Spreading of Sir3 protein in cells with severe histone H3 hypoacetylation

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Heterochromatin formation in yeast involves deacetylation of histones, but the precise relationship between acetylation and the association of proteins such as Sir3, Sir4, and the histone deacetylase Sir2 with chromatin is still unclear. Here we show that Sir3 protein spreads to subtelomeric DNA in cells lacking the transcription-related histone acetyltransferases *GCN5* and *ELP3*. Spreading correlates with hypoacetylation of lysines in the histone H3 tail and results in deacetylation of lysine 16 in histone H4. De-repression of genes situated very close to the ends of the chromosomes in *gcn5 elp3* suggests that Sir3 spreads into subtelomeric DNA from the tip of the telomere. Interestingly, growth defects caused by *gcn5 elp3* mutation can be suppressed by *SIR* deletion, suggesting that Sir proteins become detrimental for growth when chromatin is severely hypoacetylated.

Core histones are subject to a variety of covalent modifications, and enzymes responsible for these modifications have been intensely studied over the past few years. However, in contrast to our basic knowledge of these enzymes and their capabilities, only little is known about the molecular effects of changes in histone modification *in vivo*. For example, it is unclear whether histone hypoacetylation in itself is inhibitory to transcription or whether it merely enables downstream processor proteins, such as transcriptional repressors, to associate with chromatin and decrease the efficiency of the transcription process. Likewise, whether histone hypoacetylation is a prerequisite for, or a result of, heterochromatin formation is also not entirely clear.

Heterochromatin in complex eukaryotes such as fruit flies and mammals is condensed, stains darkly cytologically, and has the ability to silence nearby genes epigenetically (1, 2). Budding yeast also has chromosomal regions with many of the features of heterochromatin: the silent mating (*HM*) loci and chromatin domains adjacent to telomere ends. The chromatin in these regions is condensed, late replicating, and associated in foci that localize to the nuclear periphery (1, 3, 4). Yeast heterochromatin is also hypoacetylated on all four core histones relative to that packaging active genes (5–7).

The products of the *SIR* genes are important factors for the establishment and maintenance of yeast heterochromatin. *SIR2*, *SIR3*, and *SIR4* are important for silencing both at telomeres and at the silent mating loci, whereas *SIR1* is only required for the establishment of silencing at the silent mating loci. Other components with important roles in silencing include DNA-interacting factors such as Rap1, Abf1, the origin recognition complex (ORC), and histones H3 and H4 (4). A large number of pairwise interactions between these components have been reported. For example, Sir3 and Sir4 interact with each other, as well as with Rap1 and the amino-terminal tails of histones H3 and H4 (8, 9). Sir4 also interacts directly with Sir2 (10, 11). Such results have given rise to models for heterochromatin formation at telomeres in which Rap1 (bound to its cognate recognition site found repeated at telomere ends) recruits Sir3 and Sir4. Subsequent recruitment of Sir2 and association with histones H3 and H4 in the underlying chromatin then create a structure with the characteristics of heterochromatin (3, 4).

The precise role of histones and histone hypoacetylation in heterochromatin formation and maintenance is still not entirely clear. Sir3 and Sir4 bind to the tails of histones H3 and H4, and mutation of histone H4 amino-terminal lysine residues to uncharged residues (thought to mimic acetylation) is sufficient to disrupt H4–Sir3 interactions *in vitro* and significantly reduce silencing *in vivo* (9). This indicates that Sir3 (and Sir4) binds to chromatin-containing hypoacetylated histone H4 (and H3). Such binding might play a role in the initial Sir recruitment to chromatin. On the other hand, chromatin at silent mating loci is no longer hypoacetylated in *sir* strains (6, 7) and Sir2 is a histone deacetylase (12–14), raising the possibility that histone hypoacetylation could primarily be a consequence of the presence of Sir proteins, rather than a prerequisite for their initial recruitment.

We studied the consequence of deleting the genes encoding two transcription-related HATs, *GCN5* and *ELP3*, for histone acetylation and formation of heterochromatin-like structures. We recently reported that *gcn5 elp3* has widespread histone H3 hypoacetylation in chromatin, and that there is a correlation between reduced transcription and severe histone H3 hypoacetylation at specific genes in these cells (15). Here, we provide evidence that *gcn5 elp3* mutation leads to the spreading of Sir3 into subtelomeric DNA and the establishment of repressive chromatin structures. Our data suggest that Sir3 protein is mobilized in response to severe histone H3 hypoacetylation and that this can affect cellular growth.

Materials and Methods

Yeast Strains and DNA Constructs. All *Saccharomyces cerevisiae* strains used for genetic analysis were congenic with strain W303 (16) and were grown and manipulated as described (17, 18). The genotypes of the strains are as follows: *MATa elp3*Δ::*LEU2 gcn5*Δ::*HIS3* (JSY143) (18), *MATa elp3*Δ::*LEU2 gcn5*Δ::*HIS3 tup1*Δ::*TRP1* (JSY478), *elp3*Δ::*LEU2 gcn5*Δ::*HIS3 sir1*Δ::*TRP1* (JSY617), *elp3*Δ::*LEU2 gcn5*Δ::*HIS3 sir2*Δ::*TRP1* (JSY491), *elp3*Δ::*LEU2 gcn5*Δ::*HIS3 sir3*Δ::*TRP1* (JSY455), *elp3*Δ::*LEU2 gcn5*Δ::*HIS3 sir4*Δ::*TRP1* (JSY854), *MATα SIR3-9xMyc*::*TRP1* (*JSY820*), *MATα SIR3–9xMyc*::*TRP1 elp3*Δ::*LEU2 gcn5*Δ::*HIS3* (*JSY825*), *MATα SIR3-HA3*::*TRP1* (JSY814), *MATα SIR3-HA3*::*TRP1 elp3*Δ::*LEU2 gcn5*Δ::*HIS3* (JSY818). Plasmids from which PCR products were derived for epitope-tagging by homologous recombination were kindly provided by Kim Nasmyth (19, 20). Details are available on request. Plasmids expressing *MATa* or *MATα* were kindly provided by Lorraine Pillus (University of California at San Diego, La Jolla) and Ann

Abbreviation: ChIP, chromatin immunoprecipitation.

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Chromatin Immunoprecipitation (ChIP) and Antibodies. Chromatin immunoprecipitation was performed as described (15). Briefly, cells were fixed in 1% formaldehyde for 15 min at room temperature and 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 then chromatin was fragmented by extensive sonication (average fragment size was always <500 bp). Whole cell extract from 1×10^7 cells was used for immunoprecipitations with acetylation-specific antibodies against histones H3 or H4 [antibodies α332, α294, α410, α300, α339, and α268 (7)], antibody 4H8 directed against RNAPII [BD Biosciences Pharmingen or Upstate Biotechnology (Lake Placid, NY)], or anti-Myc antibody 9E10 to precipitate myc-tagged Sir3 (or Rpa43). Then, 1/30 of immunoprecipitated and 1/20,000 of input DNA was used for analysis by quantitative PCR in presence of 0.1 mCi/ml [α-^{32}P]dCTP (1 Ci = 37 GBq). The sizes of the different PCR products were in the range 215–360 bp. Sequences of primers are available on request. PCR products were separated in 6% polyacrylamide gel and quantified by PhosphorImager (Molecular Dynamics). Results were normalized according to the amount of input DNA.

The 9E10 antibody was also used for the Western blot shown in Fig. 3*B*.

Microarray Analysis of Transcription in *gcn5 elp3*. Microarray analysis was performed essentially as described in ref. 21. Briefly, mRNA (2 μg) from a control (WT) or experimental (*gcn5 elp3*) strain was labeled with Cy3-dCTP or Cy5-dCTP, respectively, in reverse transcription reactions. Competitive hybridizations were performed on ≈6,000 amplified ORFs of *S. cerevisiae* (Research Genetics, Huntsville, AL) arrayed in duplicate on silane-treated glass slides, and the fluorescent (Cy5 and Cy3) signal intensity from each was quantified. Arrayed fragments (gene spots) with extremely low signal intensities (≈5% of total genes) were removed from the dataset. Cy3 signal intensities were then normalized to Cy5 signal intensities by multiplying the Cy3 intensity of each gene spot by the average Cy5/Cy3 intensity of all gene spots, thus making the average Cy5/Cy3 ratio equal to 1. Data for individual genes (values) was expressed as the log(2) of these normalized Cy5/Cy3 ratios. For example, values of −1, 0, or 1 correspond to genes that are "down-regulated 2-fold," "unchanged," or "up-regulated 2-fold," respectively. Each experiment was performed in duplicate (a separate labeling/hybridization), and the shown values are the average values of the two experiments (four gene spots).

Results

Deletion of SIR Genes Suppresses the *gcn5 elp3* Phenotype. A global decrease in the level of histone acetylation, such as that observed in *gcn5 elp3* (15), might cause gene repression by directly leading to a more compact chromatin structure; for example, by allowing stronger histone–DNA and histone–histone interactions (22, 23). Additionally, however, a decrease in the level of acetylation might cause an alteration of chromatin structure and gene repression or other DNA-related processes by aberrant recruitment of proteins such as Tup1/Ssn6 or Sir proteins. These proteins have previously been shown to bind preferentially to the nonacetylated tails of histones H3 and H4 *in vitro* (9, 24–26). If the action of these proteins contributes significantly to the severe *gcn5 elp3* phenotypes, then it might be possible to relieve at least some of the phenotypes by mutating *SIR* genes or *TUP1*. Fig. 1*A* shows that mutation of *SIR4*, but not *TUP1*, indeed suppressed the temperature sensitivity and the inability to grow on galactose observed for *gcn5 elp3* cells. Reintroducing *SIR4* on a centromeric (CEN) plasmid into *gcn5 elp3 sir4* restored the original *gcn5 elp3* phenotype (data not shown), showing that suppression was due to the loss of *SIR4*. We furthermore found that mutation of *SIR1*, *SIR2*, and *SIR3* also suppressed phenotypes associated with the double HAT mutation (Fig. 1*B*). *sir* mutation also significantly improved the growth rate of *gcn5 elp3* cells in rich media at normal temperature (data not shown), pointing to a general detrimental effect of Sir proteins in cells lacking Gcn5 and Elp3. By contrast, deletion of *SIR* genes failed to suppress the phenotypes arising by deletion of *GCN5* or *ELP3* individually (data not shown).

Aberrant regulation of mating type genes has many indirect effects on cellular processes. However, such effects are unlikely to be solely responsible for the growth characteristics of *gcn5 elp3* cells and the suppression by *SIR* deletion for the following reasons. First, *gcn5 elp3* cells are capable of mating, and the density of RNAPII at the active and silent mating loci as measured by RNAPII ChIP experiments is not changed dramatically in these cells (data not shown). Second, plasmid-driven expression of the α gene in *MATa* cells mimics the pseudodiploid state of *sir* cells. However, although such expression did improve the growth of *gcn5 elp3* cells somewhat, deletion of *SIR3* had a much stronger effect (Fig. 1*C*). Finally, a *MAT*α *gcn5 elp3 sir3* mutant was constructed that completely lacked *a* genes (by deletion of *HMR*) and therefore behaved as a true haploid despite lacking *SIR3*. This modification did not annul the *sir3* suppression of the severe *gcn5 elp3* phenotype (data not shown), again indicating that a significant part of the suppressing effect of *SIR3* deletion was direct.

Deletion of SIR3 Fails to Relieve the Effect of *gcn5 elp3* Mutation on Transcription of Euchromatin Genes. The suppression of *gcn5 elp3* phenotypes by *sir* mutation could indicate that Sir proteins are the actual cause of the decreased transcription previously reported for severely hypoacetylated genes in *gcn5 elp3* cells (15). If so, then transcriptional inhibition in *gcn5 elp3* cells should be reversed by deletion of *SIR3*. We used RNAPII ChIP experiments to investigate whether the density of transcribing polymerases in the coding region of genes previously shown to be affected to different extents by *gcn5 elp3* mutation (15) was affected by the presence of *SIR3*.

Comparisons of data obtained by using ChIP with data obtained by Northern blotting and DNA microarray data have repeatedly shown that although RNAPII ChIP of the coding region of active genes with the 4H8 antibody (15) may not have the dynamic range of the other techniques, it is a highly reliable measure for relative transcription levels. As indicated by RNAPII ChIP analysis, Sir proteins were unlikely to be responsible for the lower level of transcription observed at several genes in *gcn5 elp3*, as deletion of *SIR3* had little or no beneficial effect on transcription in these cells (Fig. 2). Likewise, we failed to detect significant differences between rDNA (RNAPI) transcription in WT, *gcn5 elp3*, and *gcn5 elp3 sir3* strains when using RNAPI ChIP (from strains in which Rpa43 carried an 18xmyc tag) or reverse PCR to detect newly synthesized rDNA transcripts (data not shown). Taken together, these data strongly indicate that Sir proteins do not generally affect transcription of euchromatin genes in *gcn5 elp3* cells.

Spreading of Sir3 from the Telomere in *gcn5 elp3*. Sir proteins are known to primarily associate with telomeres and the silent mating type loci. If global changes in acetylation result in aberrant recruitment of Sir proteins and heterochromatin formation, then we might expect to see changes in Sir protein distribution at telomeres. We examined Sir3 association with chromatin at different distances from the end of the right arm telomere of chromosome VI by ChIP (Fig. 3). This chromosome

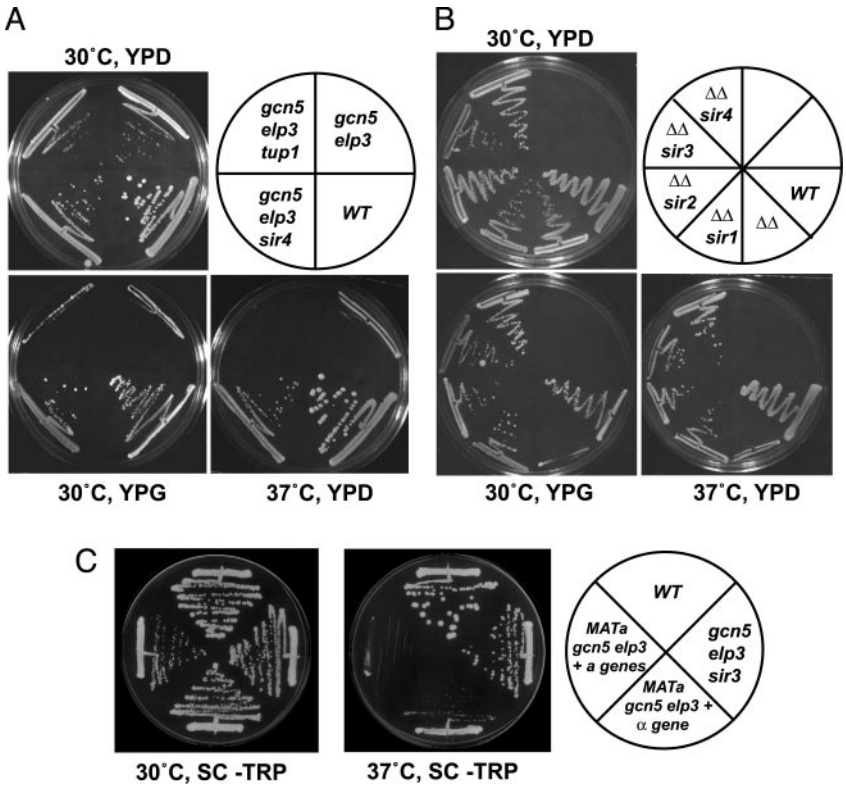

Fig. 1. *gcn5 elp3* phenotypes can be suppressed by deletion of *SIR* genes. (*A*) Deletion of *SIR4*, but not *TUP1*, suppresses the severe consequences of *gcn5 elp3* mutation. Cells of the indicated genotype were streaked on yeast extract peptone/galactose (YPG) or yeast extract peptone/glucose (YPD) and incubated for 3–4 days at 30°C or 37°C as indicated. (*B*) Deletion of other *SIR* genes also suppresses the severe consequences of *gcn5 elp3* (ΔΔ) mutation. (*C*) Expression of plasmid-borne mating type genes and their effect on the growth of *MATa gcn5 elp3* cells. To maintain plasmids, cells were grown on synthetic complete selective media (SC -TRP) for 3 days at 30°C or 37°C as indicated. As expected, *MATa* cells expressing α genes from plasmids were incapable of mating (data not shown).

was chosen because it lacks the highly repetitive Y′ and X sequences found at the ends of the other chromosomes (11).

Remarkably, a large number of internally controlled ChIP experiments showed that Sir3 is associated with DNA much further from the telomere end in the *gcn5 elp3* double mutants than in WT (Fig. 3*A*). Sir3 was only detected at distances up to 6 kb from the telomere end in WT cells, whereas it could be detected even at 10 and 15 kb in *gcn5 elp3*. At distances between 3 and 6 kb from the end of the chromosome, there was up to 3-fold more Sir3 in *gcn5 elp3* than in WT, whereas at 20 kb from the chromosome end and further into the genome (such as at the genes studied in Fig. 2), levels were similar in the two strains (Fig. 3*A* and data not shown). This is reminiscent of the effects of Sir3 overexpression reported by Grunstein and colleagues (27). However, spreading of Sir3 from the telomeres in *gcn5 elp3* was not due to overexpression of the protein in these cells, because Sir3 levels were similar in WT and *gcn5 elp3* (Figs. 3*B* and 6). Sir3–9xmyc itself was also immunoprecipitated with similar efficiency from these different cell types (Fig. 7, which is published as supporting information on the PNAS web site).

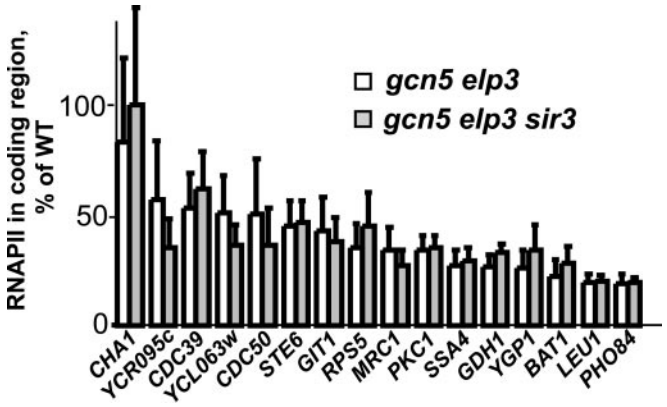

Fig. 2. Effect of *SIR3* deletion on transcription in euchromatin in *gcn5 elp3* cells. Shown are the results of transcription in *gcn5 elp3* containing or lacking *SIR3* revealed by RNAPII ChIP in the coding region of the indicated genes (15). RNAPII density in WT cells was set to be 100%, and the graphs indicate the density in *gcn5 elp3* and *gcn5 elp3 sir3* cells relative to this, as determined by multiplex PCR of immunoprecipitated samples. The results shown are averages of two to four independent experiments.

To investigate whether histone H3 acetylation near the telomere was affected by *gcn5 elp3* mutation, ChIP experiments using acetylation-specific antibodies (7) were performed. Sir3 spreading correlated with a dramatic decrease in histone H3 acetylation at lysines K9, K18, and K27, and to a much lesser extent at positions K14 and K23 in the same telomeric region in the *gcn5 elp3* double mutant (Fig. 3*C* and data not shown).

Effect of Sir3 Spreading on Transcription Near Telomere Ends. We next used data from genome-wide microarray experiments comparing transcription in the *gcn5 elp3* double mutant to WT cells to try and shed some light on the effect on transcription near telomere ends. If spreading of Sir3 protein from the telomeres in *gcn5 elp3* had functional consequences for transcription, then we might expect to observe a higher frequency of transcriptional inhibition in regions adjacent to the telomere in these cells. On the other hand, our previous data showed that severe hypoacetylation of the histone H3 tail invariably results in repression of

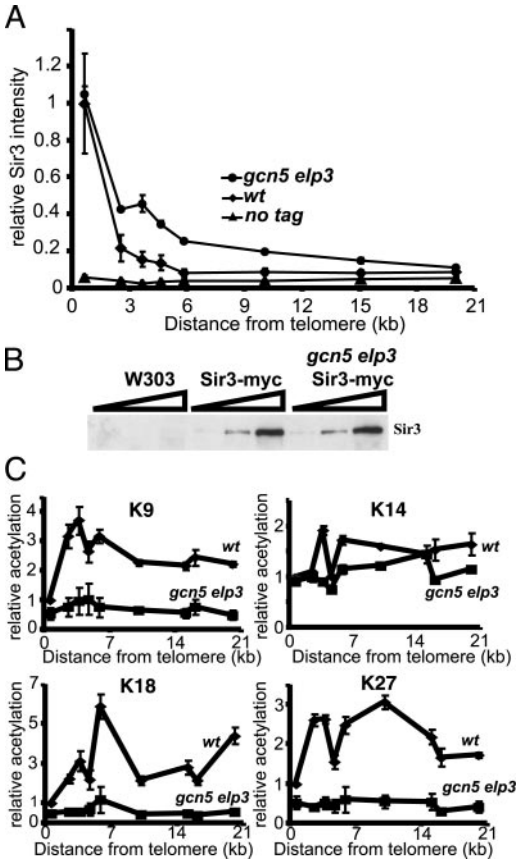

Fig. 3. Spreading of Sir3 protein and acetylation of histone H3 near the telomere in *gcn5 elp3*. (*A*) Multiplex PCR ChIP analyses of the relative level of tagged Sir3 at different distances from the right arm telomere of chromosome VI in WT and *gcn5 elp3* cells (and nontagged cells as control). The Sir3 density at the telomere end (0.5 kb) in WT cells was set to 1, and the level in *gcn5 elp3* and WT elsewhere in the genome was expressed relative to that. Strains in which Sir3 carried different tags (9xMyc and 3xHA, respectively) gave similar results, and averages from experiments with these strains were used to compile the graph. (*B*) Western blot of extract dilution series using 9E10 antibody to measure the relative level of Sir3–9xmyc in the indicated cell types. Equal loading was first ensured by Coomassie staining of titrations of the extracts (Fig. 6, which is published as supporting information on the PNAS web site, www.pnas.org). Similar results were obtained for 3xHA-tagged Sir3. (*C*) ChIP analyses of the relative level of acetylated histone H3 at different distances from the end of chromosome VI-R in WT and *gcn5 elp3* cells, using antibodies specific for the indicated acetylated lysines of H3 (7). The acetylation level at the telomere end (0.5 kb) in WT cells was set to 1. Acetylation relative to that in WT and *gcn5 elp3* further into the genome is reflected in the graph. Standard deviations are indicated for all data points but are occasionally so small that they are not clearly visible.

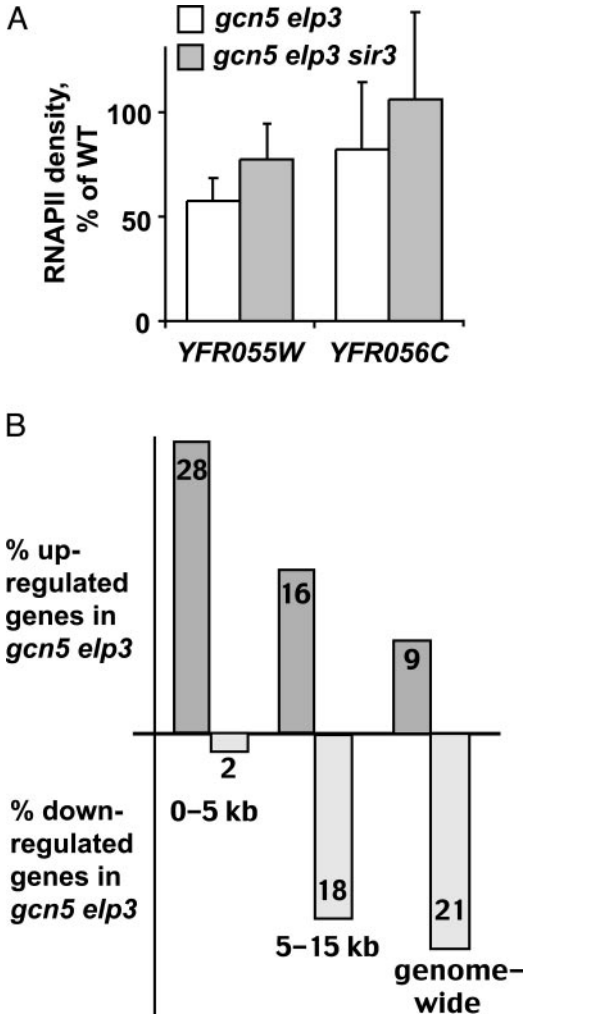

Fig. 4. Analysis of transcription near the telomere in *gcn5 elp3*. (*A*) ChIP assay of RNAPII density on coding region of chromosome VI subtelomeric genes *YFR055W* and *YFR056C*. The amount of RNAPII in WT cells was set as 100%, and RNAPII levels in *gcn5 elp3* and *gcn5 elp3 sir3* strains are shown relative to that. The results shown are averages of three independent experiments. (*B*) The percentage of genes whose transcription is changed >0.5 log(2) units in *gcn5 elp3* compared with WT in the indicated regions was plotted. Bars above baseline indicate the relative number of genes that have become up-regulated, and bars below baseline indicate genes that are down-regulated in *gcn5 elp3*.

transcription (15). An effect of Sir3 spreading might therefore be of secondary importance to an effect of the severe H3 hypoacetylation observed near the telomere.

We first looked at transcription on the right arm of chromosome VI, where Sir3 was shown to spread from the telomere end (Fig. 3). Data from genome-wide microarray experiments with *gcn5 elp3* indicated that transcription was indeed reduced at the subtelomeric genes *YFR055W* (>4-fold) and *YFR056C* (>2.5-fold). RNAPII ChIP of *YFR055W* and *YFR056C* further supported the notion that these genes were down-regulated but also indicated that repression of transcription was only partially *SIR3*-dependent (Fig. 4*A*).

To obtain a more global view of the consequence of *gcn5 elp3* mutation for transcription near the telomeres we next focused on transcription in "telomeric" (here loosely defined as 1–5,000 bp from the tip of the chromosome) and "subtelomeric" (5,000–15,000 bp) DNA in the entire genome. The number of genes in subtelomeric DNA that were down-regulated >0.5 log(2) units in *gcn5 elp3* was 24/134 (18%) compared with 1,303/6,251 (21%) globally (Fig. 4*B*). The number of genes up-regulated >0.5 log(2) units in the same region was 21/134 (16%) compared with 582/6,251 (9%) genome-wide, all in all indicating that spreading of Sir3 to this region in *gcn5 elp3* did not generally have a more detrimental effect on transcription than that imposed by the hypoacetylation in the genome in general (15). Interestingly, the microarray data also suggested that transcription near the very tip of the telomere (1–5,000 bp from the end) was slightly more efficient in *gcn5 elp3* than in WT: here, the number of genes up-regulated >0.5 log(2) units in *gcn5 elp3* was 13/46 (28%) compared with 582/6,251 (9%) genome-wide, and the number of genes similarly down-regulated was 1/46 (2%) compared with 1,303/6,251 (21%) genome-wide. These data indicate that transcription close to the very end of the chromosome has become somewhat de-repressed in *gcn5 elp3* cells.

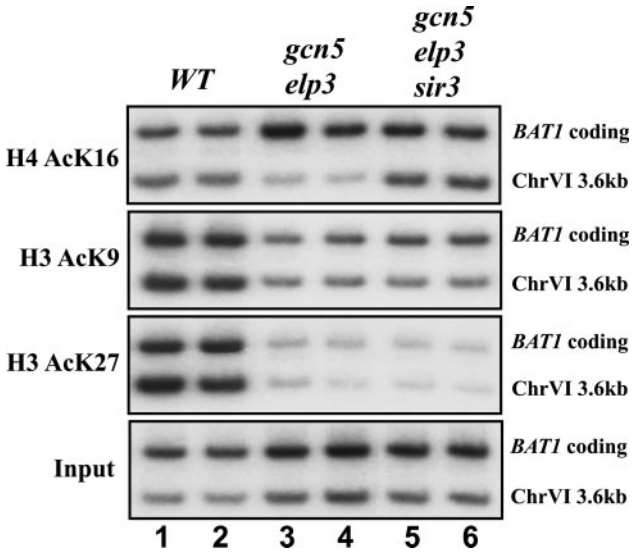

Fig. 5. Sir-dependent histone H4 and H3 acetylation characteristics close to the telomere in *gcn5 elp3*. ChIP analyses of acetylation at the indicated lysines of histones H3 and H4 3.6 kb from the end of chromosome VI-R compared with a euchromatic gene *BAT1* (≈45 kb from the end of chromosome VIII-R) are shown. The different lanes represent pairwise, independent repeats of the experiment with the indicated strains. Note the slight under-loading of WT samples.

Sir3 Spreading Is Not Caused by Deacetylation of Histone H4 Lysine 16. The relatively minor effect of *SIR3* deletion on the density of RNAPII at the subtelomeric genes of chromosome VI could be explained by a dominant effect of low histone H3 acetylation. However, it raised the important question as to whether spreading of Sir3 in *gcn5 elp3* actually led to the establishment of structures similar to those responsible for telomere silencing. Moreover, recent reports have provided convincing evidence of a dominant role for histone H4 lysine 16 in the formation of Sir-dependent structures at telomeres (28, 29), raising the possibility that Sir3 spreading in *gcn5 elp3* was not caused by severe histone H3 hypoacetylation (15), but rather by deacetylation of histone H4 K16.

We compared histone H4 lysine 16 acetylation in WT, *gcn5 elp3*, and *gcn5 elp3 sir3* cells by ChIP (Fig. 5). Histone H4 lysine 16 was indeed dramatically hypoacetylated in *gcn5 elp3* at a position 3.6 kb from the end of chromosome VI-R relative to an internal position such as the *BAT1* gene (≈45 kb into chromosome VIII-R; Fig. 5, top panel, compare lanes 1 and 2 with lanes 3 and 4, as well as with lower input DNA panel). As expected from our previously published data (15) and the data presented in Fig. 3*C*, histone H3 lysines 9 and 27 were dramatically hypoacetylated at both positions (Fig. 5, middle two panels, compare lanes 1 and 2 with lanes 3 and 4, as well as with lower input DNA panel).

It is important to note that histone hypoacetylation near the telomere in *gcn5 elp3* cells might be a secondary effect caused by the recruitment and action of the Sir2/3/4 complex rather than directly by the absence of the Gcn5 and Elp3 histone acetyltransferases. We therefore also tested whether mutation of *SIR3*, which disrupts assembly of the Sir complex at telomeres, had an effect on the level of acetylation at the relevant lysine sites. Strikingly, mutation of *SIR3* reversed histone H4 K16 hypoacetylation at the telomere (from 11% of WT in *gcn5 elp3* to 83% of WT in *gcn5 elp3 sir3*; Fig. 5, top panel, compare lanes 3 and 4 with lanes 5 and 6) but had a negligible effect on the level of histone H3 K9 or K27 acetylation (Fig. 5, middle two panels, compare lanes 3 and 4 with 5 and 6). These data support the notion that histone H3 hypoacetylation in *gcn5 elp3* is due to the absence of the Gcn5 and Elp3 HAT activities, whereas histone H4 K16 hypoacetylation is not. Rather, the severe H4 K16 hypoacetylation observed in subtelomeric DNA may be caused by spreading of Sir3 in these cells.

Discussion

Previous studies of histone acetylation and transcription in cells lacking the transcription-related histone acetyltransferase Gcn5 and Elp3 showed that genes with severe histone H3 hypoacetylation in the coding region also invariably have reduced levels of transcribing RNAPII (15). Here, we report the surprising finding that the severe *gcn5 elp3* phenotype can be suppressed by deletion of *SIR* genes. However, Sir proteins are not responsible for the reduced transcription levels observed at several genes in these cells. We show that Sir3 protein spreads inward from the telomere in *gcn5 elp3* and there supports the establishment of chromatin structures with the acetylation signature characteristic of telomeric heterochromatin; namely, histone H4 K16 hypoacetylation. Our data also indicate that transcription of genes embedded in telomeric DNA is slightly de-repressed in *gcn5 elp3*, suggesting that the source of the spreading Sir3 protein is the abundant pool at the tip of the chromosome.

Suppression of the *gcn5 elp3* Phenotype by *sir* Mutation. This study was initiated by the intriguing observation that deletion of *SIR* genes can suppress the severe *gcn5 elp3* phenotypes. This finding shows that the presence of Sir proteins becomes detrimental for growth in the absence of the transcription-related HATs Gcn5 and Elp3 and raises the possibility that substantial decreases in histone acetylation levels might give rise to the establishment of aberrant chromatin structures involving Sir proteins. It is important to note that the suppression of *gcn5 elp3* phenotypes is unlikely to be caused by spreading of Sir proteins into subtelomeric regions alone. For example, even more extensive spreading of repressive Sir complexes has been observed in cells lacking the HAT Sas2 (which targets histone H4 K16) and in cells carrying an H4 K16R mutation (28, 29), yet such cells show little or no growth defects. Second, deletion of *SIR1* also rescues the *gcn5 elp3* phenotype, and Sir1 does not play a role in telomere silencing. However, the fact that expression of both types of mating type information in *gcn5 elp3* cells fails to phenocopy *SIR* deletion means that suppression by *sir* mutation cannot be simply be explained by indirect effects arising from de-repression of silent mating type genes. We favor the idea that Sir proteins form aberrant chromatin structures also away from the telomere in *gcn5 elp3* cells (perhaps only at certain stages of the cell cycle) that repress transcription of a limited number of genes or affect processes such as chromosome condensation/decondensation, DNA replication, or other important DNA-related reactions. This and other effects of histone H3 hypoacetylation in *gcn5 elp3* (such as *SIR*-independent reduction of transcription of many genes) might combine to severely compromise growth in these cells. To our knowledge, the data presented here represent the first example of Sir proteins having a severely detrimental effect on cell growth and we therefore find it unlikely that the growth defects are related to DNA damage such as strand breaks. Indeed, the Rad53 checkpoint protein is not activated in *gcn5 elp3* cells [as measured by its phosphorylation (data not shown), a sensitive read-out for check-point activation (30)], in support of the notion that these cells do not experience significant spontaneous DNA damage.

Recruitment of Sir3 Protein and Establishment of Repressive Sir Complexes. Previous studies have shown that the initial recruitment of Sir proteins to telomere regions and silent mating loci occurs via sequence-specific proteins such as Rap1. The finding that Sir3 spreads from the telomere in *gcn5 elp3* provides further

supports for the idea that the subsequent spreading of heterochromatin from the site of origin (Rap1 binding sites) then requires that the adjoining chromatin not be highly acetylated (4, 28, 29, 31). The spreading of Sir3 in *gcn5 elp3* also underscores the important role played by transcription and transcription-related HATs in keeping heterochromatin spreading under control, as has also been suggested by work on boundary elements (32). Recently published results have shown that inhibition of heterochromatin spreading can also occur by a specific mechanism involving the histone acetyltransferase Sas2 (28, 29). Sas2 targets lysine 16 in histone H4, and in the absence of *SAS2* (or in strains carrying a phenocopy H4 Lys16Arg mutation) Sir3 spreads from the telomere ends. Interestingly, the loss of Sir3 chromatin binding observed in a *sir2*Δ strain can be suppressed by *sas2*Δ (28), indicating that Sir3 can bind to histone H4 in the absence of continued H4 K16 deacetylation by Sir2 as long as the H4 K16-specifc HAT Sas2 is absent. Sir2 and Sas2 thus have opposing effects on histone H4 acetylation and heterochromatin formation, by specifically modulating the acetylation of histone H4 lysine 16 (28, 29). Significant to the present work, Sir3 (and Sir4) is also capable of interacting with the tail of histone H3 (9), which is the primary target for the Gcn5 and Elp3 histone acetyltransferases studied here. Indeed, *gcn5 elp3* mutation does not affect the level of histone H4 K16 acetylation in the genome, but only in the region near the telomere to which Sir3 has spread. Our data on histones H3 and H4 K16 acetylation in subtelomeric DNA thus support the idea that Sir3 protein is spreading from the telomere in *gcn5 elp3* cells in response to severe histone H3 hypoacetylation, and that this recruitment in turn results in hypoacetylation of histone H4 K16, conceivably because of the action of Sir2 histone deacetylase during assembly of the entire Sir complex.

We presently favor a model in which a fraction of Sir3 proteins leave the tip of the telomere in response to severe histone H3 hypoacetylation in *gcn5 elp3* cells. This would explain where the additional Sir3 protein detected away from the telomere-end in *gcn5 elp3* is coming from, and would also help explain the slight but consistent increase in transcription 1–5,000 bp from the telomere ends observed by microarray analysis of transcription in *gcn5 elp3* cells. A decrease in Sir3 occupancy near telomeres in *gcn5 elp3* was not uncovered by ChIP, possibly because the chromatin fragments from the tip of the chromosome still contained a large amount of Sir3 and thus remained saturated with the epitopes required for efficient immunoprecipitation. The data of Grunstein and colleagues on the more severe Sir3 spreading observed in *sas2* strains indicate that depletion of Sir3 from the telomere end can indeed take place (28) and thus support this interpretation.

Several mechanisms such as histone H4 K16 acetylation by Sas2 and histone H3 K79 methylation by Dot1 have recently been shown to play a role in demarcating the boundary between hetero- and euchromatin (33, 34). The data presented here suggest that histone H3 acetylation by Gcn5 and Elp3 can be added to the growing list of mechanisms that help restrict the access for Sir proteins to euchromatin.

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