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Transcriptional Inhibition of Genes with Severe Histone H3 Hypoacetylation in the Coding Region

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Summary

Changes in histone acetylation at promoters correlate with transcriptional activation and repression, but whether acetylation of histones in the coding region of genes is important for transcription is less clear. Here, we show that cells lacking the histone acetyltransferases Gcn5 and Elp3 have widespread and severe histone H3 hypoacetylation in chromatin. Surprisingly, severe hypoacetylation in the promoter does not invariably affect the ability of TBP to bind the TATA element, or transcription of the gene. By contrast, similar hypoacetylation of the coding region correlates with inhibition of transcription, and inhibition correlates better with the overall charge of the histone H3 tail than with hypoacetylation of specific lysine residues. These data provide insights into the effects of histone H3 hypoacetylation in vivo and underscore the importance of the overall charge of the histone tail for transcription.

Introduction

The effect of histone acetylation on transcription has been the subject of intense study over the past few years (Eberharter and Becker, 2002; Mizzen and Allis, 1998). However, in spite of great advances in the understanding of the enzymes and mechanisms that govern this modification, only little is known about the molecular consequences of changes in histone acetylation in vivo.

Several studies have indicated a positive correlation between the level of acetylation of specific regulated gene loci and their transcriptional activity, and recruitment of histone acetyltransferases and hyperacetylation of histones in promoters often correlates with activation of genes (Eberharter and Becker, 2002; Kuo and Allis, 1998; Turner, 2000; Urnov and Wolffe, 2001). Histone hyperacetylation is thought to “open up” chromatin structure (Luger et al., 1997; Luger and Richmond, 1998) and allow access of transcription

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factors to promoters. Indeed, the association of DNA binding transcription factors with regulatory sequences can be affected by histone acetylation in vitro (Hassan et al., 2001; Vettese-Dadey et al., 1996) and in vivo (Sewack et al., 2001; Verdone et al., 2002), and upon mutation of histone deacetylases, increased acetylation in the promoter correlates with higher transcription of a limited number of specific genes (Robyr et al., 2002), suggesting that histone deacetylation by these enzymes represses transcription.

In sharp contrast to the many studies on promoter acetylation, the role of histone acetylation in the coding region of a gene in vivo is poorly understood. In vitro, nucleosomes in the path of elongating RNAPII pose a considerable obstacle to transcription (Izban and Luse, 1991; Orphanides et al., 1998), and a highly conserved histone acetyltransferase (HAT), Elongator, was originally isolated via its association with hyperphosphorylated, elongating RNAPII (Otero et al., 1999; Wittschieben et al., 1999). Moreover, nucleosomes formed from histone H3 or H4 with intact amino-terminal tails inhibit transcript elongation through a nucleosome much more than their tail-less counterparts in vitro, and acetylation of the tails suppresses this effect (Protacio et al., 2000). Recent data have provided evidence for a specific involvement of Elongator HAT activity in this process in vitro (Kim et al., 2002). Taken together, these in vitro results argue that histone acetylation in the coding region of active genes should play an important role for transcription in vivo, yet data to support this are lacking.

Until recently, it was thought that histone acetylation in response to positive transcription regulatory cues operates from a low basic level of acetylation. However, evidence from yeast indicates that regulation by induced histone hyperacetylation can be superimposed on chromatin that is already highly acetylated (Kuo et al., 2000; Vogelauer et al., 2000). This “global,” or “nontargeted,” histone acetylation by transcription-related HATs is likely to underlie the surprisingly high level of overall acetylation in bulk yeast chromatin (Waterborg, 2000), as it is significantly affected by mutations in the genes encoding the HATs Gcn5 and Esa1 (Kuo et al., 2000; Reid et al., 2000; Vettese-Dadey et al., 1996; Vogelauer et al., 2000). Whether the high level of acetylation in yeast chromatin is of any importance for the activity of the overall transcriptome has been unclear, especially because mutation of *GCN5* and *ESA1* does not appear to generally affect transcription (Holstege et al., 1998; Reid et al., 2000).

A general regulatory role for global histone acetylation is suggested by the characteristics of dosage compensation in metazoans (Cohen and Lee, 2002). In *Drosophila*, the transcription activity of the single male X chromosome is upregulated 2-fold due to the activity of the male-specific-lethal (MSL) complex, which acetylates at least histone H4 lysine 16 along more or less the entire length of this chromosome (Bone et al., 1994; Smith et al., 2001). In mammals, dosage compensation is brought about in a distinctly different manner, namely by inactivation of one of the female X chromosomes. Here, the transcriptionally inactive state correlates with chromosome-wide histone hypoacetylation of both H3 and H4 (Boggs et al., 1996; Jeppesen and Turner, 1993).

Arguing for a more sophisticated and specific regulatory role for histone acetylation in gene transcription is the idea that distinct acetylation sites in the tails of histones perform

different roles. In its most complex form, the “histone code” model proposes that distinct modifications in the tails of histones can act sequentially or in combination to form a code that is read by other proteins to bring about downstream events (Jenuwein and Allis, 2001; Strahl and Allis, 2000; Turner, 2000). The existence of such a code could provide the mechanistic tools to explain and support the long-held view that distinct acetylation patterns typify nucleosome depositioning and transcription, respectively (reviewed by Strahl and Allis, 2000). The general acceptance of these ideas is underscored by the fact that contemporary studies on the importance of histone modification during transcription regulation almost invariably make conclusions based on this model.

We used yeast cells lacking the genes encoding two transcription-related HATs, *GCN5* and *ELP3*, as a model to investigate the effects on un-induced (ongoing) transcription of significantly reducing histone acetylation in chromatin. Cells lacking both these genes display a number of growth defects that are not observed in the single mutants, and these phenotypes are due to the absence of the HAT activities associated with the Elon-gator and SAGA complexes (Wittschieben et al., 2000). These genetic data raised the possibility that histone acetylation in the *gcn5 elp3* double mutant—in contrast to the single mutants—has been brought to a critically low level with consequential reductions in gene transcription. Here, we show that yeast cells lacking both *GCN5* and *ELP3* indeed have widespread histone H3 hypoacetylation of chromatin relative to either single mutant and that active genes with severe reductions in acetylation also have reduced transcription. Interestingly, lowered transcription activity correlates very well with the average charge of the histone H3 tail on the coding region of affected genes, rather than with hypoacetylation of specific lysine residues or with hypoacetylation in the promoter of the gene. Our data suggest that histone hypoacetylation can severely affect events downstream from association of proteins with the promoter, and underscore the importance of the overall charge of the histone tail for transcription.

Results

Genetic experiments previously demonstrated that individual mutation of the histone acetyltransferase genes *GCN5* and *ELP3* only results in fairly mild phenotypes (Georgakopoulos and Thireos, 1992; Wittschieben et al., 1999), while deletion of both genes confers a number of severe growth defects. These defects include slow growth, temperature sensitivity, and an inability to ferment galactose and sucrose (Wittschieben et al., 2000). Similar phenotypes are conferred by point mutations that debilitate the HAT activities of these enzymes without affecting their incorporation into the SAGA and Elongator complex, respectively, suggesting that growth defects in *gcn5 elp3* cells occur because the HAT activities of the complexes are missing (Wittschieben et al., 2000). In agreement with an overlapping role for the HAT activities of Gcn5 and Elp3, both SAGA and Elongator target histone H3 in vitro (Grant et al., 1997; Winkler et al., 2002).

Synergistic Effects on Histone Acetylation by Mutation of *GCN5* and *ELP3*

We investigated the level of histone acetylation in wild-type, *gcn5*, *elp3*, and *gcn5 elp3* double mutants by the use of chromatin immunoprecipitation (ChIP) and Western blotting

using acetylation-specific antibodies (Figure 1). First, antibodies raised against multiply acetylated histone H3 or H4 were used to probe the “overall level of acetylation” at more than 20 randomly chosen promoters and open reading frames (ORFs) by ChIP (Winkler et al., 2002). In Figure 1A, the level of multiply acetylated H3 and H4 in chromatin in each strain is depicted. As can be seen, the use of these antibodies indicated that the single mutants had approximately 20% less di-acetylated histone H3, whereas the level in the double mutant was less than half of that in wild-type. The difference between the strains was less pronounced in the case of tetra-acetylated histone H4. Because the effect of *gcn5 elp3* double mutation as expected was more pronounced at histone H3, subsequent experiments focused entirely on this histone.

In order to achieve an even more global view of the histone H3 acetylation level in the strains, crude cell extracts from mid-log phase cells were fractionated by SDS-PAGE (Kushnirov, 2000). An antibody specific for the C terminus of histone H3 was then used to ensure that equal amounts of this histone were tested, before comparison of the acetylation level by re-probing the Western blot with acetylation-specific antibodies (Figure 1B). Using this technique, a striking decrease in the histone H3 acetylation was observed in *gcn5 elp3* double mutants, while the decrease in the *elp3* and *gcn5* single mutants was less pronounced, yet clearly detectable. Taken together, these data indicate that the level of histone H3 acetylation in *gcn5 elp3* is significantly lower than in either of the single mutants.

We next used five different highly acetylation- and site-specific histone H3 antibodies (Suka et al., 2001) to investigate the level of H3 acetylation by ChIP at individual lysine residues in individual genes. Using these antibodies, dramatic effects on acetylation were invariably observed at the large number of genes tested in the mutant cells. Generally, the *gcn5 elp3* double mutant always had significantly less H3 acetylation at one or more lysines than either single mutant. Two examples of the obtained results are shown in Figure 1C. In some cases, such as *SSA4* H3 lysine 27 (Figure 1C, upper panel), the level of acetylation was reduced in both *elp3* and *gcn5* but significantly more reduced in the double mutant. At other genes (or in the same gene at another lysine residue), the level of acetylation in *elp3* was similar to or actually higher than wild-type, yet the *gcn5 elp3* double mutant had a significantly lower level of acetylation than the *gcn5* strain (*SSA4* H3 lysine 18 is shown as an example in Figure 1C, lower panel). This indicates that the HAT activity of Gcn5 is able to (over)-compensate for the loss of Elp3 activity at least at some genes.

The data support the idea that histone H3 acetylation in *gcn5 elp3* cells has reached a critically low level, which affects basic cellular processes such as RNAPII transcription. These mutant cells might therefore represent a good model system for investigating the connection between histone acetylation and transcription in vivo.

Decreased RNAPII Density on Genes in *gcn5 elp3* Double Mutants

To investigate whether histone hypoacetylation had functional consequences for RNAPII transcription, we performed ChIP experiments with antibodies directed against RNAPII and TBP in wild-type and mutant cells (Figure 2). At the active *STE6* gene in MATa cells, RNAPII density on the coding region was only slightly reduced by individual *gcn5* or

elp3 mutation but was significantly reduced in the double mutant (Figure 2A, left panel). Interestingly, TBP density, and also RNAPII density, on the *STE6* TATA-element was less affected by the concomitant mutations (Figure 2A, right panels). However, acetylation levels at all tested lysine residues were lowered both on the promoter and ORF (examples shown in Figure 2B; see also Table 1). These data suggest that the reduced RNAPII density in the *STE6* coding region in *gcn5 elp3* cells is primarily caused by histone hypoacetylation in the coding region, leading to deficiencies in reactions downstream from loading of transcription factors on the promoter.

The conclusions drawn from the results in Figure 2 might be specific for the *STE6* gene, but could also be more general. Thus, in order to be able to better generally correlate histone H3 acetylation and transcription efficiency, we first identified genes whose transcription was affected by *gcn5 elp3* mutation. Transcription was only slightly reduced at the majority of genes and severely reduced at merely a few genes in *gcn5 elp3* cells (data not shown). Because we wanted to study the connection between histone H3 hypoacetylation and transcription, we now further characterized transcription at a mixture of affected and unaffected genes by RNAPII ChIP (Figure 3A). As expected from previous reports using ChIP for studying RNAPII elongation/transcription (Kulish and Struhl, 2001; Pokholok et al., 2002), the density of RNAPII in the coding region (ORF) of a gene as detected by ChIP nicely reflected the mRNA level as measured by Northern blotting (Figure 3B and data not shown).

Genes with Severe Histone Hypoacetylation in Their Coding Region Have Reduced RNAPII Transcription

The extensive histone H3 hypoacetylation observed in *gcn5 elp3* cells might be more or less uniformly distributed in the genome, but only affect ongoing transcription at a subset of “acetylation-sensitive” genes. Alternatively, acetylation levels might be very low only at a subset of genes, with a correlating reduction in transcription of these genes. To investigate if a reduction in acetylation at individual genes correlated with decreased transcription, we examined the level of acetylation at the five individual histone H3 lysine residues in the coding region of a number of the genes whose transcription/RNAPII ORF occupancy had previously been determined by RNAPII ChIP. Table 1 lists these genes according to decreasing transcription and the corresponding acetylation levels at individual lysine residues in *gcn5 elp3* cells compared to wild-type. Individual lysine residues that were severely hypoacetylated (less than 25% acetylation compared to wild-type) are underlined. Several key observations should be noted. First, large reductions in acetylation levels were observed at one or more lysines in all the genes tested. Second, in most genes (9 of 14), lysine 9 and lysine 27 were severely hypoacetylated in *gcn5 elp3* compared to wild-type, whereas lysine 14 acetylation was not severely reduced at any of the genes tested. Third, of the five genes whose expression was most significantly affected (bottom five genes in Table 1), all showed severe hypoacetylation at two or more of the lysines (two genes had two, two had three, and one gene had four sites of severe hypoacetylation). By contrast, of the five genes whose expression was least affected (top five genes in Table 1), only two showed severe histone hypoacetylation at one lysine, and in marked contrast to transcriptionally affected genes none were severely hypoacetylated at two or more lysines.

In general, very good correlations between acetylation and transcription were observed for lysine 9, 18, and 27, whereas the correlation between acetylation at K14 or K23 and transcription was less striking but still apparent. The “average H3 acetylation level” in the individual genes was also calculated (last column in Table 1). When plotted on a graph, transcription and H3 K27 acetylation showed an almost linear relationship (Figure 4A). Significantly, the same was true for transcription and the average acetylation level, especially at low acetylation values (Figure 4B).

Taken together, these data suggest that severe histone H3 hypoacetylation in the coding region of active genes can severely inhibit transcription of these genes in vivo.

Genes with Severe Histone Hypoacetylation in the Promoter Do Not Necessarily Have Reduced TBP Occupancy of the TATA Element

The finding that the *STE6* gene in *gcn5 elp3* cells showed reduced transcription as well as reduced acetylation in the promoter yet did not have significantly reduced TBP- or RNAPII-occupancy at the TATA element was surprising. We therefore also expanded the analysis of histone H3 promoter acetylation and TBP occupancy to a larger number of genes. Table 2 lists the results of this analysis according to decreasing TBP promoter occupancy and shows the corresponding histone H3 promoter acetylation at individual lysine residues in *gcn5 elp3* cells compared to wild-type. Not surprisingly, genes whose transcription was dramatically reduced (such as *YPG1* and *BAT1*; see Table 1) also tended to have a lower TBP promoter occupancy. However, in no case did it drop more than 2- to 2.5-fold. This was in contrast to inactive, regulated genes such as *GAL1*, whose TBP promoter occupancy was reduced more than 8-fold compared to the active state (data not shown).

As previously observed in the coding region, lysines 9 and 27 were the most dramatically hypoacetylated, but in this case to approximately the same level for all genes tested. Moreover, the average overall acetylation—whether the promoter in question had high (80% −90% of wild-type) or low TBP occupancy (40%−50%)—was also severely reduced, 4- to 5-fold, again to approximately the same level for all genes tested (last column of Table 2). All the tested promoters thus showed severe histone H3 hypoacetylation, but with no apparent correlation to TBP promoter occupancy or even the acetylation level in the coding region of the same gene (compare to Table 1). Significantly, histone H3 hypoacetylation in the promoter clearly also did not correlate with reduced transcription levels, in contrast to what had been observed for acetylation in the coding region of the same genes.

These data indicate that severely reduced histone H3 acetylation in the promoter region of a gene does not necessarily impair the ability of TBP to associate with the TATA box, or reduce transcription of an active gene.

Discussion

The results reported here add to the understanding of histone acetyltransferases and the connection between histone acetylation and transcription in the following ways. First, the activities of the transcription-related HATs Gcn5 and Elp3 are overlapping, so that the absence of one of the proteins is compensated for by the activity of the other. The data

indicate that histone H3 acetylation is brought to a critically low level in *gcn5 elp3* mutants, which affects transcription. Second, our data indicate that histone hypoacetylation inhibits transcription: a significantly lower level of histone acetylation in the coding region of several genes invariably correlated with reduced transcription of the same gene in *gcn5 elp3* cells. Third, the level of H3 acetylation in the promoter and the coding region of a gene can be dramatically different, but in our experiments only hypoacetylation in the coding region persistently correlated with reduced transcription. Fourth, transcription impairment does not correlate with hypoacetylation of any individual H3 lysine alone but rather with average overall acetylation of the tail. Fifth, the same low levels of histone acetylation that in the coding region of a gene correlated with inhibition of transcription failed to always impede the access of TBP to the TATA box of genes when imposed on the promoter region. This suggests that histone H3 hypoacetylation of promoters in itself does not necessarily create an obstacle to the association of TBP with the TATA box.

Functional Redundancy at the Molecular Level

During chromatin assembly, acetylated histones are delivered to the DNA by chromatin assembly factors. After nucleosome deposition, the acetylation mark is then rapidly removed by HDACs (Verreault, 2000). Because the dynamics of histone acetylation and deacetylation are extremely rapid in yeast (reversal of targeted acetylation occurs within 1.5 min (Katan-Khaykovich and Struhl, 2002), constant maintenance of histone acetylation in chromatin is likely to be both a consequence of and a requirement for transcription, especially in the very active genome of budding yeast. The data showing a severe effect of double *gcn5 elp3* mutation on histone acetylation in chromatin and, more importantly, ongoing transcription, thus suggest that an important global role for SAGA and Elongator is to keep chromatin transcription-permissive. For the Elongator complex, this role is more likely fulfilled via its interactions with elongating RNA polymerase II (Wittschieben et al., 1999) than via a proposed function in the cytoplasm (Pokholok et al., 2002).

Our characterization of histone H3 acetylation levels in cells lacking Gcn5 or/and Elp3 also provides a rare demonstration of functional redundancy at the molecular level. Most strikingly, acetylation at certain lysine residues in some genes was not reduced by *ELP3* deletion. Rather, it seemed to increase. However, acetylation at the same positions in the *gcn5 elp3* double mutant was much lower than observed in the *gcn5* single mutant. This indicates that Gcn5 and Elp3 are redundant for acetylation of histone H3 in chromatin and that Gcn5 activity can sometimes compensate for the absence of *ELP3*. The opposite might also be true. The recent finding that cells lacking both *GCN5* and *SAS3* are inviable further supports the notion that functional redundancy between nonessential H3 HAT activities occurs in yeast (Howe et al., 2001). It is interesting to note that a *sas3 elp3* double mutant does not have a striking new phenotype (data not shown), but the molecular implications of this finding are unclear.

Severe Hypoacetylation of Chromatin in *gcn5 elp3* Cells and the Effect on Transcription Processes

Our data suggest that only very severe H3 hypoacetylation in the coding region of an active gene correlates with effects on transcription. For example, reducing the average overall

acetylation level in the coding region to about half of that of wild-type did not show a relationship with reduced transcription, while the 4- to 5-fold reduction introduced at some genes invariably correlated with a dramatic transcription effect. Interestingly, similarly severe reductions in histone H3 promoter acetylation did not always correlate with reduced transcription, and the levels of promoter acetylation and coding region acetylation did not change together. For example, transcription at genes such as *FAB1* and *TRA1* was not significantly affected, yet their average promoter acetylation was reduced 5-fold. In contrast to the promoter, acetylation in the coding region of these genes was only slightly reduced. This suggests that histone H3 acetylation in the promoter and coding region can be deposited independently and that it can differentially affect the initial association of TBP with promoters and "postrecruitment events" such as, for example, the movement of RNAPII through DNA.

It is important to note that our results do not argue against histone acetylation being used as a regulatory cue to allow binding of TBP and other proteins to their cognate recognition sites in promoters. Indeed, several examples of such regulation exist (Sewack et al., 2001; Verdone et al., 2002). A recent study using ChIP and intergenic microarrays to generate genome-wide HDAC enzyme activity maps also clearly shows that HDACs such as Rpd3 and Hda1 deacetylate a limited number of distinct promoters and gene classes where they repress transcription (Robyr et al., 2002). What our data indicate is that although TBP occupancy of certain promoters might be regulated via changes in their H3 histone acetylation level, this is not a feature of all promoters. Interestingly, previous results showed that deletion of either the H3 or H4 tail significantly affects activation of *GAL1* and *PHO5* in vivo but that only H4 deletion exerts its effect through the TATA box (Wan et al., 1995). Taken together with the present results, this could suggest that TBP promoter occupancy is primarily regulated through acetylation of histone H4, rather than H3, but more direct evidence for such a connection is clearly required. It should furthermore be noted that although in this study we focused on histone H3, mutation of *GCN5* and *ELP3* does also affect acetylation of histone H4 (Figure 1; Winkler et al., 2002; Zhang et al., 1998), and this might contribute to some of the effects described here.

Transcription-Associated Acetylation: Site-Specific or General?

SAGA and Elongator primarily target histone H3 lysine 14 in vitro (Grant et al., 1997; Winkler et al., 2002). It is therefore somewhat surprising that acetylation at this specific position in a large number of genes studied was the position that was least affected by *gcn5 elp3* mutation among the five acetylation sites studied in the tail of histone H3. A similar result has previously been obtained at the *INO1* gene in *gcn5* cells (Suka et al., 2001). Acetylation at lysine 14 was only affected significantly at a small number of genes in *gcn5 elp3* cells (such as in the coding region of *GDH1*), whereas acetylation at (for example) positions K9 and K27 was dramatically affected in both the promoter and coding region of most genes. Whether lysine 14 is only an important site for H3 acetylation by SAGA and Elongator in vitro, or if Sas3 (which targets this position; Howe et al., 2001) and/or other HATs carry out acetylation at this residue in the absence of *GCN5* and *ELP3* remains an open question. The intriguing possibility that acetylation at lysine 14 is "reserved" for regulatory events should also be mentioned. In any case, the dramatic acetylation decrease

in *gcn5 elp3* cells at lysine sites that had not been predicted to be targets for SAGA and Elongator by in vitro work might suggest that the site-specificity of histone modifying enzymes in general should not be predicted from in vitro experiments alone, although it is important to note that predictions from in vivo work should also be taken with caution because of the possibility of indirect effects. Equally important, our results question the validity of the idea that acetylation of H3 K14 is a particular mark for transcription activity as has been the traditional view (reviewed by Strahl and Allis, 2000). As a matter of fact, our data suggest that histone acetylation at other lysines—and of the tail overall—correlates better with transcription activity than acetylation specifically at K14.

Overall Histone Tail Charge and Higher Order Chromatin Structure

Most studies on acetylation so far have focused on nucleosomes in the promoter of regulated genes, comparing the “on” and “off” states of the gene. By contrast, this study focused on the consequences of altering the H3 acetylation state (by *gcn5 elp3* mutation) in the promoter and coding regions of genes whose transcription states were not changed by regulatory signals during the experiment. The experimental aims and the results obtained are thus markedly different from those on correlations between acetylation and transcription previously reported in several studies (Eberharter and Becker, 2002; Mizzen and Allis, 1998).

Although our data cannot be used to argue for or against the existence of a histone code for transcriptional regulation, they do convey an important reminder that histone modifications, such as acetylation, are also important through other effects, such as on nucleosome mobility or the folding of higher order chromatin structure. The finding that the overall charge of the histone H3 tail correlates better with transcription than any individual modification alone might be reminiscent of the recent evidence that it is the synergy of clustered positive charges that is important for histone H1 function in gene regulation (Dou and Gorovsky, 2002). In vitro work on folding/compaction of chromatin has shown that hyperacetylated chromatin fibers are in a less folded state than their un-acetylated counterpart (Krajewski et al., 1993; Luger and Richmond, 1998; Ridsdale et al., 1990; Wang et al., 2001). Moreover, a study from Davie and coworkers has suggested that transcript elongation is required to form, and histone acetylation is needed to maintain, an unfolded structure of transcribing nucleosomes (Walia et al., 1998). Viewing our functional data in the light of these results raises the possibility that the HAT activity of Gcn5 and Elp3 plays an important role for transcription by promoting the unfolding of the chromatin fiber and/or increased nucleosome mobility over the coding regions of active genes.

Experimental Procedures

Yeast Strains and DNA Constructs

All *S. cerevisiae* strains used for genetic analysis were congenic with strain W303 (Thomas and Rothstein, 1989) (Table 3) and were grown and manipulated as previously described (Otero et al., 1999; Wittschieben et al., 2000). The parental yeast strain expressing 3xHA-tagged TBP (Kuras and Struhl, 1999) was kindly provided by Kevin Struhl. The MATa

offspring (Table 3) of a cross between this strain (YLK4) and a *gcn5 elp3* MATα strain were used for most ChIP experiments.

Western Blotting

Freshly grown yeast cells were treated with sodium hydroxide prior to further lysis in SDS-gel running buffer to produce whole-cell extract proteins as described (Kushnirov, 2000). The antibodies to detect various histone H3 forms in Western blots were: AcK9,14 (Upstate Biotechnology), AcK9 (α332) (Suka et al., 2001), and an antibody directed against the last 13 amino acids in the C terminus of histone H3 (a kind gift from Alain Verreault).

Chromatin Immunoprecipitation

Strains were grown in 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, and protease inhibitors), and chromatin was fragmented by extensive sonication (average fragment size always <500 bp). Whole-cell extract from 1 × 10^7 cells was used for immunoprecipitations. 1/30 of immunoprecipitated and 1/20,000 of input DNA was used for analysis by quantitative PCR in presence of 0.1 μCi/μl [α-^{32}P]dCTP. Because we wanted to be able to make quantitative as well as qualitative conclusions from these experiments, great care was taken to ensure that the PCR reactions were always in the linear range. The validity of key experimental procedures was also confirmed by real-time PCR. For the experiments shown, PCR products were quantified by Phosphor Imager analysis and results were normalized according to the amount of input DNA. Antibodies against tetraacetylated (K5, K8, K12, K16) histone H4 or di-acetylated (K9, K14) histone H3 (Upstate Biotechnology) were used for the experiments in Figure 1A. In this experiment, PCR reactions were done with 20 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.

Antibodies (α332, α294, α410, α300, α339) against individual lysine residues in histone H3 (Suka et al., 2001) were used in experiments where acetylation at specific lysine residues was studied. 4H8 antibody (Pharmingen or Upstate Biotechnology; recognizes both hypo- and hyperphosphorylated RNAPII [B. Winkler and J.Q.S., unpublished data]) and 12CA5 (anti-HA antibody) (Wilson et al., 1984) were used to precipitate RNAPII and TBP (HA-tagged [Kuras and Struhl, 1999]), respectively. The PCR products for studying association of proteins with promoter and coding region sequences, respectively, were as follows (numbered relative to AUG): *Gene* (coding region PCR product, promoter PCR product): *FAS1* (2897 to 3177, −253 to −13); *CHA1* (505 to 714, −); *FAB1* (3372 to 3611, −240 to −30); *TRA1* (5801 to 6015, −279 to −2); *POL2* (3303 to 3516, −286 to −1) *GCN1* (3900 to 4139, −238 to −27); *YCR095C* (483 to 693, −); *CDC39* (3501 to 3772, −220 to 13); *YCL063W* (549 to 787, −); *CDC50* (571 to 803, −); *GIT1* (711 to 990, −); *ACT1* (1263 to 1568, −); *HXK1* (851 to 1124, −); *MRC1* (1653 to 1928, −); *PKC1* (1710 to 1924, −); *STE6* (1085 to 1323, −266 to 77); *RPS5*, (415 to 636, −); *GDH1* (702 to 941, −); *SSA4* (930 to 1143, −99 to 143); *YGP1* (502 to 742, −290 to −20); *BAT1* (501 to 775, −228 to −13);

LEU1 (1002 to 1277, −). Sequences of all primers used in this study are available upon request.

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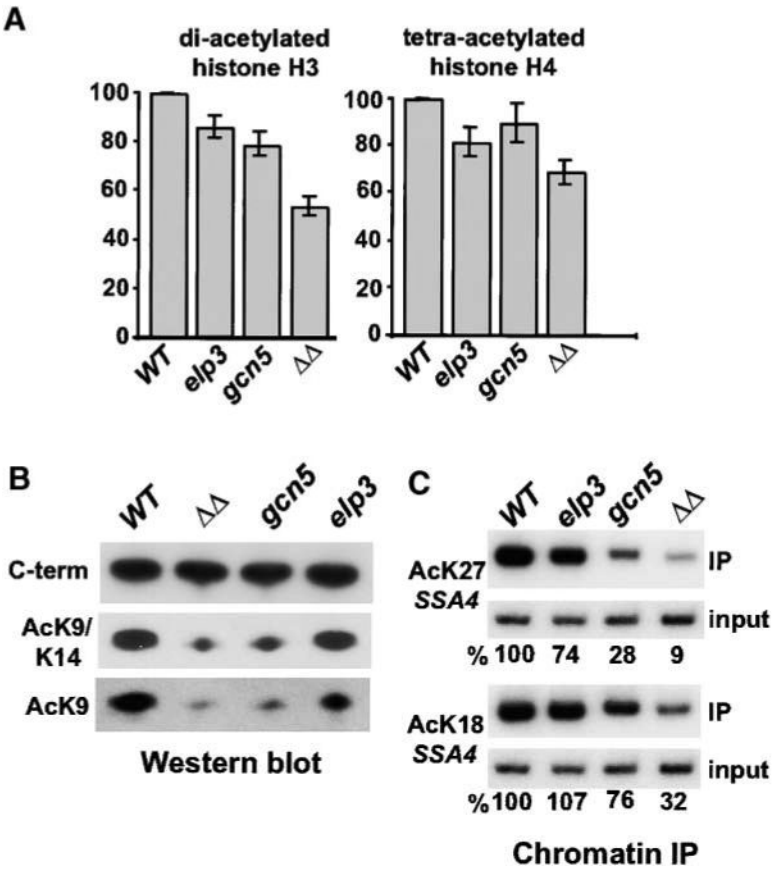

Figure 1. Histone Hypoacetylation in Cells Lacking *GCN5* and *ELP3*

(A) ChIP analyses of the relative level of multiply acetylated histone H3 (left panel) and H4 (right panel) in 20−22 different chromosomal regions using antibodies specific for di-acetylated histone H3 and tetra-acetylated H4, respectively. The results shown are averages of at least three independent experiments with standard deviations. ΔΔ denotes the *gcn5 elp3* double mutant.

(B) Western blot analysis of the global acetylation level in the indicated strains. Results from the use of other acetylation-specific antibodies (Suka et al., 2001) showed similar trends, but only the H3 K9-specific antibody worked well in Western blots of these crude extracts.

(C) Examples of ChIP analyses of the relative level of acetylated histone H3 in *SSA4*, using antibodies specific for lysine 18 and 27 of H3. See Experimental Procedures for details of probes used.

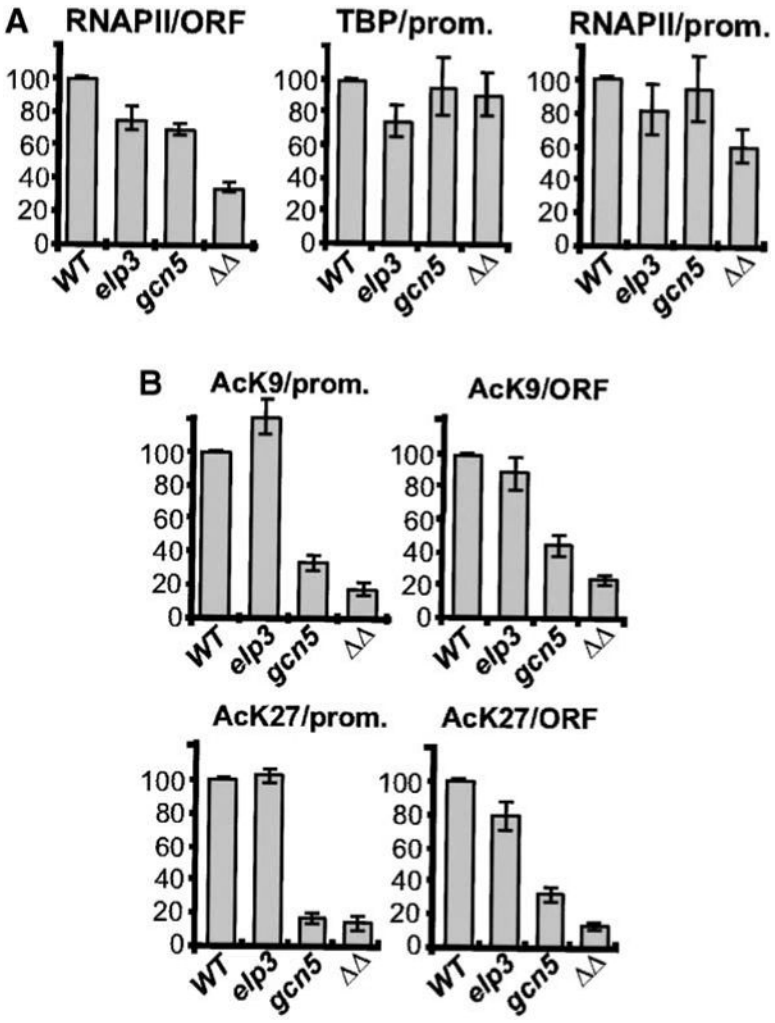

Figure 2. Transcription and Acetylation in the Active *STE6* Gene

ChIP analyses of the relative level of RNAPII and TBP (A) and histone H3 K9 and K27 acetylation (B) in the coding region (ORF) and at the TATA element (prom.) of *STE6* in wild-type, *gcn5*, *elp3*, and *gcn5 elp3* (ΔΔ) cells. The results shown are averages of at least three independent experiments.

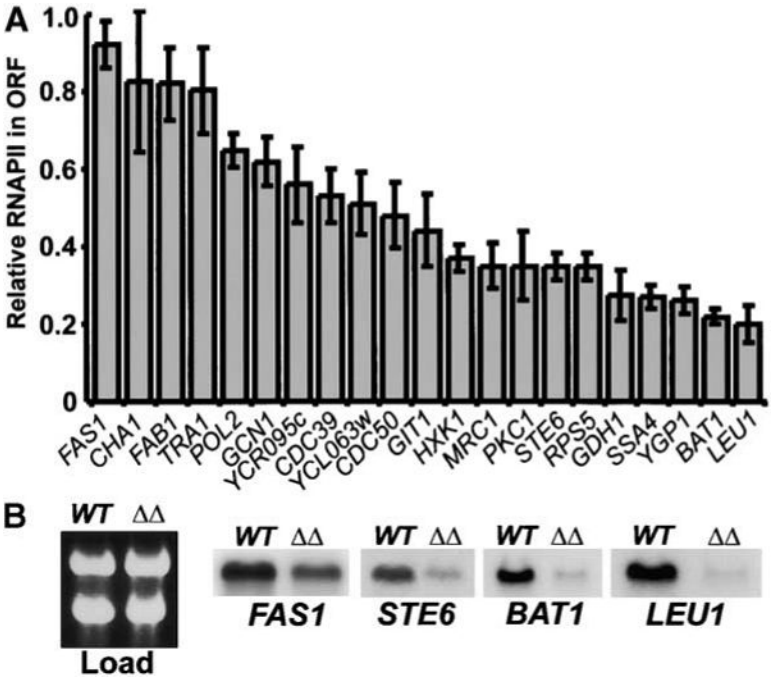

Figure 3. Transcription in *gcn5 elp3* Cells

(A) The density of RNAPII in the coding region of different genes in *gcn5 elp3* (ΔΔ) relative to wild-type. The RNAPII density in wild-type cells was set to be 100%, and the graphs indicate the density in *gcn5 elp3* cells relative to this. The results shown are averages of two to four independent experiments. Note that Western blotting of crude cell extracts with the IP-antibodies showed that there were no changes in the overall abundance of RNAPII in *gcn5*, *elp3*, or *gcn5 elp3* cells relative to wild-type (data not shown).

(B) Northern blots confirming that RNAPII density is a good measure of transcription efficiency in the cells.

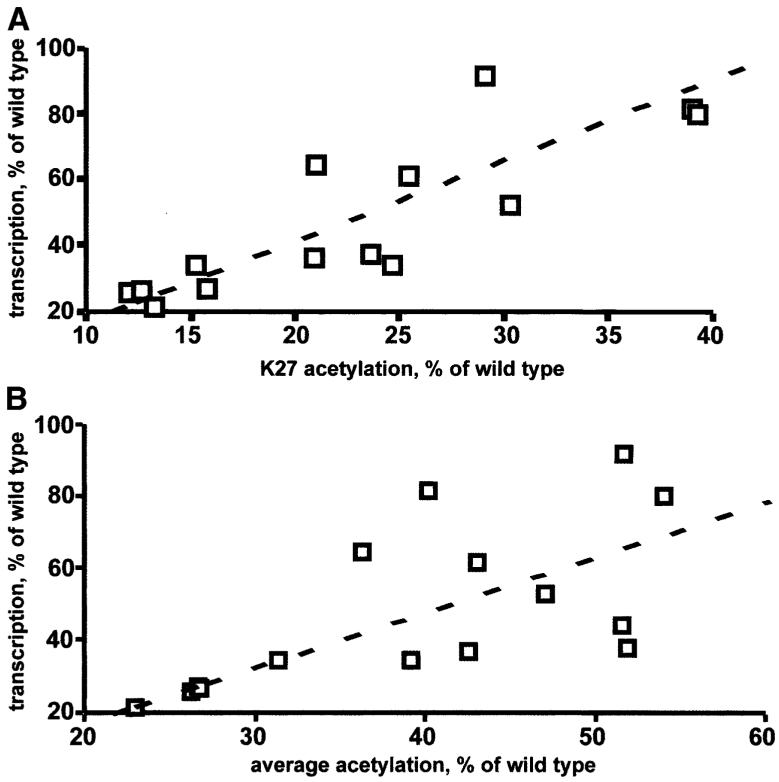

Figure 4. A Linear Correlation between Transcription and Acetylation in Individual Genes

Transcription in *gcn5 elp3* was plotted against K27 (A) and average acetylation (B) in the coding region of the genes from Table 1. The correlation coefficients between acetylation and transcription in these plots are 0.79 (K27) and 0.74 (average acetylation), respectively (a value of 1 indicates perfect correlation, 0 indicates no correlation, and −1 indicates an inverse correlation).

Table 1

Transcription and Histone H3 Acetylation of the Coding Region in Several Genes

	RNA Pol II	K9-orf	K14-orf	K18-orf	K23-orf	K27-orf	Average
FAS1	92.0 ± 6.0	66.3 ± 6.1	46.7 ± 6.7	73.2 ± 33.2	42.8 ± 7.1	29.0 ± 4.0	51.6
FAB1	82.0 ± 9.3	*21.6 ± 2.0*	45.2 ± 9.7	53.9 ± 4.6	41.0 ± 3.9	38.9 ± 2.5	40.1
TRA1	80.2 ± 11.2	32.0 ± 8.1	58.4 ± 18.8	89.4 ± 30.0	50.7 ± 7.7	39.2 ± 0.5	53.9
POL2	64.9 ± 4.5	36.6 ± 5.2	56.8 ± 11.2	35.3 ± 7.1	31.7 ± 5.6	*20.9 ± 0.5*	36.2
GCN1	61.8 ± 6.4	29.3 ± 1.4	55.0 ± 8.1	71.3 ± 26.5	33.8 ± 6.2	25.3 ± 5.1	42.9
CDC39	53.1 ± 7.1	*24.8 ± 2.9*	69.4 ± 21.0	70.3 ± 19.6	41.0 ± 11.1	30.2 ± 2.7	47.0
ACT1	38.1 ± 9.1	27.1 ± 2.4	115.5 ± 20.8	*14.7 ± 7.3*	78.1 ± 6.2	*23.7 ± 2.3*	51.8
HXK1	37.0 ± 3.4	*22.8 ± 5.2*	76.4 ± 6.6	*24.9 ± 2.0*	67.1 ± 5.2	*20.8 ± 1.7*	42.4
PKC1	34.9 ± 8.9	*24.0 ± 1.7*	34.4 ± 5.5	37.5 ± 8.4	36.1 ± 11.2	*24.5 ± 6.1*	31.3
STE6	34.8 ± 3.4	*24.3 ± 1.4*	66.2 ± 1.6	41.2 ± 5.3	48.5 ± 3.2	*15.1 ± 0.5*	39.1
GDH1	27.3 ± 6.4	*21.0 ± 1.1*	26.7 ± 8.7	50.7 ± 3.0	*18.5 ± 0.5*	*15.6 ± 1.3*	26.5
SSA4	27.0 ± 3.0	*20.1 ± 0.8*	40.2 ± 8.0	32.2 ± 2.3	27.8 ± 0.2	*12.5 ± 0.4*	26.6
YGP1	26.2 ± 3.4	*21.2 ± 0.5*	36.1 ± 10.1	*24.0 ± 2.0*	37.1 ± 11.1	*11.9 ± 2.4*	26.1
BAT1	21.8 ± 2.0	*23.0 ± 1.3*	34.4 ± 15.4	*23.6 ± 8.5*	*19.9 ± 3.4*	*13.1 ± 0.2*	22.8

ChIP analyses of transcription (RNAPII density) and the relative level of acetylated histone H3 in the coding region of 14 different genes using antibodies directed towards RNAPII and specific, individual acetylation sites. The numbers shown are for *gcn5 elp3* cells in percent of wild-type and are averages (with standard deviations) of at least three independent experiments. The column to the right shows the average acetylation of the gene. Lysine residues showing severe hypoacetylation (less than 25% of wild-type) are underlined.

Table 2

TBP Promoter Occupancy and Acetylation of the Promoter Region in Several Genes

	TBP	K9-prom	K14-prom	K18-prom	K23-prom	K27-prom	Average Acetylation (prom)
STE6	90.6 ± 13.3	*18.5 ± 1.7*	44.8 ± 13.3	31.2 ± 4.5	34.5 ± 0.7	*14.3 ± 2.9*	28.7
FAB1	84.6 ± 10.1	*12.6 ± 1.3*	38.1 ± 1.4	*19.4 ± 3.8*	28.6 ± 1.0	*11.9 ± 1.2*	22.1
TRA1	70.7 ± 7.7	*12.5 ± 1.6*	34.3 ± 3.1	*17.8 ± 10.8*	31.6 ± 2.7	*10.3 ± 0.5*	21.3
CDC39	64.1 ± 7.8	*11.4 ± 1.5*	38.1 ± 3.8	*13.5 ± 0.7*	29.4 ± 1.5	*12.7 ± 3.6*	21.0
YGP1	46.0 ± 6.3	*16.0 ± 0.1*	35.0 ± 6.6	28.9 ± 4.9	38.8 ± 4.8	*10.7 ± 0.0*	25.9
BAT1	41.5 ± 4.4	*13.9 ± 0.9*	48.4 ± 20.2	*20.8 ± 7.6*	45.3 ± 0.3	*9.7 ± 0.9*	27.6

ChIP analyses of TBP promoter occupancy and the relative level of acetylated histone H3 in the promoter region of six different genes using antibodies directed towards TBP (TBP-3xHA), and specific, individual acetylation sites. The numbers shown are for *gcn5 elp3* cells in percent of wild-type and are averages (with standard deviations) of at least three independent experiments. Lysine residues showing severe hypoacetylation (less than 25% of wild-type) are underlined.

Table 3

Yeast Strains

Strain	Genotype	Reference/Source
W303−1A	*MATa ura3−1 leu2−3,112 his3−11,15 trp1−1 ade2−1 can1−100*	Thomas and Rothstein, 1989
W303−1B	*MATα ura3−1 leu2−3,112 his3−11,15 trp1−1 ade2−1 can1−100*	Thomas and Rothstein, 1989
JSY130	W303−1A, except *elp3*Δ::*LEU2*	Wittschieben et al., 1999
JSY141	W303−1A, except *gcn5*Δ::*HIS3*	Wittschieben et al., 2000
JSY143	W303−1A, except *elp3*Δ::*LEU2 gcn5*Δ::*HIS3*	Wittschieben et al., 2000
YLK4	W303−1A, except *TBP3xHA*::*URA3*	Kuras and Struhl, 1994
JSY653	JSY130, except *TBP3xHA*::*URA3*	This study
JSY654	JSY141, except *TBP3xHA*::*URA3*	This study
JSY647	JSY143, except *TBP3xHA*::*URA3*	This study