Overlapping roles for the histone acetyltransferase activities of SAGA and Elongator *in vivo*

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Elp3 and Gcn5 are histone acetyltransferases (HATs) that function in transcription as subunits of Elongator and SAGA/ADA, respectively. Here we show that mutations that impair the *in vitro* HAT activity of Elp3 confer typical *elp* phenotypes such as temperature sensitivity. Combining an *elp3Δ* mutation with histone H3 or H4 tail mutations confers lethality or sickness, supporting a role for Elongator in chromatin remodelling *in vivo*. *gcn5Δelp3Δ* double mutants display a number of severe phenotypes, and similar phenotypes result from combining the *elp* mutation with mutation in a gene encoding a SAGA-specific, but not an ADA-specific subunit, indicating that Elongator functionally overlaps with SAGA. Because concomitant active site alterations in Elp3 and Gcn5 are sufficient to confer severe phenotypes, the redundancy must be specifically related to the HAT activity of these complexes. In support of this conclusion, *gcn5Δelp3Δ* phenotypes are suppressed by concomitant mutation of the *HDA1* and *HOS2* histone deacetylases. Our results demonstrate functional redundancy among transcription-associated HAT and deacetylase activities, and indicate the importance of a fine-tuned acetylation–deacetylation balance during transcription *in vivo*.
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Introduction

In order to facilitate transcription in a repressive chromatin environment, the RNA polymerase II (RNAPII) transcription machinery can enlist chromatin-remodelling complexes (Workman and Kingston, 1998). Conversely, transcriptional repression often entails recruitment of factors stabilizing chromatin, such as histone deacetylases (HDACs) (Ayer, 1999). Remodelling factors that allow transcriptional activators and RNAPII transcription factors to work in chromatin have been divided into two major groups: ATP-dependent chromatin-remodelling machines (the SWI/SNF group of proteins) (Cairns, 1998) and histone acetyltransferases (HATs) (Mizzen and Allis,

1998; Struhl, 1998; Brown *et al.*, 2000). The discovery that a well known transcriptional co-activator, Gcn5, also possesses intrinsic HAT activity provided the first direct link between histone acetylation and gene activation (Brownell *et al.*, 1996). Since then, many studies have confirmed and extended the initial findings in different eukaryotic systems (Wade *et al.*, 1997; Mizzen and Allis, 1998; Workman and Kingston, 1998). In yeast, Gcn5 is the catalytic component of at least two multi-protein complexes, SAGA and ADA (Grant *et al.*, 1997; Eberharter *et al.*, 1999). Consistent with a role for Gcn5 in chromatin remodelling of promoters, recruitment of SAGA by a transcriptional activator leads to histone acetylation and concomitant activation of transcription *in vitro* (Utley *et al.*, 1998). Moreover, mutation of sequences encoding residues critical for Gcn5 HAT activity *in vitro* is sufficient to significantly affect the function of the protein in transcriptional activation *in vivo* (Kuo *et al.*, 1998; Wang *et al.*, 1998), and to generate localized alterations in the chromatin structure of target gene promoters (Gregory *et al.*, 1998; Kuo *et al.*, 1998).

Recently, we isolated the form of RNAPII that is responsible for transcriptional elongation (Otero *et al.*, 1999). This form of RNAPII is distinct from previously characterized RNAPII holoenzymes in that it lacks Mediator/Srb components, but instead carries a novel multi-subunit complex, termed Elongator. One of the subunits of Elongator was identified as being a novel HAT (Otero *et al.*, 1999; Wittschieben *et al.*, 1999). This protein, Elp3, is highly conserved among eukaryotes and can acetylate all four histones *in vitro*. The function of Elp3 HAT might be to confer to elongating RNAPII holoenzyme the ability to act as a 'chromatin conditioner' in generally establishing and maintaining active chromatin in eukaryotes (Travers, 1999; Wittschieben *et al.*, 1999).

The genome of the yeast *Saccharomyces cerevisiae* also encodes other HATs, such as Esa1, Sas2, Sas3, Hpa2 and Hat1 (Kleff *et al.*, 1995; Reifsnyder *et al.*, 1996; Neuwald and Landsman, 1997; Smith *et al.*, 1998; Angus-Hill *et al.*, 1999; Brown *et al.*, 2000). Among these HATs, only Esa1 is encoded by an essential gene (Smith *et al.*, 1998). Indeed, the phenotypes resulting from the deletion of any of the others are rather mild. For example, cells lacking *ELP3* and *GCN5*, the only HATs that have been connected directly to transcription both *in vitro* and *in vivo*, have doubling times comparable to those of wild type, and the strains only fail to grow under very specific conditions that are distinct for each mutation. While *gcn5* mutants grow poorly during amino acid starvation (Georgakopoulos and Thireos, 1992) and fail to undergo high-frequency mating-type inter-conversion (switching-defective) (Breeden and Nasmyth, 1987; Cosma *et al.*, 1999), *elp3* mutants display a peculiar 'slow-start' phenotype, manifested as a pronounced delay in adaptation to new growth conditions.

Elongator mutants are also salt- and temperature-sensitive (Otero *et al.*, 1999; Wittschieben *et al.*, 1999). The molecular basis for these growth phenotypes appears to be problems with activation of a few, specific genes. *gcn5* mutant strains fail to activate the *HIS4* gene in response to low nitrogen (Lucchini *et al.*, 1984), and also show reduced activation of *HO* and some additional genes (Pollard and Peterson, 1997; Cosma *et al.*, 1999; Krebs *et al.*, 1999). Likewise, *elp* strains show significant delays in the activation of genes whose expression is required for the cells to grow under the relevant conditions. For example, activation of the genes *GAL1–10*, *ENA1* and *PHO5* is delayed and often reduced in *elp3* mutants (Wittschieben *et al.*, 1999).

The rather limited phenotypic consequences of mutating the transcription-related HATs described above could be explained by the existence of functional redundancy. As a consequence, cellular functions such as transcriptional activation might still proceed normally when one HAT complex is non-functional, but not when two (or more) are non-functional. Similar redundancies might underlie the limited consequence of mutating genes encoding HDACs (Rundlett *et al.*, 1996). Here we show that single-site alterations in the HAT domain of Elp3 confer typical *elp* phenotypes such as slow growth adaptation and temperature sensitivity. A role for Elongator in chromatin modification and remodelling *in vivo* is supported by the finding that the *elp3* mutation in combination with histone tail alterations confers sickness or lethality. In support of the existence of functional overlaps between HATs, Elongator and SAGA, or, specifically, the acetyltransferase activities of Gcn5 and Elp3, can indeed not be abrogated at the same time without severe consequences for cell growth. The severe consequences of concomitant *gcn5Δelp3Δ* mutation can, however, be counteracted by also mutating the genes encoding the HDACs, *HDA1* and *HOS2*. Deletion of these genes individually fails to suppress the *gcn5Δelp3Δ* phenotypes, indicating functional redundancy among HDACs as well.

Results

The HAT activity of Elp3 is essential for its function in vivo

The presence of Elp3 protein in elongating RNAPII holoenzyme could potentially serve to couple histone modification to a DNA-tracking engine, leading to more or less genome-wide potentiation of transcriptional activation in yeast, and domain-specific changes in higher eukaryotes (Travers, 1999; Wittschieben *et al.*, 1999). In order to investigate the structure–function relationship of Elp3 HAT *in vitro*, mutations were made in *ELP3* that changed two conserved tyrosine residues to alanine in the B motif of the putative catalytic HAT domain (Y540A and Y541A, respectively; Figure 1A). These residues are likely to be directly involved in acetyl-CoA binding (Dutnall *et al.*, 1998; Rojas *et al.*, 1999). Similar mutations in yeast Gcn5 (Y220A and F221A, respectively) have previously been shown to reduce the HAT activity of this protein to <10% of wild-type levels *in vitro*, and to significantly affect its function in transcription *in vivo* (Kuo *et al.*, 1998; Wang *et al.*, 1998). Baculoviruses that enable expression of mutated Elp3 protein in insect cells were generated. As
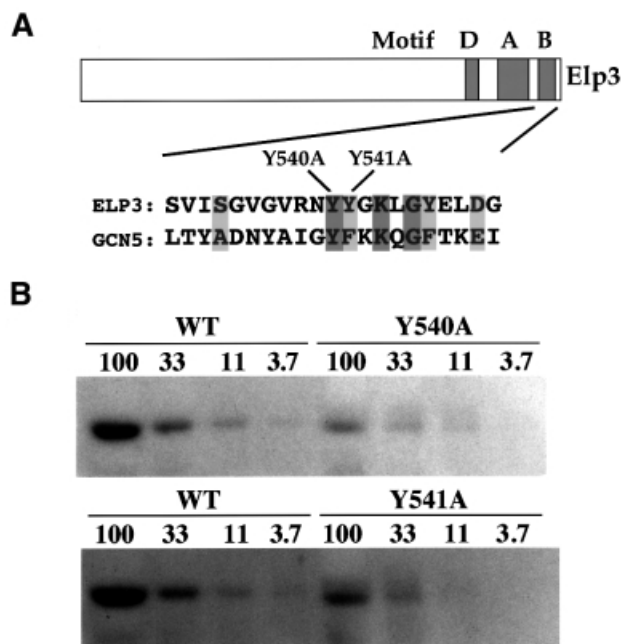

Fig. 1. Elp3 mutants have reduced HAT activity *in vitro*. (**A**) Alignment of Elp3 and Gcn5 HAT motif B based on Neuwald and Landsman (1997) with the positions of the changes introduced in Elp3 indicated. (**B**) The HAT activity of Elp3 mutants is significantly reduced. Titrations of equal amounts of wild-type and mutant proteins were fractionated in 10% SDS–PAGE gels containing either BSA (not shown) or 1 mg/ml histone. After renaturation of separated proteins, the gels were incubated with tritium-labeled acetyl-CoA. Auto-radiograms of dried gels are shown. Relative amounts of protein loaded are indicated above the lanes. Based on two independent titration experiments, the Y540A mutant had 22–25% activity, while Y541A had 34–38% activity compared with wild type.

reported previously by Wittschieben *et al.* (1999), Elp3 protein was insoluble, making it necessary to employ an in-gel HAT assay for measuring enzyme activity (Brownell and Allis, 1995; Wittschieben *et al.*, 1999). As expected, the mutant proteins had a significantly reduced activity compared with wild type: a reduction to <25% for Y540A and ~35% for Y541A (Figure 1B).

DNA fragments encoding the HAT domain mutations were exchanged for wild-type fragments in CEN plasmids carrying *ELP3* expressed from its native promoter. In order to test their ability to complement *elp* phenotypes, these plasmids were then transformed into *elp3Δ* cells (Figure 2). Wild-type and mutant versions of Elp3 expressed to roughly the same level as endogenous Elp3 (Figure 2A), and could be co-immunoprecipitated with Elp1 and Elp2 from extracts with similar efficiency (data not shown), showing that they were incorporated into Elongator complexes. As expected, expression of wild-type Elp3 rescued the mutant phenotype. In contrast, Elp3 Y541A failed to fully complement both the slow adaptation to growth on galactose (Figure 2B) and the temperature-sensitive phenotype of the strain (Figure 2C), while cells expressing Elp3 Y540A displayed phenotypes that were indistinguishable from the strain that did not express Elp3. The *in vivo* effects of these mutations correlate with the lower level of *in vitro* HAT activity observed with Elp3 Y540A compared with Elp3 Y541A (Figure 1B). The data thus indicate that the acetyltransferase activity of Elp3 is essential for Elongator function *in vivo*.
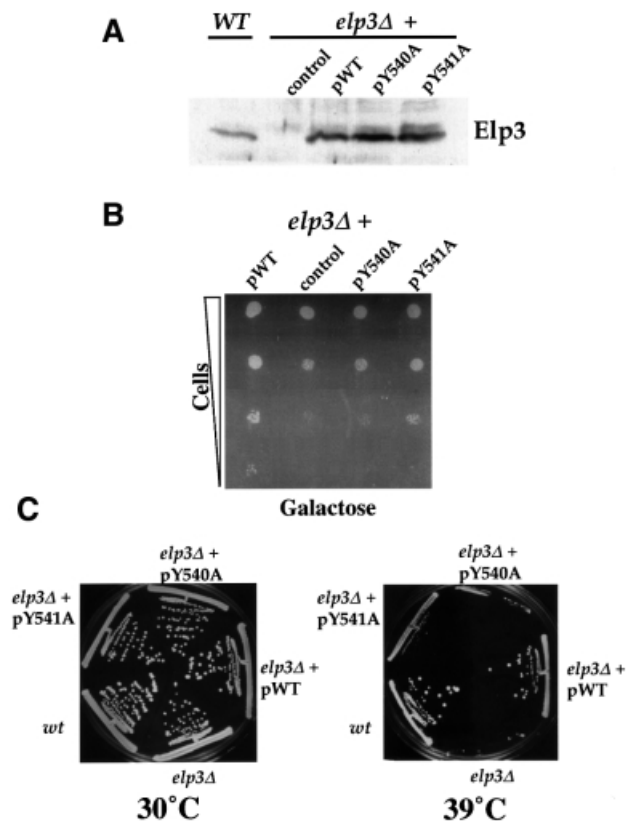
Fig. 2. Elp3 HAT mutants display *elp* phenotypes. (**A**) Similar levels of wild-type and mutant Elp3 protein are detected in whole-cells extracts. Extracts from *elp3Δ* cells carrying CEN plasmids expressing wild-type Elp3 (pWT), no protein (control), Elp3 Y540A (pY540A) or Elp3 Y541A (pY541A) were compared with wild-type (*ELP3*) extracts. The result of a western blot probed with anti-Elp3 antibodies is shown. (**B**) Elp3 protein-carrying altered HAT domains are unable to complement the slow adaptation phenotype typical of *elpΔ* strains. Strains carrying the above CEN plasmids were grown in YPD and plated in a dilution series onto YPG (galactose) plates and incubated at 30°C for 2–3 days. (**C**) Elp3 protein-carrying altered HAT domains are unable to complement the temperature-sensitive phenotype of an *elp3Δ* strain. Strains were treated as in (**B**), but streaked on YPD plates and incubated for 3–5 days at 30 or 39°C.

Synthetic phenotype of strains lacking ELP3 and the tails of histones H3 and H4

gcn5 mutation is synthetic lethal with mutations encoding changes in the N-terminal tails of histones H3 and H4 (Zhang *et al*., 1998), indicating that histones are targets for the HAT activity of Gcn5 *in vivo*. To investigate whether histones could also be genetically linked to Elp3 *in vivo*, an *elp3Δ* strain was constructed in which the endogenous H3 and H4 genes (*HHT1-HHF1* and *HHT2-HHF2*) were deleted so that survival was dependent on a plasmid borne copy of *HHT2-HHF2*. Plasmids carrying different mutated forms of the same histone genes were then introduced, and viability in the presence of these mutations was tested by plasmid shuffle experiments (Zhang *et al*., 1998) (Table I). Deletion of the N-terminal tail of histone H4 (Δ4–19) was lethal in the *elp3Δ* strain. An obvious interpretation of this result would be that Elp3 is involved in H3 acetylation *in vivo*. In the absence of H4 tails, this acetylation might become essential for viability. *elp3Δ* cells carrying a deletion of the N-terminal (Δ3–29) tail of histone H3 grew

Table I. Plasmid shuffle of histone alleles

	ELP3	*elp3Δ*
H4 Δ4–19	viable	dead
H3 Δ3–29	viable	ts–
H3 K14R *H4 K8,16R*	viable	ts–
H3 K14Q *H4 K8,16Q*	viable	viable
H3 K9R *H4 K5,12R*	viable	viable

The effects of mutated histone alleles were tested in *ELP3* or *elp3* cells carrying wild-type histones on a *URA3*-marked plasmid. After growth in URA+ media, the ability to grow in the absence of wild-type histones was tested by plating on 5-FOA-containing media. 5-FOA-resistant cells were plated on SD plates lacking uracil to confirm the loss of the *URA3*-marked plasmid, and were then plated on YPD plates and incubated at 30 and 37°C for 3–5 days to test for temperature sensitivity (ts–).

normally at 30°C, but were temperature-sensitive (37°C), pointing to a less important Elp3-mediated effect on histone H4 tails.

Distinct acetylation patterns typify nucleosome deositioning and transcription, respectively (Strahl and Allis, 2000), making synthetic phenotypes with mutations in sequences encoding specific lysine residues in the histone tails useful as tools to indicate the involvement of gene products in transcription-coupled chromatin modification (Zhang *et al*., 1998). We found that the *ELP3* mutation combined with concomitant point mutations in sequences encoding the 'transcription-related' histone H3 lysine residue 14 and H4 lysine residues 8 and 16 to arginine (H3 K14R, H4 K8,16R) also conferred temperature sensitivity. In contrast, this phenotype was not observed when *elp3Δ* was combined with changes at these sites to glutamine (which might mimic constitutive acetylation of the sites), or when combined with changes in residues primarily thought to be related to nucleosome deposition (H3 K9R, H4 K5,12R). These results indicate that Elp3 is involved in transcription-associated chromatin modification and re-modelling, and support the notion that histones are an important target for Elp3 activity *in vivo*.

Synthetic phenotype of strains lacking both GCN5 and ELP3

If Elp3 is involved in potentiation of transcriptional activation by histone acetylation, its *in vivo* function might at least partly overlap with that of the well characterized Gcn5 protein. To test this hypothesis, one copy of the *GCN5* gene was deleted in *ELP3/elp3Δ* diploid yeast cells. The resulting strain was induced to sporulate and spores dissected. *gcn5Δelp3Δ* cells generated only very small, slow growing colonies (Figure 3A). Moreover, while *gcn5Δ* and *elp3Δ* single mutants grew on galactose, raffinose and sucrose as the sole carbon source, *gcn5Δelp3Δ* cells did not (Figure 3B and data not shown). Likewise, *gcn5Δelp3Δ* cells were unable to grow in the absence of inositol or at 37°C (Figure 3B). These results clearly demonstrate that *GCN5* and *ELP3* cannot be deleted at the same time without severe effects
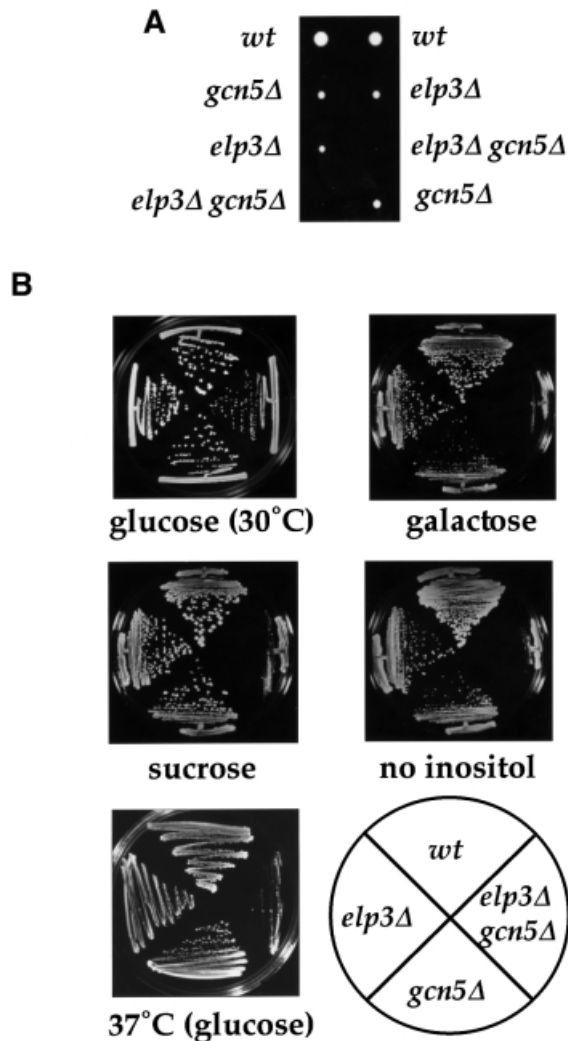
Fig. 3. Synthetic phenotype of the *elp3Δgcn5Δ* strain. (**A**) *elp3Δgcn5Δ* double mutants are slow growing. Tetrad dissections of *gcn5Δ/GCN5 elp3Δ/ELP3* diploids are shown after 2 days of incubation at 30°C. (**B**) *elp3Δgcn5Δ* double mutants are unable to grow on a number of media. Cells of the indicated genotype were streaked on YPD [glucose (30°C)], YPG (galactose), YPS (sucrose) or inositol starvation (no inositol) plates, and incubated for 3–5 days at 30°C. To demonstrate temperature sensitivity, cells of the indicated genotype were streaked on YPD [37°C (glucose)] and incubated for 3–5 days at 37°C.

on cell growth, and thus indicate that the encoded proteins perform an overlapping function or co-operate during RNAPII transcription. The results gain further significance from the finding that *ELP3* or *GCN5* deletion in combination with deletion of *HAT1* [encoding the only known B type (cytoplasmic) HAT (Kleff *et al.*, 1995; Parthun *et al.*, 1996)] does not lead to a new phenotype (data not shown; Ruiz-Garcia *et al.*, 1998). Likewise, the *ELP3* deletion in combination with deletion of genes encoding other HATs, such as *SAS3* (Reifsnyder *et al.*, 1996) and *HPA2* (Angus-Hill *et al.*, 1999), also does not result in new, dramatic phenotypes (data not shown). This indicates that the functional overlap is specifically related to transcription and not simply generally observed between HAT-encoding genes.

The phenotype of gcn5 elp3 is linked to the functions of SAGA and Elongator

While Gcn5 is the catalytic component of at least two different HAT complexes, SAGA and ADA (Grant *et al.*, 1997; Eberharter *et al.*, 1999), Elp3 appears to exert its function mostly, if not exclusively, through the Elongator complex (Wittschieben *et al.*, 1999). In agreement with this notion, mutation of the genes encoding the other subunits of Elongator (*ELP1* and *ELP2*, respectively) in conjunction with a *GCN5* mutation yielded phenotypes similar to those of the *elp3Δgcn5Δ* strain (Figure 4A and E, and data not shown). To investigate whether Elp3 (and therefore Elongator) functionally overlaps with Gcn5 in the context of the ADA or SAGA complex, we also tested whether combining mutations in *ELP3* with mutations in the ADA-specific *AHC1* gene, as well as in the SAGA-specific *SPT20* and *SPT3* genes, would confer phenotypes similar to those of *elp3Δgcn5Δ*. The *ahc1* mutation disrupts the ADA complex, the *spt20Δ* mutation is believed to abolish all SAGA functions, and *spt3Δ* is only required for a HAT-independent function of SAGA (Grant *et al.*, 1997; Roberts and Winston, 1997; Sterner *et al.*, 1999). While *elp3Δahc1Δ* and *elp3Δspt3Δ* failed to confer a new phenotype (Figure 4B, C and E, and data not shown), *elp3Δspt20Δ* strains were, like *gcn5Δelp3Δ*, very slow growing and temperature-sensitive (Figure 4D and E). We conclude that the synthetic phenotype of the *gcn5Δelp3Δ* strain is due to functional overlap or redundancy between Elongator and SAGA. The finding that the *spt3 elp3* mutation does not result in a new phenotype (these cells also grew on galactose, sucrose and in the absence of inositol; data not shown) furthermore suggests that the partial redundancy between the complexes is specifically related to the HAT functions of SAGA.

The functional overlap between Gcn5 and Elp3 is due to their intrinsic HAT activity

The basis for the dramatic consequences of lacking both Elongator and SAGA function could be a disturbance of the integrity (or stability) of SAGA and Elongator resulting from the removal of one of their subunits, and/or an effect on the functions of Gcn5 and Elp3 that is distinct from their intrinsic HAT activity. Alternatively, the basis could be a specific requirement for Elp3- and Gcn5-catalyzed acetylation by these complexes. To distinguish between these possibilities, we investigated whether merely abrogating the HAT activity of Gcn5 and Elp3 would give rise to severe phenotypes. As expected, the *gcn5Δelp3Δ* strain was dependent on a plasmid-borne copy of *ELP3* or *GCN5* for normal growth on a number of media. To investigate the effect of concomitant HAT mutation in Gcn5 and Elp3, plasmids carrying different mutated forms of the *GCN5* and *ELP3* genes were introduced, and the consequences for growth were tested by plasmid shuffle experiments (see Materials and methods for details) (Figure 5). The different versions of Elp3 expressed to similar levels and incorporated into Elongator when expressed from such constructs (Figure 2A), and the same was true for Gcn5 and SAGA (Kuo *et al.*, 1998). As expected, expression of just one wild-type version of either Gcn5 or Elp3 partially rescued the slow-growth phenotype of the *gcn5Δelp3Δ* strain, while expression of both led to wild-type-like growth.
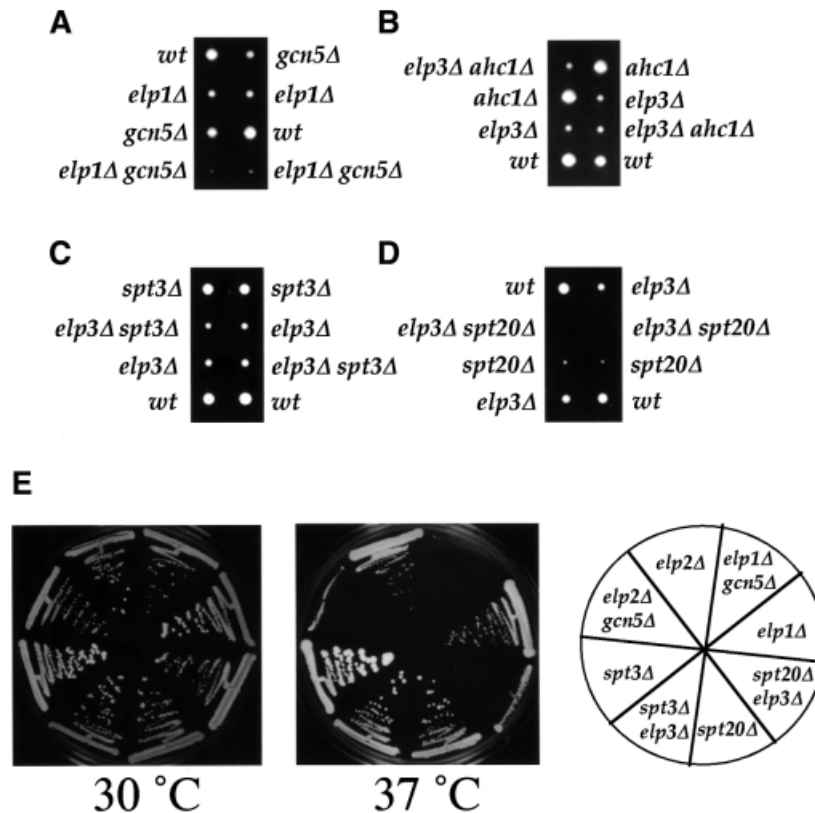
Fig. 4. Severe phenotypes of cells with concomitant disruptions of Elongator and SAGA genes. (**A–D**) *elp1Δgcn5Δ* and *elp3Δspt20Δ*, but not *elp3Δahc1Δ* and *elp3Δspt3Δ* double mutants are slow growing. Tetrad dissections of the respective diploids are shown after 2–3 days of incubation at 30°C. (**E**) *elp3Δspt3Δ*, but not *elp1Δgcn5Δ*, *elp2Δgcn5Δ* and *elp3Δspt20Δ* double mutants grow at 37°C. Cells of the indicated genotype were streaked on YPD and incubated for 3–5 days at 30 or 37°C. As expected, *elp3Δahc1Δ* cells are not temperature-sensitive either (data not shown).

However, if the HAT activity of both Gcn5 and Elp3 was impaired by a point mutation in the sequence encoding the respective catalytic domains (Kuo *et al.*, 1998), cells continued to grow very poorly. These data strongly indicate that the severe phenotypes of the *gcn5Δelp3Δ* strain are specifically due to the concomitant absence of the enzymatic activities of Gcn5 and Elp3.

Mutation of specific deacetylases can suppress the *gcn5Δelp3Δ* phenotype

Mutation of the gene encoding the HDAC Rpd3 has previously been shown to suppress phenotypes resulting from the *gcn5* mutation (Perez-Martin and Johnson, 1998). The *rpd3* mutation did not suppress *elp3* phenotypes and *rpd3Δgcn5Δelp3Δ* cells had an even more severe growth defect than *gcn5Δelp3Δ* cells (data not shown). We also found that mutation of genes encoding other deacetylases, *HDA1* and/or *HOS2* (Rundlett *et al.*, 1996), failed to suppress phenotypes resulting from single *elp3Δ* or *gcn5Δ* mutations (data not shown). In contrast, *gcn5Δelp3Δ* phenotypes could be partially suppressed by the mutation of *HDA1* and almost entirely by further mutating *HOS2* (Figure 6). These results support two conclusions: first, that HDACs are also functionally redundant; and secondly, that there is a fine-tuned, enzyme-regulated balance between acetylation and deacetylation in living cells, which can be shifted by mutating genes encoding the relevant enzymes.

Discussion

The findings reported here add to the understanding of the roles of HAT and HDACs in transcription in the following ways. First, they demonstrate a role for Elp3 acetyltransferase activity in chromatin modification and transcription *in vivo*. Secondly, they show that Gcn5 and Elp3 cannot be deleted simultaneously without severe effects on yeast growth and transcriptional activation. The partially redundant function of Elp3 and Gcn5 is exerted through the SAGA and Elongator complexes, as combinations of mutations in genes encoding other subunits of these complexes confer similar phenotypes. Moreover, it is the acetyltransferase activities of Gcn5 and Elp3 that are the determining factors for the phenotypes observed. The concomitant absence of these activities confers phenotypes similar to those observed after disruption of the entire open reading frames of the genes. Finally, the consequences of mutating both HATs can be counteracted by mutation of two, but not one, specific HDACs. This indicates the importance of a fine-tuned balance between acetylation and deacetylation *in vivo*, and points to the existence of functional redundancy among HDACs as well.

A role for Elongator in chromatin remodelling *in vivo*

Previously, we showed that Elp3 is an integral component of elongating RNAPII holoenzyme as a subunit of Elongator (Wittschieben *et al.*, 1999). Motifs in Elp3
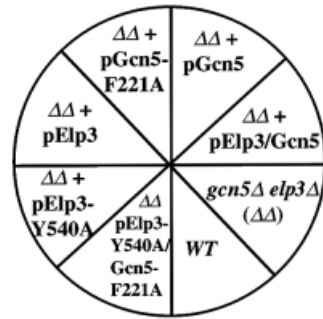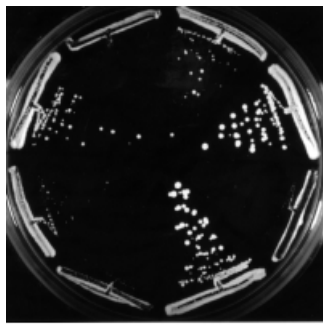
Fig. 5. Severe phenotypes of cells with concomitant disruption of Elp3 and Gcn5 HAT activity. The effects of altered HAT domains in *GCN5* and *ELP3* were tested in *gcn5Δelp3Δ* cells carrying wild-type *ELP3* on a *URA3*-marked plasmid. Cells capable of losing the *URA3* plasmid were grown on 5-FOA-containing synthetic media for 4–6 days at 30°C. See Materials and methods for details. The slight growth of *gcn5Δelp3Δ* cells carrying pELP3-Y540A was not observed in other similar experiments. *gcn5Δelp3Δ* cells expressing altered versions of the HAT domains were also temperature-sensitive and unable to grow on alternative carbon sources (data not shown).

indicated that the protein is a HAT, and this was confirmed by an *in vitro* activity assay. The data presented here show that the acetyltransferase activity of Elp3 is essential for elongator function *in vivo*. Moreover, *elp3* mutation is synthetically lethal with deletion of the tail of histone H4 (Δ4–19), and point mutation of sequences encoding transcription-related lysine residues results in temperature sensitivity in the *elp3Δ* background. A straightforward interpretation of the synthetic lethality resulting from *ELP3* and H4 tail mutation would be that Elp3 primarily acetylates the H3 tail *in vivo*.

Our demonstration of a partially overlapping function for Elongator and SAGA might provide an attractive explanation as to why these complexes have remained highly conserved throughout evolution, yet are not encoded by essential genes in yeast: an important role for either may be at least partly substituted by that of the other. Cells lacking either *GCN5* or *ELP3* grow with a doubling time comparable to wild type (doubling times 90–110 min), but *gcn5Δelp3Δ* cells grow very slowly (doubling time >4 h). These cells are also unable to grow at elevated temperature, in the absence of inositol, or on galactose, sucrose and raffinose as the sole carbon source, indicating widespread transcription defects.

Functional redundancy among chromatin remodelling complexes: similarities and differences

The genetic interaction between Elongator and SAGA reported here provides evidence for redundancy specific-
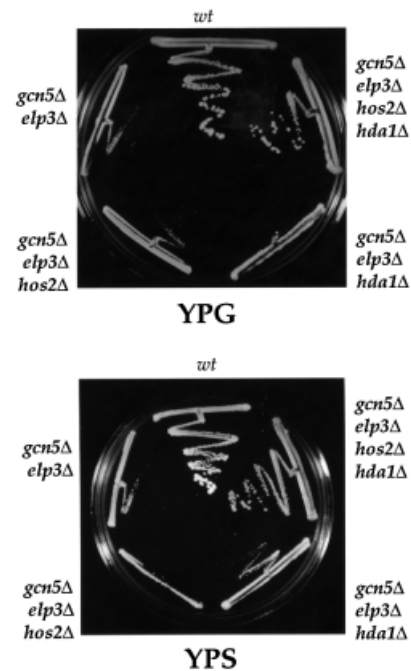

Fig. 6. The severe consequences of the *gcn5Δelp3Δ* mutation can be suppressed by mutation of specific HDACs. Mutation of *HDA1* and *HDA1 HOS2* suppresses the severe consequences of the *gcn5Δelp3Δ* mutation. Cells of the indicated genotype were streaked on YPG or YPS and incubated for 3–4 days at 30°C. Mutation of *HDA1* and *HOS2* also suppresses the temperature sensitivity (37°C) as well as the slow growth on YPD (30°C) of *gcn5Δelp3Δ* cells (data not shown).

ally among HATs. A more widespread redundancy between chromatin remodellers has previously been suggested based on the results obtained by deleting *SWI* and *ADA/GCN5* genes in the same cell (Pollard and Peterson, 1997; Roberts and Winston, 1997; Biggar and Crabtree, 1999). SWI/SNF and SAGA are likely to co-operate during promoter opening to allow activators and the basal transcription machinery access to DNA (Roberts and Winston, 1997; Pollard and Peterson, 1998; Biggar and Crabtree, 1999; Gregory *et al.*, 1999; Sudarsanam *et al.*, 1999). Several large transcription complexes, such as Mediator, Spt proteins, SWI/SNF, SAGA and Elongator, might well be partially redundant, or act in synergy, a concept previously articulated by Roberts and Winston (1997).

While the results of this study indicate that Gcn5 and Elp3 have a partially redundant function, there is also evidence to suggest that their normal role in transcription must be fundamentally different. First, the combination of *swi/snf* and *gcn5/ada* mutations gives rise to dramatic phenotypes (Pollard and Peterson, 1997; Roberts and Winston, 1997; Biggar and Crabtree, 1999), whereas the *swi2ΔelpΔ* strains display no clear, additional growth defect (data not shown). Secondly, while both *swi* and *gcn5* phenotypes can be suppressed by deletion of one of the two sets of genes that encode histones H2A and H2B [(*hta1-htb1*)Δ] (Hirschhorn *et al.*, 1992; Perez-Martin and Johnson, 1998), *elp* phenotypes cannot (data not shown). Thirdly, deletion of *RPD3*, encoding an HDAC that can be recruited to promoters by interactions with DNA-bound repressors (Kadosh and Struhl, 1997), leads to suppression of *gcn5* phenotypes (Perez-Martin and Johnson, 1998), but

Table II. Yeast strains

Strain	Genotype	Reference/source
JSY130	MATa *elp3Δ::LEU2.*	Wittschieben *et al.* (1999)
JSY131	MATα *elp3Δ::LEU2.*	Wittschieben *et al.* (1999)
JSY141	MATa *gcn5Δ::HIS3*	this study
JSY142	MATα *gcn5Δ::HIS3*	this study
JSY143	MATa *elp3Δ::LEU2 gcn5Δ::HIS3*	this study
JSY144	MATα *elp3Δ::LEU2 gcn5Δ::HIS3*	this study
JSY411	MATα *elp1Δ::LEU2 gcn5Δ::HIS3*	this study
JSY150	MATa *elp2Δ::LEU2 gcn5Δ::HIS3*	this study
JSY151	MATα *elp2Δ::LEU2 gcn5Δ::HIS3*	this study
JSY330	MATα *elp3Δ::LEU2 spt3Δ::HIS3*	this study
JSY331	MATa *elp3Δ::LEU2 spt3Δ::HIS3*	this study
JSY332	MATa *spt3Δ::HIS3*	this study
JSY456	MATa *ahc1Δ::HIS3*	this study
JSY457	MATα *ahc1Δ::HIS3*	this study
JSY458	MATa *elp3Δ::LEU2 ahc1Δ::HIS3*	this study
JSY459	MATα *elp3Δ::LEU2 ahc1Δ::HIS3*	this study
JSY407	MATa *spt20Δ::HIS3*	this study
JSY408	MATα *spt20Δ::HIS3*	this study
JSY409	MATa *elp3Δ::LEU2 spt20Δ::HIS3*	this study
JSY410	MATα *elp3Δ::LEU2 spt20Δ::HIS3*	this study
DY5939	MATa *elp3::LEU2 gcn5::HIS3 hda1::URA3*	this study
DY5937	MATa *elp3::LEU2 gcn5::HIS3 hos2::TRP1*	this study
DY5935	MATa *elp3::LEU2 gcn5::HIS3 hos2::TRP1 hda1::URA3*	this study
DY5993	MATα *elp3::LEU2 gcn5::HIS3 rpd3::KanMX*	this study
DY5733	MATa *ade2 can1 his3 leu2 lys2 trp1 ura3 hht1-hhf1Δ::LEU2 hht2-hhf2Δ::kanMX3 +* YCp50:HHT2-HHF2 (URA3)	this study
DY5734	MATα *ade2 can1 his3 leu2 lys2 trp1 ura3 hht1-hhf1Δ::LEU2 hht2-hhf2Δ::kanMX3 +* YCp50:HHT2-HHF2 (URA3)	this study
JSY316	MATα *ade2 can1 his3 leu2 lys2 trp1 ura3 elp3::ADE2 hht1-hhf1Δ::LEU2 hht2-hhf2Δ::kanMX3 +* YCp50:HHT2-HHF2 (URA3)	this study

does not result in suppression of the effects of the *elp3* mutation. Taken together, these data, as well as functional data obtained *in vitro* (Cote *et al.*, 1994; Imbalzano *et al.*, 1994; Kwon *et al.*, 1994; Utley *et al.*, 1998; Otero *et al.*, 1999), are consistent with the notion that the SWI/SNF complex and SAGA are primarily involved in assisting transcriptional activators and basal factors in their association with promoter sequences (Pollard and Peterson, 1998), while Elongator is likely to function mainly in elongation (Otero *et al.*, 1999). It cannot be ruled out, however, that Elongator also plays a role during promoter remodelling, for example during its loading onto RNAPII at the initiation–elongation transition. Likewise, it is also possible that SAGA might play an as yet undefined role during transcript elongation through chromatin.

An acetylation–deacetylation balance and its importance for transcription in living cells

Our data indicate that the most likely explanation for the severe consequences of concomitantly disrupting *GCN5* and *ELP3* is the reduced acetylation of transcription-related lysine residues in histone tails. First, severe phenotypes are also observed in cells concomitantly expressing Gcn5 and Elp3 with single residue alterations in their HAT domains. Secondly, mutation of the deacetylases *HDA1* and *HOS2* in combination is sufficient to suppress *gcn5Δelp3Δ* phenotypes. These data suggest a significance of overall acetylation levels for transcription proficiency. It might indeed be relevant to think of the effects of deleting both Gcn5 and Elp3 in the light of the proposed existence of an enzyme-maintained acetylation–deacetylation equilibrium. In *gcn5Δelp3Δ* cells, this

equilibrium may have become shifted too far towards deacetylation at certain genes. A shift towards deacetylation might create a local chromatin structure, a 'chromatin micro-environment', which is detrimental to the efficient opening of the chromatin structure required for access by transcription factors, as well as for efficient progress of the transcription machinery through the coding region at these genes.

Materials and methods

Yeast strains, media and DNA constructs
All the *S.cerevisiae* strains used for genetic analysis (Table II) were congenic with strain W303 (*ade2 can1 his3 leu2 trp1 ura3*) (Thomas and Rothstein, 1989), and were grown and manipulated essentially as described previously (Otero *et al.*, 1999). Deletion of the open reading frame of yeast genes was performed as described (Lorenz *et al.*, 1995). *ELP2* is described elsewhere (Fellows *et al.*, 2000). Plates used to investigate the ability of strains to ferment galactose, raffinose and sucrose contained 1 µg/ml antimycin A (Sigma).

ELP3 coding sequence with upstream regulatory sequences as well as downstream terminator sequences was copied from yeast genomic DNA using *Pfu* polymerase (Stratagene) and primers containing *Bam*HI and *Kpn*I sites, respectively. Products were cloned into the *Bam*HI and *Kpn*I sites of pRS316 (Sikorski and Hieter, 1989) to create the plasmid pBOP60-13 (pWT). Single point mutations in sequences encoding the HAT domain of Elp3 were created by PCR mutagenesis using primers encoding the mutation as well as *Bam*HI or *Sal*I sites. Mutated versions of the HAT catalytic domain were then exchanged with wild-type fragments, yielding pBOP60-14 (pY540A) and pBOP60-15 (pY541A). Mutations that had been introduced were confirmed by sequencing. *TRP1*-marked plasmids concomitantly expressing Gcn5 and Elp3 alleles from their cognate promoters were constructed by cloning PCR products generated from pBOP60-13 and pBOP60-14 with *Eag*I ends into the *Eag*I site of pRS314 (Sikorski and Hieter, 1989), pMK125 and pMK125 F221A (Kuo *et al.*, 1998). These plasmids were named pBOP60-26 (wild-

type Elp3), pBOP60-31 (Elp3 Y540A), pBOP60-32 (wild-type Elp3; wild-type Gcn5) and pBOP60-35 (Elp3 Y540A; Gcn5 F221A). The experiments described were performed using plasmids where the *ELP3* gene pointed towards the *GCN5* gene, but similar results were obtained with either orientation. Details of cloning procedures are available on request.

Plasmid shuffle experiments

Plasmid shuffle of histone alleles carried on *TRP1*-marked plasmids into *ELP3* (DY5734) or *elp3Δ* (JSY316) cells carrying wild-type histones on a *URA3*-marked plasmid was performed as described (Zhang *et al.*, 1998). Concomitant experiments with similar *gcn5Δ* cells confirmed the results previously reported using another parental strain (Zhang *et al.*, 1998). S.Roth (University of Texas) kindly provided all the histone plasmids and the parental histone shuffle strain used to construct a W303 counterpart. After growth in URA⁺ media, cells that had lost the *URA3* plasmid were selected on 5-fluoroorotic acid (5-FOA)-containing media. Survivors were checked for the loss of ability to grow on ura⁻ plates. For temperature sensitivity assays, cells that had lost the wild-type *URA3*-marked histone alleles on 5-FOA were plated on yeast extract/peptone/dextrose (YPD) and incubated at 37°C for 3–4 days.

We repeatedly observed that the *gcn5Δ elp3Δ* strain very rapidly picked up suppressor mutations. This was further enhanced when experiments with point-mutated versions of the genes were attempted, and we therefore adopted the plasmid shuffle technique to circumvent the problem. *gcn5Δ elp3Δ* cells carrying wild-type *ELP3* on a *URA3*-marked plasmid were transformed with *TRP1*-marked plasmids concomitantly expressing *ELP3* and *GCN5* HAT alleles. These cells were thus phenotypically *ELP3 gcn5Δ*. Cells that allowed the loss of the *URA3* plasmid were selected on 5-FOA-containing media. This approach allowed a reproducible comparison of the severity of deleting *GCN5* and *ELP3* with merely mutating the respective HAT domains.

In-gel HAT assay

HAT activity assays of Elp3 expressed in insect cells were performed as described (Wittschieben *et al.*, 1999) using either bovine serum albumin (BSA) or core histones (Sigma). Gels were typically exposed to autoradiograms for 3–6 days to reveal Elp3-positive HAT signals. Relative amounts of wild-type and mutant Elp3 to be used for the activity comparisons were determined by western blotting of dilution series from the respective HAT fractions. Quantification of acetylation levels was performed by scanning autoradiographs with a Umax Powerlook 2000 scanner, using NIH Image 1.61 software for data processing. The relatively modest decreases in the activity of Elp3 HAT mutants compared with those obtained by a similar mutation in Gcn5 (Kuo *et al.*, 1998) might at least partly be a reflection of the different assays used (in-gel assay at high protein concentration versus liquid assay).

Acknowledgements

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