the distinct functions of Vps75 and Rtt109.

Bioinformatics analysis indicated that the Vps75 regulon is strikingly similar to that of Haa1, a transcriptional activator that controls numerous stress-related membrane proteins (27). However, whereas loss of *HAA1* resulted in downregulation of these genes, the opposite was the case in *vps75*Δ cells. Five of the ten genes most significantly downregulated in *haa1*Δ cells, namely, YGR138C (*TPO2*), YPR157W, YLR297W, YER130C, and YER037W (*PHM8*), were upregulated by a factor of ≥2.5-fold in cells lacking *VPS75*. Moreover, expression of *SPI1*, another gene whose expression was increased in *vps75*Δ cells, was also shown to require Haa1 in a separate study (63). These data could potentially be explained if expression of *HAA1* itself was increased in *vps75*Δ. However, *HAA1* expression was largely unaltered (1.38-fold upregulated) in the mutant cells. Many of the genes controlled by Haa1, including those upregulated in response to *VPS75* deletion, are required for adaptation to growth in the presence of weak acids (13, 63). Interestingly, the *VPS75*-regulated *HSP30* gene is also induced by sorbic acid, in a manner that is likely to be independent of Haa1 (56). To test the idea that *VPS75* is somehow involved in regulating the acid response, we analyzed the growth of cells lacking *VPS75* or *HAA1* on medium containing acetic acid. As expected, the *haa1*Δ strain was very sensitive to acetic acid, but in contrast, *vps75*Δ cells grew better than the wild-type control (Fig. 5A). Interestingly, a *vps75*Δ *haa1*Δ double mutant exhibited essentially wild-type growth rates on 80 mM acetic acid (Fig. 5A). These data support the idea that Vps75 and Haa1 have opposing roles in controlling the expression of genes responsible for resistance to acid stress and, more specifically, that Haa1 is required to overcome the negative effect of Vps75 on expression of these genes.

When analyzing the genomic location of genes affected in *vps75*Δ cells, we noticed that 9 of the 30 genes (30.0%) changed by ≥2.5-fold were clustered together (Table 5). This phenomenon is most striking on chromosome 4, where three consecutive genes (YDL037C, YDL038C, and YDL039C) and another gene within 14 kb (YDL048C) were upregulated in *vps75*Δ (Fig. 5B). This observation was not pursued further, but it is tempting to speculate that the modified transcription rates in the affected gene clusters reflect localized changes in chromatin architecture.

Vps75 functions in replication-independent histone exchange. Given its role as a histone chaperone, we hypothesized that transcriptional changes in cells lacking Vps75 might cor-

TABLE 3. Genes differentially expressed between *vps75*Δ and wild-type yeast cells (change of ≥2.5-fold)

ORF	Gene	Fold change in expression[a]				Description
		Expt 1	Expt 2	Expt 3	Avg	
Upregulated						
YCR021C	*HSP30*	24.8	24.2	4.1	17.7	Plasma membrane protein induced by various stresses
YDL037C	*BSC1*	21.7	13.2	2.4	12.4	Transcript shows a high level of stop codon bypass
YGR249W	*MGA1*	13.5	15.4	2.1	10.3	Similar to heat shock transcription factor
YDL038C		13.8	13.1	3.1	10.0	Putative protein of unknown function
YGR052W	*FMP48*	9.2	9.2	2.4	6.9	Unknown; localizes to mitochondria
YDL039C	*PRM7*	6.5	8.6	3.0	6.0	Pheromone-regulated membrane protein
YPL014W		6.6	7.7	3.2	5.8	Putative protein of unknown function
YGR138C	*TPO2*	5.8	6.1	3.3	5.1	Polyamine transport protein of the major facilitator superfamily
YPR157W		5.2	5.3	3.6	4.7	Putative protein of unknown function
YDL048C	*STP4*	3.9	4.5	3.1	3.8	Kruppel-type zinc-finger-domain-containing protein
YER150W	*SPI1*	4.5	5.7	0.8	3.7	GPI-anchored cell wall protein involved in weak acid resistance
YOR107W	*RGS2*	3.6	4.3	3.1	3.7	Regulator of G-protein signaling for Gpa2
YLR297W		4.0	3.3	3.0	3.4	Putative protein of unknown function
YOL016C	*CMK2*	4.7	4.2	1.2	3.4	Calmodulin-dependent protein kinase
YMR316W	*DIA1*	4.3	4.2	1.6	3.4	Unknown, involved in invasive and pseudohyphal growth
YMR081C	*ISF1*	3.4	4.3	1.0	2.9	Serine-rich, hydrophilic protein with similarity to Mbr1p
YOL014W		3.4	3.5	1.0	2.7	Putative protein of unknown function
YER130C		3.1	2.8	2.0	2.6	Putative protein of unknown function
YOR306C	*MCH5*	3.0	2.8	1.8	2.5	Plasma membrane riboflavin transporter
YNL065W	*AQR1*	2.7	2.7	2.2	2.5	Multidrug transporter of the major facilitator superfamily
YER037W	*PHM8*	2.7	2.8	2.0	2.5	Unknown, possibly involved in phosphate metabolism
YOR032C	*HMS1*	3.3	2.8	1.4	2.5	Basic helix-loop-helix protein
Downregulated						
YHL048W	*COS8*	−1.8	−1.6	−4.2	−2.5	Nuclear membrane protein, member of the DUP380 subfamily
YJR079W		−2.4	−3.1	−2.1	−2.5	Putative protein of unknown function
YLR327C	*TMA10*	−2.7	−2.6	−2.2	−2.5	Unknown; associates with ribosomes
YPR158W		−2.9	−2.3	−2.4	−2.5	Putative protein of unknown function
YIL037C	*PRM2*	−4.0	−1.6	−2.5	−2.7	Pheromone-regulated protein; regulated by Ste12p
YHR177W		−3.0	−3.2	−2.2	−2.8	Putative protein of unknown function
YNL277W	*MET2*	−3.0	−3.4	−3.2	−3.2	L-homoserine-*O*-acetyltransferase
YJR078W	*BNA2*	−4.3	−3.4	−2.3	−3.3	Putative tryptophan 2,3-dioxygenase
YNL246W	*VPS75*	−16.8	−12.7	−11.6	−13.7	NAP domain histone chaperone

[a] Three independent experiments (1 to 3) were done.

relate with an aberrant chromatin structure, a notion supported by the observation that some genes affected by *VPS75* deletion were clustered together. To test this idea, we first analyzed histone levels at affected loci by ChIP assays using antibodies against endogenous histones. In all cases tested, steady-state levels of H2B and H3 were essentially unchanged at genes with modified transcription (Fig. 6A), indicating that an altered nucleosome density is not the cause of aberrant transcription at these genes in cells lacking Vps75.

TABLE 4. Validation of *vps75*Δ microarray data with reverse transcription and real-time (quantitative) PCR and comparison with expression in *rtt109*Δ cells

Gene	Fold change in expression in:[a]		
	*vps75*Δ cells		*rtt109*Δ cells (QPCR)
	Microarray	QPCR	
HSP30	17.7	14.2	1.6
BSC1	12.4	7.4	0.2
MGA1	10.3	12.9	1.4
BNA2	−3.3	−2.3	1.3
MET2	−3.2	−2.5	1.2

[a] Change in expression from that in wild-type cells. QPCR, real-time (quantitative) PCR.

Besides being deposited during replication, histones are also turned over in a replication-independent manner that appears to be at least partly proportional to the transcription rate (10, 52). We therefore speculated that Vps75 might affect histone turnover, rather than density, at certain genes. To test this idea, we performed histone exchange assays using strains containing two sources of histones H2B and H3: the untagged, endogenous protein and a HA-tagged, galactose-inducible form (^{HA}H2B and H3^{HA}) (23). In order to restrict the analysis to replication-independent exchange of histones, cells were arrested in G_1 with α-factor and induced with galactose, and the amounts of the relevant histone protein at specific loci were determined by ChIP analysis. Given that Vps75 affects the density of RNAPII in at least one galactose-inducible gene (Fig. 4), an important preliminary control experiment was to determine whether the production of ^{HA}H2B and H3^{HA} protein was affected in cells lacking this factor. Western blotting of cell extracts after galactose treatment revealed that there was no observable difference in ^{HA}H2B and H3^{HA} production between wild-type and *vps75*Δ cells (Fig. 6B).

As reported by others previously (23), the induced, HA-tagged version of H2B is rapidly incorporated into the coding region and promoter of genes in a manner that is largely independent of gene activity (Fig. 6C, left graph). Interestingly,

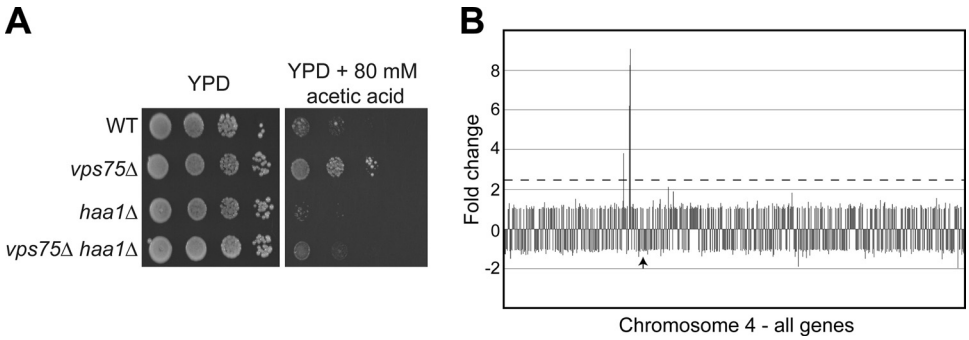

FIG. 5. Genes affected by deletion of *VPS75* belong to an acid-responsive regulon and cluster together. (A) Deletion of *VPS75* confers resistance to acetic acid. The indicated strains were grown overnight in YPD, spotted as 10-fold serial dilutions on YPD or YPD containing 100 mM acetic acid, and incubated at 30°C for 1 to 3 days. WT, wild-type. (B) Clustering of genes affected by deletion of *VPS75* in chromosome IV. The *x* axis represents the chromosome on which each gene is shown successively from left to right. The expression ratio of each gene, compared with expression of a wild-type strain, is shown on the *y* axis. The dotted horizontal line represents a 2.5-fold upregulation. The arrow indicates the centromere.

at two genes upregulated in response to loss of *VPS75*, *HSP30* and *BSC1*, a small but significant and highly reproducible increase in H2B incorporation was observed in the *vps75*Δ strain. In contrast, the much lower histone exchange in nontranscribed, telomeric DNA within chromosome VI (TEL) was unaffected by loss of *VPS75*. Moreover, H2B dynamics were also largely unaffected by *VPS75* at the induced *GAL10* gene and at *TDH3*, a constitutive, highly expressed gene. Note, however, that highly expressed genes typically have a low nucleosome occupancy, which might affect our ability to detect small changes in this assay. These results indicate that Vps75 affects histone dynamics so that in its absence, new H2B incorporation is increased at certain genes.

Interestingly, the profile of new $H3^{HA}$ incorporation was significantly different from that for ^{HA}H2B at the same loci (Fig. 6C, right graph). New H3 incorporation at *HSP30*, *BSC1*, *TDH3* and TEL was essentially the same in wild-type and *vps75*Δ cells. In contrast, deletion of *VPS75* caused a significant reduction in the incorporation of new H3 molecules at the *GAL10* gene following galactose induction. This indicates that Vps75 can positively influence H3 turnover in some situations.

Taken together, these data indicate that Vps75 affects the transcription-dependent dynamics of both histones H2B and H3. The observation that Vps75 is physically associated with each of the genes exhibiting changes in new histone incorporation (Fig. 2A and 6D) and the fact that the changes at *HSP30* and *BSC1* correlate with altered transcription levels suggest that this regulation could be direct.

Given the putative involvement of Vps75 in regulating the incorporation of new H3 molecules into chromatin at the active *GAL10* gene, we compared our Vps75 chromatin occupancy map to a published map of H3 exchange (26). The rate of H3 exchange was slightly higher at sites of Vps75 occupancy (Fig. 6E), but this difference was not statistically significant as determined by a Wilcoxon rank sum test ($P = 0.5708$). This result, combined with our observation that new H3 incorporation is not affected at most genes tested in mutant cells, suggests that Vps75 does not play a significant role in regulating H3 exchange. However, we cannot rule out that in certain situations where the requirement for histone exchange factors is great, for example, when a gene is rapidly induced to a high level of expression (e.g., *GAL10*), Vps75 might have an auxiliary function in the assembly/disassembly of H3/H4.

Reassembly of chromatin in the wake of transcription is

TABLE 5. Clustering of genes affected by deletion of *VPS75*

Cluster	ORF	Gene	Fold change[a]	Chr[b]	Position[c]		Type[d]
					Start	End	
1	YDL039C	*PRM7*	6.0	4	382330	381983	Consecutive
	YDL038C		10.0	4	384078	382327	Consecutive
	YDL037C	*BSC1*	12.4	4	385584	384598	Consecutive
2	YJR078W	*BNA2*	−3.3	10	578853	580214	Consecutive
	YJR079W		−2.5	10	580198	581232	Consecutive
3	YOL016C	*CMK2*	3.4	15	296121	294778	Clustered
	YOL014W		2.7	15	299694	300068	Clustered
4	YPR157W		4.7	16	841262	842665	Consecutive
	YPR158W		−2.5	16	843258	844016	Consecutive

[a] Positive values signify upregulated genes, while negative values signify downregulated genes.
[b] Chromosome.
[c] Nucleotide position on the chromosome.
[d] Genes immediately adjacent were designated "consecutive," while those within 5 kb were designated "clustered."

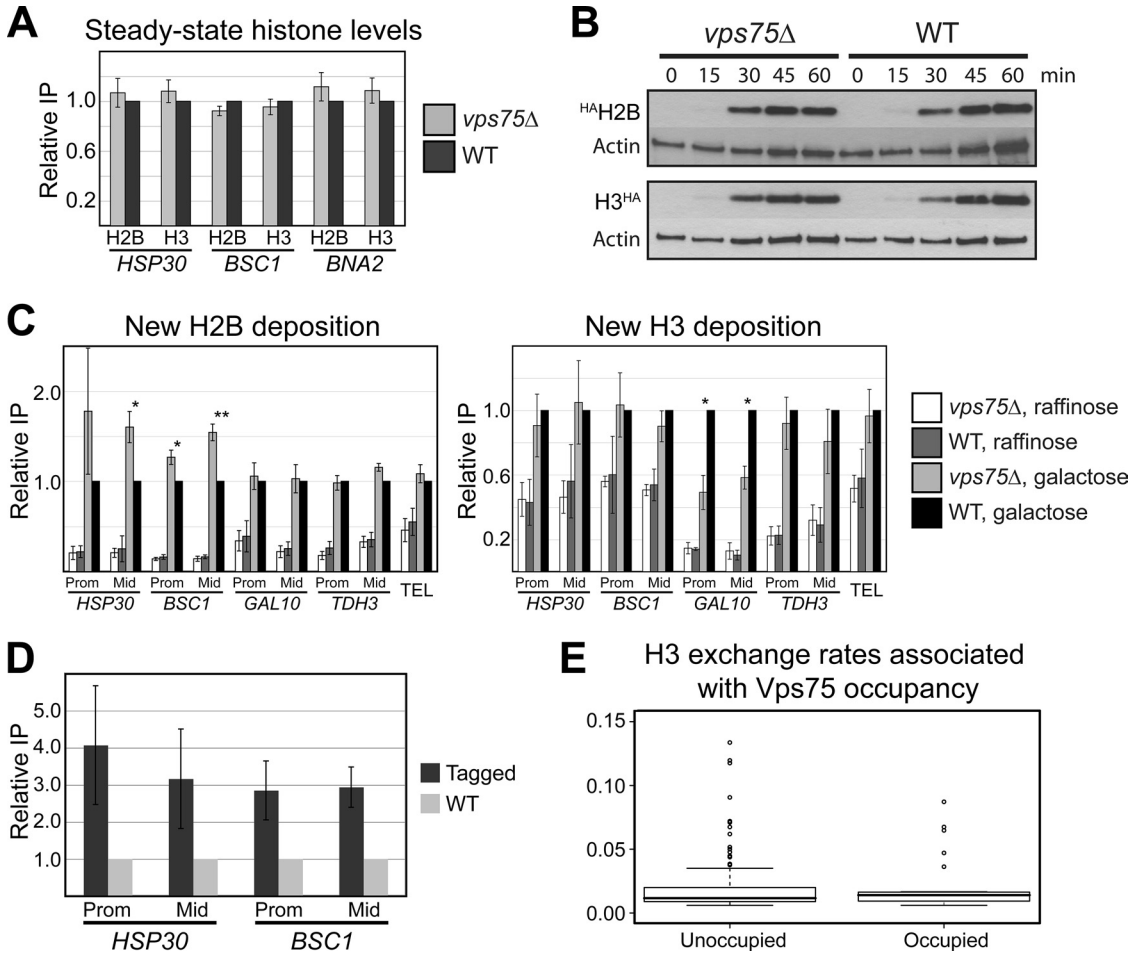

FIG. 6. Changes to chromatin in cells lacking Vps75. (A) Steady-state histone levels are not affected in cells lacking Vps75. The relative levels of histones H2B and H3 in the wild-type (WT) or *vps75*Δ strain grown in YPD were determined by ChIP assays followed by quantitative PCR. Values shown are the averages with standard errors of four independent experiments and are expressed as the amounts of immunoprecipitated DNA corrected to that of input DNA. The value for the wild-type strain was set to 1 and other values expressed relative to that. (B) Deletion of *VPS75* does not significantly affect the production of galactose-induced ^{HA}H2B or H3^{HA} proteins. Wild-type (WT) or *vps75*Δ cells were arrested in G_1 phase with α-factor for 2 h, and then galactose was added to the medium. Samples were taken at the indicated time points and cross-linked with formaldehyde. After preparation of chromatin and reversal of cross-links, proteins were fractionated on 12% polyacrylamide gels and the presence of ^{HA}H2B or H3^{HA} proteins detected by Western blotting with an antibody directed against the HA tag (12CA5). Actin is shown as a loading control. (C) Vps75 regulates replication-independent incorporation of new histones H2B and H3. Wild-type (WT) or *vps75*Δ strains containing HA-tagged, galactose-inducible forms of H2B and H3, respectively, were grown in raffinose and arrested in G_1 with α-factor. Cells were then treated with formaldehyde before (raffinose) or after (galactose) a 1-h induction with galactose. The relative level of ^{HA}H2B (left graph) or H3^{HA} (right graph) was determined by ChIP assays followed by quantitative PCR. Values shown are the averages, with standard errors, of four independent experiments and are expressed as the amounts of immunoprecipitated DNA corrected to input DNA. The value for the wild-type strain in galactose was set to 1 and other values expressed relative to that. Prom, promoter; Mid, middle; TEL, telomeric DNA on chromosome 6. A one-sample *t* test was used to determine statistical significance, with a single asterisk denoting a *P* value of <0.05 and two asterisks denoting a *P* value of <0.01. (D) Vps75 is physically associated with the *HSP30* and *BSC1* genes. Wild-type (WT) yeast or a strain expressing Myc-tagged Vps75 (Tagged) were cross-linked and subjected to ChIP using an antibody against the Myc epitope. Real-time PCR was used to detect coprecipitated DNA from the promoter (Prom) and middle (Mid) of the *HSP30* and *BSC1* genes. Values shown are the averages, with standard errors, of three independent experiments and are expressed as amounts of immunoprecipitated DNA corrected to that of input DNA. The value for the untagged strain was set to 1 and other values expressed relative to that. (E) Histone H3 exchange rates are not significantly different at sites of Vps75 occupancy on chromatin. Peaks of Vps75 occupancy were compared to a published map of histone H3 exchange (26). A box plot comparing exchange rates at Vps75-occupied and unoccupied regions is shown. The line in the center of each box represents the median value of the distribution, and the upper and lower ends of the box are the upper (25th) and lower (75th) quartiles, respectively.

required to prevent initiation from cryptic promoters within the coding regions of certain genes, with the best characterized of these being *FLO8* (25). Mutations in numerous factors involved in transcription elongation, including histone chaperones such as Spt6 and FACT, result in increased use of these promoter-like sequences, presumably by decreasing nucleosome reassembly in the wake of transcription and thereby allowing inappropriate access of transcription factors to chromatin (7, 22, 25, 39, 57). Given its role in regulating transcription-associated histone exchange, we speculated that *VPS75* might affect the level of cryptic initiation. *VPS75* was therefore disrupted in a strain expressing a galactose-inducible version of

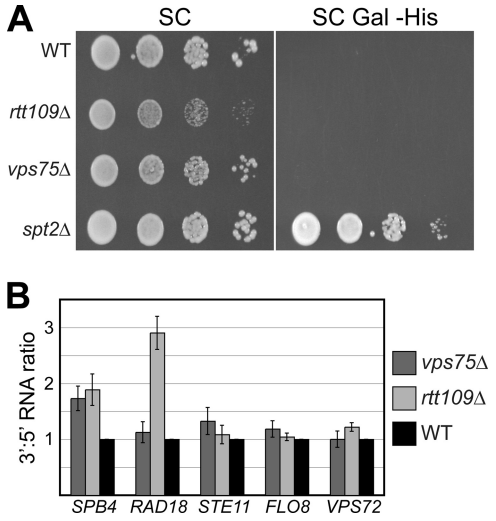

FIG. 7. Effect of deletion of *VPS75* and *RTT109* on intragenic transcription. (A) Vps75 and Rtt109 do not regulate intragenic transcription from the cryptic promoter of the *GAL1*::*FLO8-HIS3* reporter gene. The indicated wild-type (WT) and deletion strains containing the *GAL1*::*FLO8-HIS3* reporter gene were grown overnight in synthetic complete (SC) medium and spotted as 10-fold serial dilutions on SC or SC lacking histidine and containing galactose as the carbon source (SC Gal −His) and incubated at 30°C for 1 to 3 days. (B) Ratio of 3′ to 5′ RNA levels expressed from the indicated genes in wild-type (WT), *vps75*Δ, or *rtt109*Δ cells. RNA levels were normalized to an internal 18S control and represent the averages for three independent experiments, with standard errors shown. The value for the WT strain was set to 1 and other values expressed relative to that.

FLO8 in which the 3′ coding region has been replaced with the *HIS3* coding region, so that prototrophic growth on galactose medium lacking histidine is possible only if the cryptic promoter is active (44). According to this assay, *VPS75* was not required for normal chromatin reassembly within the *FLO8* coding region (Fig. 7A). Further, quantitation of mRNA from the 3′ and 5′ ends of several other genes that can initiate transcription from cryptic promoters revealed that a loss of Vps75 did not significantly increase the 3′:5′ RNA ratio at these loci either, although a weak but reproducible effect was seen at *SPB4* (Fig. 7B). These two experimental strategies were also used to assess the role of Rtt109 in transcription-coupled chromatin reassembly. The absence of this factor did not activate the cryptic promoter in *FLO8* but significantly increased the 3′:5′ RNA ratio at *RAD18* and to a lesser extent *SPB4* (Fig. 7). These data suggest that Vps75 and Rtt109 are unlikely to have a dramatic and general role in chromatin reassembly in the wake of transcription, although they may function redundantly and/or at specific loci.

Vps75 regulates activation-associated H3K56ac enrichment at *GAL10*. It was recently shown that histone eviction and transcriptional activation at *PHO5* is slowed in yeast expressing a form of histone H3 that cannot be acetylated on lysine 56 (H3K56R), suggesting that this modification can affect chromatin disassembly (69). Furthermore, two recent studies indicate that H3K56ac can aid histone deposition during replication and repair (8, 35), while global analyses of histone turnover in yeast cells lacking H3K56ac also indicate a role for this mark in stimulating histone replacement (26). While Vps75 does not appear to play an important role in stimulating H3K56ac in vivo, we could not rule out the possibility that defects in histone exchange (i.e., new histone incorporation) in cells lacking Vps75 were related to localized changes in acetylation of H3K56. To test this idea, we used ChIP with antibodies recognizing total H3 (a C-terminal epitope) or H3 acetylated at K56 to investigate enrichment of this mark at *GAL10* following activation. As reported previously (30), H3 levels were significantly reduced in both the promoter and coding region of *GAL10* following addition of galactose (Fig. 8A). The extent of chromatin disassembly was essentially the same in wild-type and *vps75*Δ cells. Interestingly, H3K56ac levels at the *GAL10* promoter increased significantly (>15-fold) following induction (Fig. 8B). This parallels the findings at the *PHO5* promoter and suggests that H3K56ac may be generally involved in chromatin assembly/disassembly during transcription. These experiments also revealed a similar although less dramatic (>5-fold) increase in H3K56ac within the *GAL10* ORF following galactose induction (Fig. 8B), indicating that this mark might play a role in histone dynamics not only during transcriptional initiation but also during elongation. Importantly, when the same ChIP assays were carried out with the *vps75*Δ strain, we observed a significant decrease in H3K56ac enrichment at both the promoter and ORF of *GAL10* compared to results for wild-type cells (Fig. 8B). These experiments indicate that Vps75 affects transcription-associated accumulation of acetylated K56 at the highly transcribed *GAL10* gene.

We next tested whether loss of Vps75 might also cause changes in H3K56ac levels at *HSP30* and *BSC1*, where changes in new H2B incorporation were observed (see Fig. 6C). However, *vps75*Δ and wild-type cells contained the same low level of acetylated K56 at these loci, and this did not change significantly following galactose induction (Fig. 8C). Therefore, the changes in H2B exchange at these genes caused by loss of *VPS75* are probably unrelated to K56 acetylation.

Vps75 and RSC can mediate removal of H2A/H2B dimers from nucleosomes. The SWI/SNF-related chromatin remodeling complex RSC can disassemble nucleosomes in vitro by a stepwise transfer of the octamer to a NAP domain histone chaperone, Nap1 (37). It has been proposed that this ability of a chromatin remodeler and a histone chaperone to remove nucleosomes might facilitate transcriptional activation (37). We previously showed that Vps75 can assemble nucleosomes in vitro (59), but given the pleiotropic effect of *vps75*Δ on nucleosome dynamics described above, we investigated another biochemical mechanism that might underlie the role of Vps75 in transcription and its apparent affect on histone/nucleosome equilibria, namely, its ability to act as a histone acceptor during chromatin remodeler-mediated nucleosome disassembly. As reported previously (37), the combined action of Nap1 and RSC resulted in a complete release of histone proteins from the nucleosome, generating naked DNA (Fig. 9A, lane 4). When incubated with nucleosomal DNA and ATP, neither Vps75 nor RSC alone could remove histone proteins from nucleosomes (Fig. 9A, lanes 1 and 3). However, when both factors were added to the reaction mixture, a band representing a nucleosome devoid of H2A/H2B dimers rapidly appeared (Fig. 9A, lane 2). The identity of this species was verified by the addition of excess dimer, which restored the

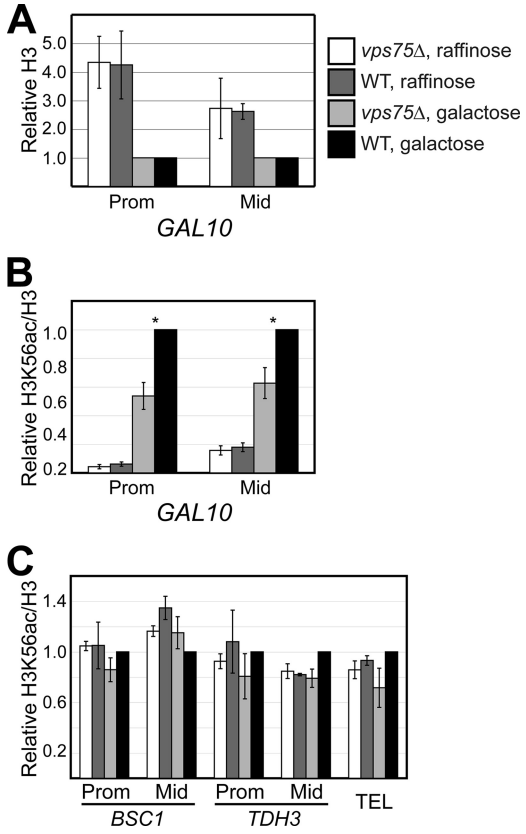

FIG. 8. Vps75 regulates transcription-dependent accumulation of histone H3 acetylated at lysine 56 (H3K56ac) at *GAL10*. (A) Loss of histone H3 at *GAL10* following induction. Wild-type (WT) or *vps75*Δ cells were grown in raffinose and arrested in G_1 phase with α-factor. Cells were then treated with formaldehyde before (raffinose) or after (galactose) a 1-h induction with galactose. The relative levels of histone H3 at the induced *GAL10* gene were then determined by ChIP assays followed by quantitative PCR. Values shown are the averages, with standard errors, of four independent experiments and are expressed as the amounts of immunoprecipitated DNA corrected to input DNA. Values in galactose were set to 1 and other values expressed relative to that. Prom, promoter; Mid, middle of ORF. (B) Defects in H3K56ac accumulation at induced *GAL10* in cells lacking Vps75. Wild-type or *vps75*Δ cells were grown and treated with formaldehyde as described for panel A. The relative levels of H3K56ac at the induced *GAL10* gene in were then determined by ChIP assays followed by quantitative PCR. Values shown are the averages, with standard errors, of four independent experiments and are expressed as the amounts of immunoprecipitated DNA corrected to that of input DNA and normalized to total histone H3 levels. The value for the wild-type strain in galactose was set to 1 and other values expressed relative to that. Prom, promoter; Mid, middle of ORF. A one-sample *t* test was used to determine statistical significance, with an asterisk denoting a *P* value of <0.05. The key is as shown in panel A. (C) Normal H3K56ac at genes exhibiting altered H2B exchange. H3K56ac levels in wild-type and *vps75*Δ cells were measured as described for panel B. The value for the wild-type strain in galactose was set to 1 and other values expressed relative to that. Prom, promoter; Mid, middle of ORF; TEL, telomeric DNA on chromosome 6. The key is as shown for panel A.

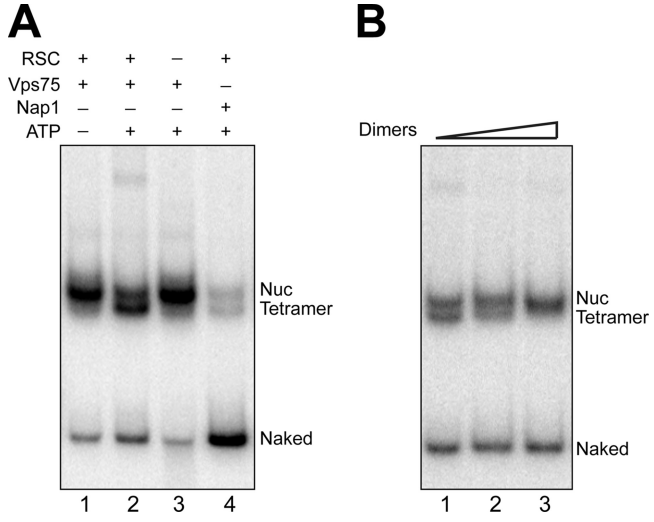

FIG. 9. Vps75 promotes loss of H2A/H2B dimers from mononucleosomes in the presence of RSC and ATP. (A) Nucleosomes were treated for 2 h at 30°C with the indicated proteins and then fractionated by gel electrophoresis. An autoradiograph of the gel is shown. Bands corresponding to nucleosomes (Nuc), nucleosomes lacking H2A/H2B dimers (Tetramer), and free DNA (Naked) are indicated. (B) Reversal of histone depletion by RSC and Vps75. After a 2-h incubation (as in lane 2 of panel A), increasing amounts of yeast H2A/H2B dimer were added and the reaction mixtures incubated for an additional 2 h at 30°C. Gel electrophoresis and autoradiography was performed as described for panel A. Bands corresponding to nucleosomes (Nuc), nucleosomes lacking H2A/H2B dimers (Tetramer), and free DNA (Naked) are indicated.

complete nucleosome (Fig. 9B). These data provide support for the idea that histone chaperones act in combination with ATP-dependent chromatin remodeling complexes to achieve transcription-associated nucleosome disassembly (37) and also provide an important biochemical basis on which to understand the diverse effects on histone dynamics observed in cells lacking *VPS75*.

DISCUSSION

An Rtt109-independent role for Vps75 in transcription. Previous studies have shown that Vps75 is a histone chaperone that acts as a potent stimulator of the Rtt109 HAT, which acetylates histone H3 primarily on lysines 9 and 56 (4, 14, 67). However, elucidating the precise function of Vps75 in vivo has been difficult. The absence of Vps75 does not affect bulk H3K56ac levels (59), suggesting that its ability to enhance Rtt109-catalyzed modification of this residue may be an in vitro artifact. This idea is supported by the finding that Asf1, another histone chaperone, is absolutely required for Rtt109-mediated H3K56ac in vivo (51). In addition, while clearly required for efficient H3K9 acetylation, it is unlikely that this is the only role of Vps75 given the pleiotropic phenotypes of *vps75*Δ cells, which include defects in telomere maintenance (3), intracellular protein sorting (6), and nonhomologous end joining (24).

In this study, we present evidence to support the idea that Vps75 functions in transcription independently of its binding partner, Rtt109. First, epistasis measurements show that it genetically interacts with many genes involved in transcription, whereas Rtt109 (and as expected Asf1) is found in a network that consists primarily of factors involved in DNA repair/replication. Second, Vps75 is recruited to genes following their

induction in the absence of Rtt109, and its genome-wide chromatin association profile correlates with transcription activity. Third, the absence of this factor deregulates transcription at a number of genes, none of which (tested thus far) are affected by deletion of *RTT109*. Finally, Vps75 affects replication-independent nucleosome dynamics at certain genes, providing a mechanism by which it might regulate transcription. This last function is also unlikely to involve Rtt109, since the absence of Vps75 promotes new H2B incorporation at certain loci (this study) whereas deletion of *RTT109* causes an overall decrease in histone exchange (26; our unpublished data). Other indirect evidence supports the idea that Vps75 is not simply a cofactor for Rtt109-catalyzed histone acetylation but has alternative cellular functions: while expression of Rtt109 is tightly regulated by the cell cycle (11), Vps75 is expressed ubiquitously (this study), meaning that it will frequently exist separately from Rtt109. This is supported by our earlier finding that purification of Vps75 yields a significant fraction of protein not complexed with Rtt109 (59).

We propose that during S phase, Vps75 exists primarily within a Rtt109/Vps75 heterodimer. At this time, the primary role of Vps75 is to enable efficient H3K9 acetylation by Rtt109, a modification that may be important for transcription of S-phase-specific genes (14). It is important to note that Vps75 facilitates histone acetylation not only by presenting the substrate to Rtt109 but also by stabilizing this enzyme (14). In addition, the possibility that the Rtt109/Vps75 HAT complex also enhances Rtt109-catalyzed H3K56 acetylation at some loci during S phase to regulate transcription and/or promote genome stability cannot be ruled out. However, during the remainder of the cell cycle, when Rtt109 levels are dramatically reduced, Vps75 functions independently in the regulation of replication-independent chromatin assembly/disassembly and possibly other, as yet undiscovered processes. Thus, future studies of Vps75 should not be limited to analyses of Rtt109-catalyzed histone acetylation.

How does Vps75 regulate transcription? Our results suggest that Vps75 functions in transcription by modifying chromatin structure. Previous biochemical data indicated that Vps75 can assemble nucleosomes (59), and here we show that it is also a potent acceptor of histone H2A/H2B dimers during RSC-mediated nucleosome remodeling. Thus, Vps75 can act as both a nucleosome assembly and disassembly factor in vitro. Our analyses of histone dynamics in yeast cells lacking Vps75 suggest that this factor also mediates equivalent changes to chromatin in vivo. At genes upregulated in response to deletion of *VPS75* (*BSC1* and *HSP30*), new histone H2B incorporation is more rapid, suggesting that in wild-type cells Vps75 promotes nucleosome stability or acts as a histone eviction factor, thereby slowing new H2B deposition. It could be argued that this observation simply reflects higher levels of transcription at these genes, which may result in increased displacement of H2A/H2B dimers (28). However, the role of Vps75 as a histone chaperone, its physical presence at these genes, and the finding that H3 turnover is not affected at *BSC1* or *HSP30* all suggest that reduced incorporation of new H2B molecules is a cause, and not simply an indirect consequence, of changes in transcription. The idea that Vps75 inhibits transcription by suppressing histone exchange is supported by a recent study, published while the manuscript of this article was undergoing review, reporting that the absence of Vps75 increased H3 turnover at certain "hot" nucleosomes (26).

Interestingly, we also identified a locus (activated *GAL10*) where loss of Vps75 reduced the rate of new H3 incorporation, suggesting that in some circumstances it facilitates H3 turnover. On the surface, this observation seems difficult to reconcile with Vps75's putative role as a negative regulator of H2B and H3 exchange (discussed above). However, accumulating evidence from in vitro assays and in vivo experiments indicates that other histone chaperones, such as Asf1, can mediate replication-independent nucleosome assembly (15, 57) and disassembly (1, 53, 57) and moreover that these seemingly opposing functions can occur even on the same gene. We propose that Vps75 can also promote both histone deposition and eviction depending on spatial context and in all likelihood associated factors. For example, Vps75 may act coordinately with chromatin remodelers at certain genes (i.e., *HSP30* and *BSC1*) to promote H2A/H2B eviction and inhibit new H2A/H2B incorporation, a hypothesis supported by our observation that RSC and Vps75 can catalyze dimer removal from nucleosomes in vitro. At other loci, Vps75 could enhance deposition of new H3/H4, either alone or in concert with other remodeling factors.

Another way that Vps75 could modify the chromatin structure to regulate transcription was revealed by our microarray data. Genes affected by deletion of *VPS75* tend to group together within the genome, implying that the transcription defects are due to localized changes in higher-order chromatin architecture. In this model, upregulation of a gene cluster would relate to a looser local chromatin structure, whereas downregulation would result from a more compact assembly. It is important to note that this idea is compatible with the observed defects in new histone incorporation in *vps75*Δ, since altered histone exchange rates may be a manifestation of changes in higher-order structure and vice versa. Interestingly, loss of Nap1 also affects clusters of genes, suggesting that proteins in the NAP domain family of histone chaperones may have a general role in maintaining higher-order chromatin structure in yeast (46). Acetylation of H4 lysine 16 is known to decompact chromatin (62), and Rtt109/Vps75 can weakly acetylate H4 tails in vitro (4), suggesting a mechanism by which this factor could also regulate the transcription of multiple consecutive genes. Future work should be aimed at investigating this possibility by genome-wide analyses of nucleosome density and positioning and histone acetylation patterns in cells lacking Vps75.

One possibility that remains to be investigated is whether the role of Vps75 in promoting new H3 incorporation at *GAL10* reflects its ability to stimulate Rtt109-catalyzed H3K56 acetylation. This is particularly pertinent since this modification was recently shown to be required for efficient promoter chromatin disassembly, and subsequent activation, of *PHO5* (69). In this study, we showed that Vps75 is required for normal enrichment of H3K56ac at the promoter and ORF of *GAL10* following galactose induction. It is tempting to speculate that Vps75-mediated deposition of H3K56ac is a direct process whereby Rtt109/Vps75 (possibly in a complex with Asf1) acetylates free H3 molecules, which are subsequently incorporated into chromatin. Such a model is particularly attractive because H3K56ac is known to facilitate nucleosome assembly on plas-

mids and replicating DNA (35) and Rtt109 was recently shown to stimulate the histone deposition activity of Vps75, albeit in a manner that appears to be independent of acetylation (4). However, given that Vps75 does not affect bulk H3K56ac levels in cells (14, 59) and that its ability to regulate H2B exchange appears to be unrelated to this modification (this study), it is equally plausible that reduced incorporation of H3K56ac at *GAL10* simply reflects the function of Vps75 as a dedicated histone exchange factor (discussed above). In other words, Vps75 may be required for disassembly of nucleosomes at *GAL10*, so that its absence would only indirectly reduce incorporation of new H3 acetylated at K56. It is important to note that these two models are not mutually exclusive and that Vps75 might regulate new H3 incorporation by stimulating histone acetylation at some genes and at others by promoting acetylation-independent histone exchange.

Another interesting observation to emerge from this study is the apparent ability of Vps75 to regulate the replication-independent dynamics of both H2B and H3. In general, histone chaperones are thought to target specific subnucleosomal components, with distinct activities being responsible for H2A/H2B and H3/H4 exchange (57). However, Vps75 can bind both H2A/H2B and H3/H4 in vitro (49, 59), and there is considerable evidence that Nap1, its closest homologue in yeast, can also act as a chaperone for all core histones and histone H1 (for a review, see reference 48). The idea that histone chaperones facilitate the transfer of both subnucleosomal histone complexes is also supported by the finding that most of these factors, including Vps75 (59), can assemble nucleosomes in vitro, a process that is likely to occur in two steps with deposition of H3/H4 (to form the tetrasome) preceding addition of H2A/H2B dimers (16). Clearly, there is still much to learn regarding the means by which histone chaperones contribute to chromatin assembly and disassembly. Having taken the first important step in identifying the majority of these factors in eukaryotes, it will now be important to elucidate these mechanistic details.

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