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Expression profiling of *S. pombe* acetyltransferase mutants identifies redundant pathways of gene regulation

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Abstract

Background: Histone acetyltransferase enzymes (HATs) are implicated in regulation of transcription. HATs from different families may overlap in target and substrate specificity.

Results: We isolated the *elp3⁺* gene encoding the histone acetyltransferase subunit of the Elongator complex in fission yeast and characterized the phenotype of an Δ *elp3* mutant. We examined genetic interactions between Δ *elp3* and two other HAT mutants, Δ *mst2* and Δ *gcn5* and used whole genome microarray analysis to analyze their effects on gene expression.

Conclusions: Comparison of phenotypes and expression profiles in single, double and triple mutants indicate that these HAT enzymes have overlapping functions. Consistent with this, overlapping specificity in histone H3 acetylation is observed. However, there is no evidence for overlap with another HAT enzyme, encoded by the essential *mst1⁺* gene.

Background

Chromatin is modulated in part by the pattern of different histone modifications, leading to the speculation that a “histone code” provides epigenetic information that facilitates chromosome activities [1,2]. Not surprisingly, the enzymes that modify chromatin have diverse roles in the cell that affect multiple DNA-dependent events. Acetylation was the first histone modification identified, and is associated with a variety of chromatin functions [2,3]. While some evidence suggests that acetylation changes association of DNA with the underlying nucleosomes [4], it also creates specific binding sites for proteins involved in a variety of DNA transactions [2,3]. Acetylation has long been linked to transcriptional activation, so that the histone acetyltransferase enzymes function generally as transcriptional activators [5].

Histone acetyltransferases are well conserved in eukaryotes and can be separated into multiple gene families based on primary sequence. The MYST family of

histone acetyltransferases was named for founding members identified in yeast and humans, MOZ, YBF2/SAS3, SAS2 and TIP60 [6]. Most species contain multiple members of this family. The Kat5 group, the most conserved family members, includes *SpMst1* (*S. pombe*), *ScEsa1* (*S. cerevisiae*) and *hTip60* (human), which acetylates residues in the tails of histones H2A and H4 [7]. These proteins are the catalytic HATs for the NuA4 complex in their respective organisms [8,9]. In the yeasts the Kat5 orthologues are the only MYST family HATs that are essential for viability [10,11].

In budding yeast there are two other MYST family members, *ScSas2* and *ScSas3*, which were originally isolated with defects in silencing the mating locus [12,13]. *ScSas2* is the catalytic HAT for the budding yeast SAS complex that acetylates H4K16 [14]. It antagonizes the histone deacetylase Sir2 [15]. *ScSas3* is the catalytic HAT for the NuA3 complex in budding yeast [16] which specifically acetylates histone H3 [17]. In *S. pombe* by contrast there is only one additional MYST family member, *SpMst2* [11]. *Mst2* shows sequence similarity to both *ScSas2* and *ScSas3*. However, its biological function, in which it antagonizes *SpSir2* in

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telomere silencing, suggests that it is a functional homologue of ScSas2 [11].

Another conserved family of HATs is the Gcn5-related N-acetyltransferase (GNAT) super-family consisting of Gcn5, Elp3, Hat1 and Hpa2, with Gcn5 (KAT2) and Elp3 (KAT9) as the most studied members [18]. The evolutionarily conserved Gcn5 is the catalytic subunit of several multi-subunit complexes including the SAGA co-activator complex [19]. Gcn5 acetylates multiple lysine residues of histones H3 and H2B and mediates both targeted and non-targeted (global) acetylation [20]. In *S. cerevisiae*, SAGA is recruited to promoter regions by transcription factors like ScGal4 and ScGcn4. Elongating RNA Pol II is associated with the Elongator complex that contains ScElp3 and has been shown to acetylate H3K14 and H4K8 in budding yeast [21]. Elp3 is highly conserved in structure and function between human and budding yeast [22].

While neither ScGcn5 nor ScELP3 is essential in budding yeast, a double mutation in $\Delta gcn5 \Delta elp3$ double mutant is significantly sicker than either single mutant [23], resulting in hypoacetylation of histone H3 in gene coding regions [24] and spreading of ScSir3 into sub-telomeric DNA [25]. Data from budding yeast suggest that ScGcn5 also overlaps with ScSas3, because a double deletion $\Delta gcn5 \Delta sas3$ is lethal [26]. This suggests that there are shared functions or targets of the NuA3, SAGA, and Elongator complexes in budding yeast, which implies that different HAT families may perform the same modifications. However, because the complement of HATs is somewhat different in *S. pombe*, it is not clear how general this overlap may be. SpGcn5 has been previously characterized for its effects on gene expression [27,28] and for chromatin binding genome-wide [29]. Surprisingly, relatively little change in gene expression profile is observed in *gcn5* mutants despite a very high association with the chromatin. This suggests that SpGcn5 overlaps with other HATs.

Here, we examine evidence for redundancy between HAT enzymes in *S. pombe*. We report the initial characterization of fission yeast *elp3*⁺ and examine phenotypes associated with single, double, and triple mutations of $\Delta mst2$, $\Delta gcn5$ and $\Delta elp3$. We show that these enzymes affect expression of overlapping but nonidentical sets of genes, suggesting overlapping contributions to gene expression. Consistent with this, we observe biochemical evidence that these proteins share common histone substrates. We also observe that these phenotypes are distinct from the gene expression effects of a temperature sensitive allele of *mst1*, and suggest that multiple histone targets regulate gene activation in fission yeast.

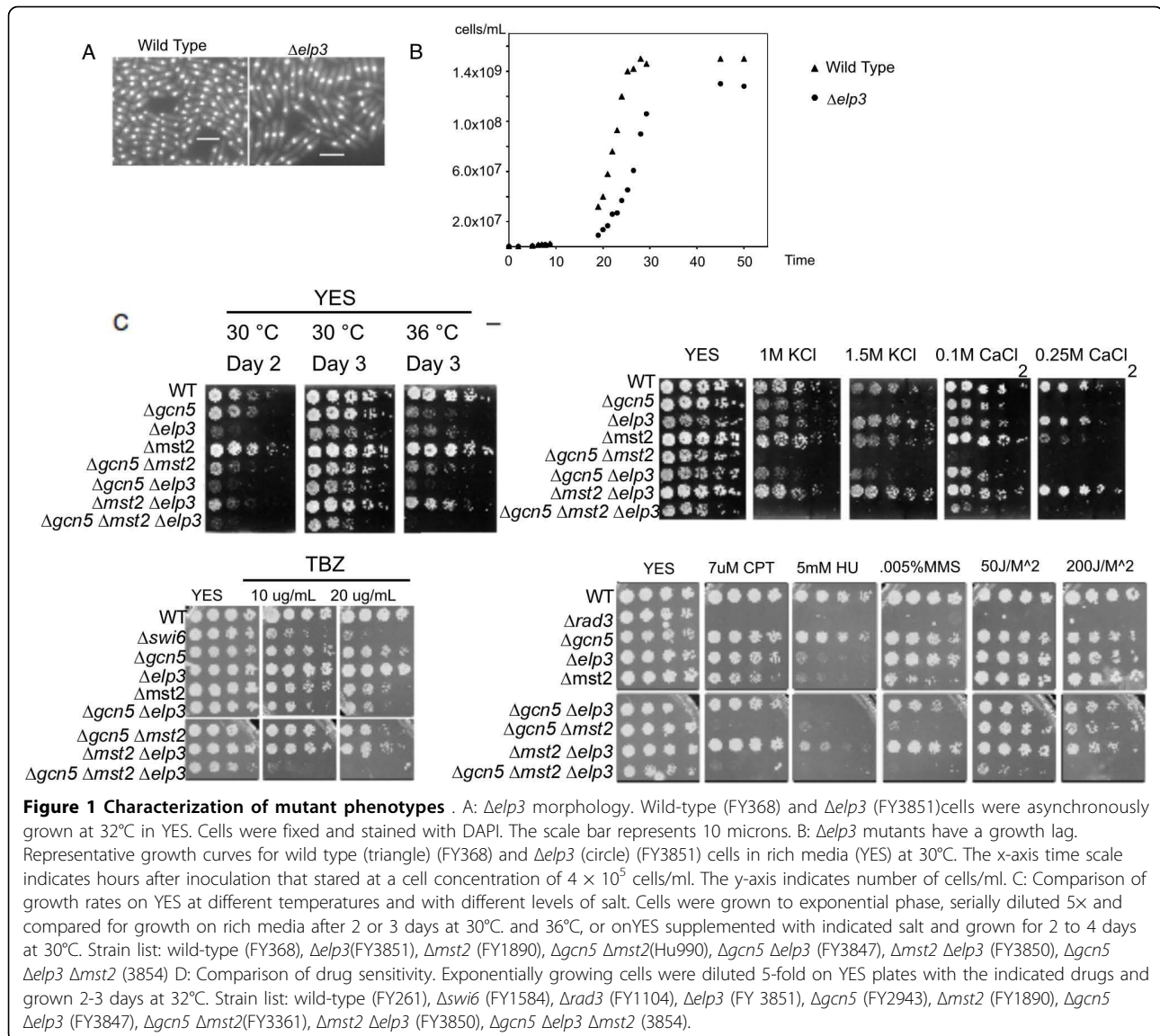
Results

Characterization of $\Delta elp3$ and interaction with other HAT mutants.

In order to compare the roles of different histone acetyltransferases on transcription in fission yeast, we first characterized the disruption of the histone acetyltransferase gene *elp3*⁺, the likely orthologue of the ScELP3 subunit of Elongator complex [21]. We found that *S. pombe elp3*⁺ is a non-essential gene, as is its budding yeast orthologue [30]. However, $\Delta elp3$ mutant cells have elongated morphology compared to wild type, indicative of cell cycle delay (Fig. 1A). The mean length of mononucleate cells is 6.7 μ m in wild type, and 10.7 μ m in $\Delta elp3$. For binucleate cells, wild type averages 9.8 μ m compared to 15.9 μ m in $\Delta elp3$. Thus $\Delta elp3$ is approximately 60% larger than wild type. Also as in budding yeast [30], fission yeast $\Delta elp3$ grows slowly due to a prolonged lag phase (Fig. 1B). Although the growth rate of $\Delta elp3$ cells once they have reached exponential phase is similar to wild type, they appear to enter stationary phase at a lower cell density. Given the presumed role of Elp3 as part of the Elongator complex, we presume that this reflects defects in one or more growth-associated transcription program.

We examined other phenotypes associated with $\Delta elp3$, and compared them to phenotypes associated with mutations in other non-essential histone acetyltransferases, $\Delta mst2$ and $\Delta gcn5$. We observed that $\Delta elp3$ has poor growth overall compared to either $\Delta gcn5$ or $\Delta mst2$ (Fig. 1C). Both $\Delta gcn5$ and $\Delta elp3$ showed slight temperature sensitivity. As reported previously [28], $\Delta gcn5$ is sensitive to salts (KCl or CaCl₂), but we observed no overlap in this phenotype with $\Delta mst2$ or $\Delta elp3$. We also assayed the sensitivities of these HAT mutants to different DNA damaging agents (Fig. 1D), since histone acetylation is necessary for proper repair from DNA damage [31]. The $\Delta mst2$ mutants are sensitive to hydroxyurea, which depletes nucleotides and results in replication fork stalling, and to high doses of the DNA alkylating agent MMS, which causes DNA damage [11]. We observed that $\Delta gcn5$ and $\Delta mst2$ show similar sensitivity to hydroxyurea. Consistent with our previous results, $\Delta mst2$ is modestly sensitive to low levels of MMS, but we observed no sensitivity in $\Delta gcn5$ or $\Delta elp3$. We also observed no sensitivity to the topoisomerase inhibitor camptothecin (which causes S phase specific DNA breaks) or UV irradiation. $\Delta mst2$ is also sensitive to the microtubule poison thiabendazole (TBZ), a phenotype typical of mutations that affect centromere function or chromosome segregation.

We next constructed double and triple mutants to see whether there was evidence for epistasis or synthetic growth defects under these conditions. All combinations



were viable, although the triple mutant $\Delta gcn5 \Delta elp3 \Delta mst2$ was very slow-growing. The slow-growth phenotype of the triple mutant strain made it difficult to distinguish pathway specific effects from general sickness in this strain.

Previously, we assigned Mst2 as the likely *ScSas2* orthologue, based on the similarity of their phenotypes, although it is also phylogenetically related to *ScSas3*. In budding yeast $\Delta sas3 \Delta gcn5$ is lethal [26], but in fission yeast $\Delta mst2 \Delta gcn5$ is viable. We assayed growth at 30° and 36°C for the single and double mutants. At 30°C, there was little effect observed. At 36°C, the $\Delta gcn5 \Delta mst2$ mutant displays a temperature sensitivity resembling $\Delta gcn5$, indicating that there is no overlap between *Gcn5* and *Mst2* with regards to heat stress. The $\Delta gcn5 \Delta elp3$ mutant, however, shows increased temperature

sensitivity compared to the single mutant strains. The $\Delta mst2 \Delta elp3$ mutant displayed a phenotype intermediate between the parents, suggesting that the faster growth associated with $\Delta mst2$ in relation to wild type is also able to overcome the slow growth associated with $\Delta elp3$ (Fig. 1C).

We examined the salt sensitivity of the double mutants, because *Δgcn5* has been associated with this phenotype [28]. The $\Delta gcn5 \Delta mst2$ double mutant is hypersensitive to salt compared to $\Delta gcn5$ alone (Fig. 1C), suggesting that for this function, these two HATs overlap. In contrast, the $\Delta gcn5 \Delta elp3$ double mutant has a phenotype similar to $\Delta gcn5$ alone suggesting that *Elp3* does not contribute to proper salt stress response.

Since $\Delta mst2$ has been linked to damage sensitivity, we also examined the response to different DNA damaging

agents (Fig. 1D). $\Delta mst2 \Delta gcn5$ cells also showed increased sensitivity to MMS and CPT treatment relative to the two single mutants, suggesting that these two HAT enzymes make redundant contributions to replication-specific DNA damage. Interestingly, $\Delta elp3$ modestly suppressed the sensitivity of $\Delta mst2$ to HU (Fig. 1D). All the double mutants showed some sensitivity to TBZ; however, $\Delta gcn5 \Delta elp3$ was more sensitive than the parent strains.

Genome wide expression studies

Previous studies showed while Gcn5 is bound to a high proportion of genes in *S. pombe* growing on rich media, it is required for the expression of only a few of its binding targets [27,28]. This together with the synthetic and additive phenotypes observed here with the different mutant combinations suggests that HAT enzymes redundantly affect common pathways at the level of transcriptional regulation. However, it is also formally possible that they independently affect regulation of separate pathways that overlap downstream at the level of cellular function. To distinguish between these possibilities, we performed genome-wide analysis of expression using microarray technology to identify target genes that show HAT-specific changes in gene expression. We examined the effects on transcription upon deletion of the GNAT family HATs Gcn5 and Elp3 and the MYST HAT Mst2 both singly and in combination. We chose a very statistically rigorous cutoff of 3.25 log₂ fold change for genes that we considered differentially expressed. This allowed us to be extremely confident that all results were biologically relevant and eliminated most false positives.

We found that there were very few genes whose expression was significantly changed by 3.25 log₂ fold or more during asynchronous growth compared to wild-type cells in $\Delta mst2$, $\Delta gcn5$ or $\Delta elp3$ cells (Table 1). Of the small number of genes with increased expression, the RecQ-type DNA helicase SPBCPT2R1.08c, located in the sub-telomere domain (*tlh2*⁺; [32,33]) was the only significantly up-regulated gene in all three mutant strains. Consistent with previously published data we found that there was an enrichment of genes involved in mating and meiosis in $\Delta gcn5$ cells (Additional File 1). Interestingly, of the few genes with decreased expression in $\Delta gcn5$ and $\Delta mst2$, most are found within 150 kb of the ends of chromosome I or II, suggesting that cells require Gcn5 and Mst2 for expression of genes localized near the sub-telomeric region (Fig. 2D).

The few genes that were differentially expressed in $\Delta elp3$ and $\Delta gcn5$ showed significant over-representation in several functional ontology groups. In agreement with prior reports [27] we observed that $\Delta gcn5$ mutants repressed mating and meiosis genes. Specifically we found that gene ontology (GO) terms [34] related to

mating and meiosis were found in the up-regulated genes, indicating that Gcn5 is required for repression of these genes. In $\Delta elp3$ mutants we found that genes significantly enriched in GO classes associated with transmembrane transport were down-regulated and those associated with ion transport were up-regulated (Fig 2, Additional File 1). Interestingly, *mei2*, which has been shown to be repressed by Gcn5 [27] was down-regulated in $\Delta elp3$ (Fig. 3). This may indicate that Gcn5 and Elp3 target the same pathway but with opposite effects. The few genes that were differentially expressed in $\Delta mst2$ mutants provided no over-represented GO classes.

Global gene expression changes in double mutants

We next asked whether the transcription profiles of the double mutants were simply additive to the single mutants, or whether they affected new genes not in common to the single mutant strains. We assayed changes in gene expression in the double mutants. The number of differentially expressed genes in the double and triple mutants was significantly increased compared to the respective single mutants for each strain tested (Fig. 2, Additional File 1, Additional File 2), suggesting overlapping regulation rather than an additive effect.

Only the helicase *tlh2*⁺ and a metabolic kinase, *dak1*⁺ were significantly up-regulated in all mutant combinations. The double mutants $\Delta gcn5 \Delta elp3$ and $\Delta mst2 \Delta elp3$ had the most overlapping up-regulated genes (Fig. 2, Additional File 3). These genes included metal transporters and the sporulation specific gene *spo6*⁺. We found that $\Delta gcn5 \Delta mst2$ cells had a greater number of up-regulated genes related to the mating pathway than $\Delta gcn5$ alone, suggesting that Gcn5 and Mst2 are functionally redundant in repressing sexual differentiation. This is also observed, although to a lesser extent, with the $\Delta gcn5 \Delta elp3$ mutant. However changes associated with $\Delta mst2 \Delta elp3$ were not significantly enriched in gene classes associated with mating or meiosis, except for *spo6*⁺, which is essential for progression of meiosis II and sporulation. When we examined down-regulated genes in the double mutants, we again found the most overlap between $\Delta gcn5 \Delta elp3$ and $\Delta mst2 \Delta elp3$ cells, however there were more differentially expressed genes in $\Delta gcn5 \Delta mst2$. A large fraction of these genes were found to be sub-telomeric (Fig. 2D) which suggests that Mst2 and Gcn5 but not Elp3 function preferentially in the sub-telomeric regions. This is consistent with previous studies suggesting that Mst2 works at the telomere [11].

Functional redundancies between HATs

To determine whether the mutations affected distinct functional groups, we categorized the affected gene targets by gene ontology (GO) classification. As was apparent for the individual genes, the genes affected by the double mutants were more significantly enriched in a

Table 1 Differentially regulated genes in single HAT mutants.

| Down-regulated genes | | | | | | | | |
|----------------------|---------------|----------|---------------|---------------|---------------|--------------|---------------|----------|
| Gene | $\Delta gcn5$ | | Gene | $\Delta mst2$ | | Gene | $\Delta elp3$ | |
| | Log change | p-value | | Log change | p-value | | Log change | p-value |
| his3 | -5.12 | 7.16E-03 | SPAC186.05c | -3.77 | 4.00E-07 | SPAC186.05c | -4.31 | 1.11E-04 |
| SPAC186.05c | -4.09 | 1.26E-07 | SPBPB2B2.06c | -3.66 | 6.60E-07 | SPAC186.06 | -3.23 | 7.69E-03 |
| gcn5 | -3.47 | 1.08E-02 | SPAC186.06 | -3.52 | 1.94E-06 | inv1 | -2.57 | 4.30E-02 |
| SPBPB2B2.06c | -3.26 | 3.11E-06 | SPAC17G8.13c | -2.76 | 2.03E-02 | str1 | -2.50 | 5.79E-03 |
| SPAC186.06 | -3.23 | 5.82E-06 | SPAC1039.02 | -2.26 | 2.58E-03 | SPBPB2B2.08 | -2.34 | 3.98E-02 |
| SPBPB2B2.01 | -2.60 | 2.00E-05 | SPBPB2B2.01 | -2.23 | 1.12E-04 | mei2 | -2.27 | 2.18E-02 |
| SPBPB2B2.05 | -2.35 | 1.46E-02 | | | ght4 | -1.90 | 1.90E-02 | |
| SPAC977.14c | -2.29 | 2.54E-02 | | | SPBC215.11c | -1.75 | 5.35E-05 | |
| SPBPB2B2.08 | -2.22 | 2.01E-02 | | | | | | |
| SPAC1039.02 | -2.17 | 3.46E-03 | | | | | | |
| SPAC977.05c | -2.10 | 2.09E-03 | | | | | | |
| SPBPB10D8.02c | -2.02 | 1.47E-03 | | | | | | |
| Up-regulated genes | | | | | | | | |
| Gene | $\Delta gcn5$ | | Gene | $\Delta mst2$ | | Gene | $\Delta elp3$ | |
| | Log change | p-value | | Log change | p-value | | Log change | p-value |
| spn6 | 2.05 | 4.71E-03 | SPBCPT2R1.08c | 2.08 | 3.59E-02 | SPAPB1A11.01 | 1.88 | 1.01E-04 |
| spk1 | 2.26 | 4.08E-03 | SPBC359.06 | 2.29 | 2.33E-02 | SPCC132.04c | 2.01 | 6.01E-08 |
| SPCC794.01c | 2.34 | 3.09E-02 | | | SPBC2G2.17c | 2.02 | 2.69E-05 | |
| mam2 | 2.45 | 3.38E-03 | | | SPBPB8B6.03 | 2.05 | 6.89E-04 | |
| mei2 | 2.57 | 5.66E-03 | | | spo6 | 2.05 | 1.63E-04 | |
| SPCC1739.08c | 2.94 | 2.22E-02 | | | air2 | 2.08 | 6.21E-05 | |
| SPBC359.06 | 3.20 | 2.79E-03 | | | SPAC750.04c | 2.08 | 3.73E-06 | |
| mfm1 | 3.23 | 4.03E-03 | | | spn5 | 2.17 | 9.14E-06 | |
| mfm3 | 3.27 | 3.68E-03 | | | ctr4 | 2.22 | 1.49E-03 | |
| ght3 | 3.87 | 8.30E-03 | | | SPAC2E1P3.02c | 2.62 | 5.60E-08 | |
| SPBCPT2R1.08c | 3.89 | 7.73E-04 | | | SPAC750.07c | 3.83 | 2.13E-03 | |
| matmc_1 | 4.18 | 8.90E-03 | | | dak2 | 3.86 | 2.68E-02 | |
| mfm2 | 4.73 | 8.99E-03 | | | SPBCPT2R1.08c | 4.50 | 1.23E-03 | |

This table lists the down and up-regulated genes of mutant HATs compared to wild-type cells using an Affymetrix microarray.

larger number of GO classes than for the single mutants. For GO classes enriched in down-regulated genes there were 23 terms for $\Delta gcn5 \Delta elp3$, 22 for $\Delta gcn5 \Delta mst2$, while $\Delta mst2 \Delta elp3$ had 10 enriched GO terms (Fig 2, Additional File 1). Curiously there is almost no overlap in enriched GO terms associated with genes down-regulated between double mutants, with the exception of cellular response to stress and response to stimulus shared between $mst2 \Delta elp3$ and $\Delta gcn5 \Delta elp3$. Therefore we conclude that Gcn5, Mst2 and Elp3 are functionally redundant in activating transcription. For GO categories enriched with up-regulated genes there were 7 categories for $\Delta gcn5 \Delta elp3$, 36 for $\Delta gcn5 \Delta mst2$, and $\Delta mst2 \Delta elp3$ had 3 enriched GO terms (Fig. 2). There are no overlapping enriched up-regulated GO categories between double mutants. When we examined the number of genes and the GO terms they represent in the double mutants compared to the single mutants,

we conclude that Gcn5, Mst2 and Elp3 are functionally redundant in repressing transcription because the number of terms enriched in the double mutants cannot be explained by adding the single mutants' terms together. There were more significantly enriched genes in the double mutants than in the single mutants.

Validation of microarray results

We used quantitative PCR (qPCR) to confirm the microarray results. The ddCt method [35] was applied to measure relative fold differences between each strain and one wild type sample with *act1*⁺ used as the loading control. For each strain, we chose genes that were predicted by the microarray to have significant changes in transcription. The fold changes of the other two wild type samples relative to the first wild type sample was small (2-fold at the most) across all the loci. The fold changes of the other two control genes relative to *act1*⁺ were also small (2-fold at the most) across all the

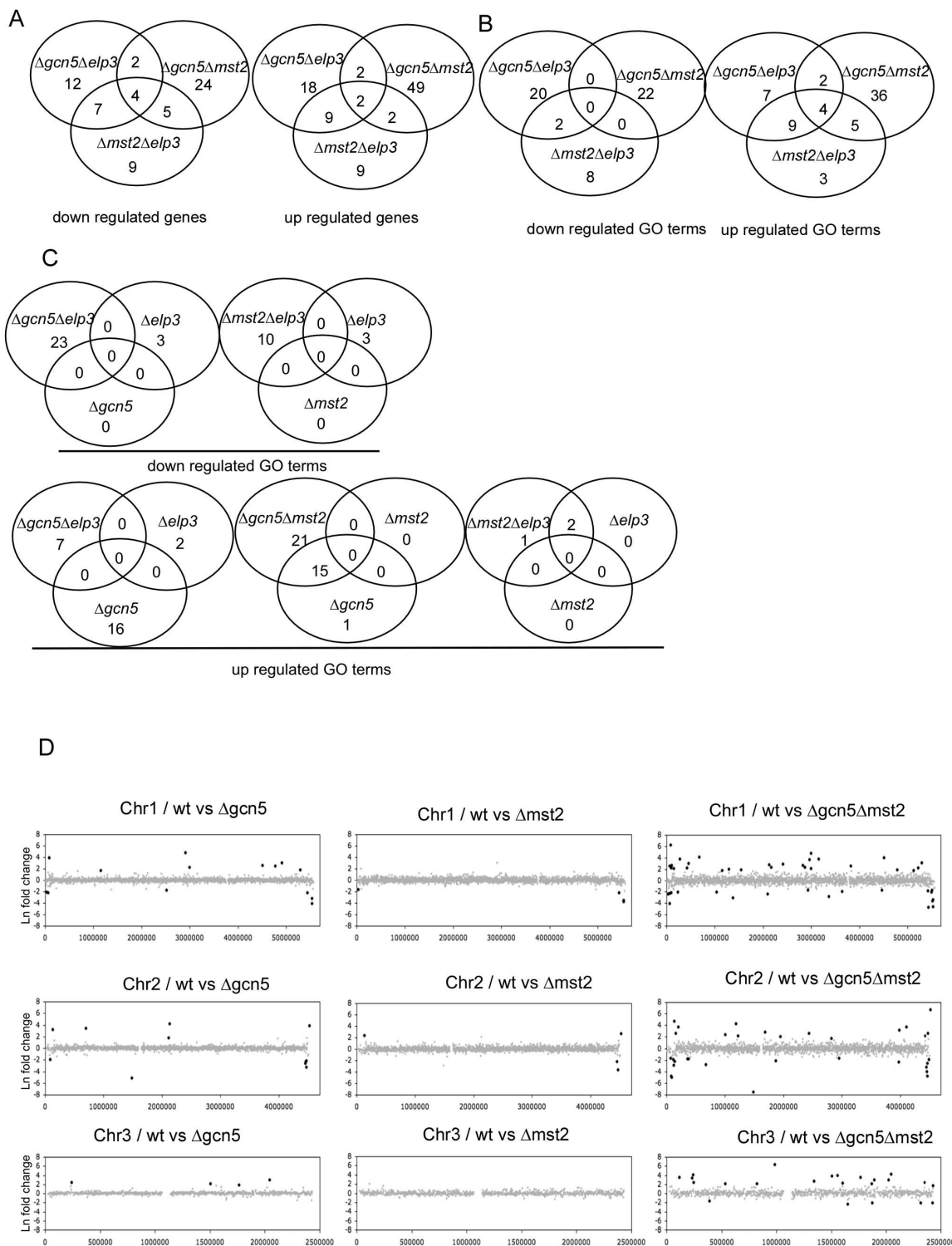


Figure 2 Overlapping expression in different mutations. A: Venn diagram of differentially down and up-regulated genes in double HAT mutants. (Additional File 5). B: Venn diagram of differentially down and up-regulated GO terms in the double HAT mutants using the affymetrix array. (Additional File 5) C: Venn diagram of differentially down and up-regulated GO terms of a double HAT mutant and the corresponding single HAT mutant. (Additional File 1). D: Localization of differentially regulated genes along chromosome 2 for the double mutant $\Delta gcn5 \Delta mst2$ (FY3361) and the corresponding single mutants, (Tables 1 and 2). Strains list: $\Delta gcn5 \Delta elp3$ (FY3847), $\Delta gcn5 \Delta mst2$ (FY3361), $\Delta mst2 \Delta elp3$ (FY3850), $\Delta gcn5$ (FY2943) and $\Delta mst2$ (FY1890).

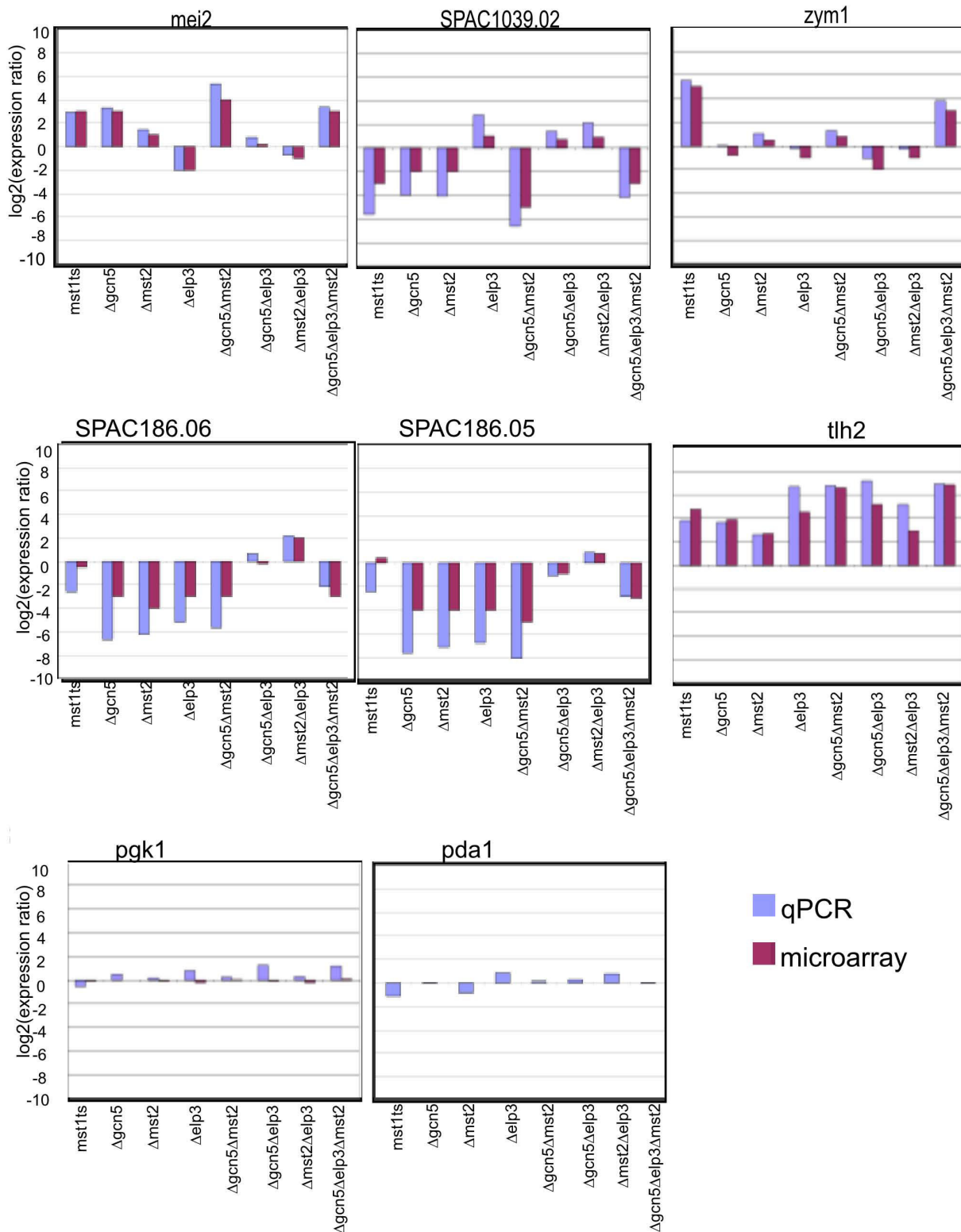


Figure 3 Validation of microarray results by qPCR. Genes that showed differential expression from microarray analysis were analyzed in different HAT mutants through qualitative PCR (qPCR). The log₂ fold expression ratios from microarray and qPCR experiments for the mutants versus wild type were plotted. qPCR results are indicated by the blue bars, microarray by the purple bars. Strain list: *Δelp3* (FY 3851), *Δgcn5* (FY2943), *Δmst2* (FY1890), *Δgcn5 Δelp3* (FY3847), *Δgcn5 Δmst2*(FY3361), *Δmst2 Δelp3* (FY3850), *Δgcn5 Δelp3 Δmst2* (3854).

strains. We found high degrees of congruence in gene expression as measured by RT-PCR and microarray analysis. (Fig 3).

Salt response in $\Delta mst2$ and $\Delta gcn5$ mutants

Since $\Delta mst2$ and $\Delta gcn5$ showed a synthetic phenotype in salt sensitivity, we performed expression profiling on $\Delta mst2$ and $\Delta gcn5\Delta mst2$ double mutant under KCl treatment and compared this to our previous results on $\Delta gcn5$ using the same platform [28]. The number of affected genes in $\Delta mst2$ increases during KCl induced stress but fewer genes are affected in $\Delta mst2$ compared to $\Delta gcn5$, consistent with the observation that $\Delta mst2$ alone has little effect on salt response. To find genes that require Gcn5 or Mst2 during KCl stress we compared the expression levels between $\Delta gcn5$ and $\Delta gcn5\Delta mst2$.

We found 99 genes that were significantly down-regulated and 191 genes significantly up-regulated in the double mutant, compared to $\Delta gcn5$ single mutant (Additional File 4). About 80% of these genes were not significantly regulated in either of the single mutants (Fig 4). This suggests that the two HAT enzymes overlap in the regulation of salt-responsive pathways, although there was no significant overlap in particular GO pathways.

Histone acetylation levels in HAT mutants

Gcn5/SAGA is a known regulator of H3 acetylation, as is Elp3/Elongator [21,36]. The phenotypes associated with $\Delta mst2$ suggest it is the functional orthologue of ScSas2, but if this is the case, there is no obvious NuA3/Sas3 homologue in *S. pombe* to overlap with Gcn5 activity, as Sas3 does in *S. cerevisiae*. Because more genes showed changes in expression in the double mutants than would be expected by a simple sum of those affected in the single mutants, we examined levels of

histone H3 acetylation in the mutant strains to see whether there is evidence for overlapping specificity at the level of global histone acetylation.

As expected, we found H3 acetylation levels on H3K9, K14 and K18 were dramatically reduced in the $\Delta gcn5$ mutant (Fig 5). In $\Delta elp3$ and $\Delta mst2$ as well as in the $\Delta mst2\Delta elp3$ double mutant there is only a modest decrease in H3K9 acetylation levels compared to wild type. In contrast, all strains lacking *gcn5* show significant loss of H3K9ac (Fig. 5A), suggesting that Gcn5 is the major contributor to H3K9 acetylation. Similar results were observed for H3K18ac.

For H3K14 acetylation we found significant reductions of acetylation levels for each single mutant compared to wild-type cells. Strikingly, in the double mutant $\Delta gcn5\Delta mst2$, the signal is much more strongly reduced (Fig. 5B). None of the deletion mutants showed any significant reduction in the overall level of histone H3 (Fig. 5D). Therefore we conclude that Gcn5 (GNAT family) and Mst2 (MYST family) each contribute to acetylate H3K14, which has been shown to be important for the response to salt stress [37]. Defective acetylation of H3K14 could explain the hypersensitivity to salt stress observed in the $\Delta gcn5\Delta mst2$ mutant.

Genes differentially expressed by the essential HAT Mst1

Finally, we examined the essential MYST family HAT Mst1 using the temperature sensitive allele *mst1^{ts}* [38]. The *mst1* mutant was grown at the semi-permissive temperature 30°C overnight and RNA was isolated for microarray analysis. There were many more differentially expressed genes in the *mst1* mutant compared to any of the non-essential HATs studied here (Table 2). There were also many more up-regulated than down-regulated genes in the *mst1* mutant, suggesting that it functions in repression of gene expression. Consistent

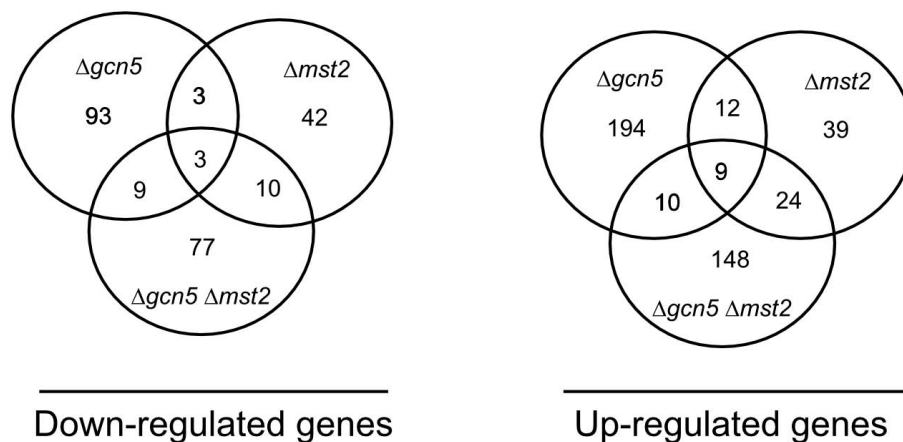
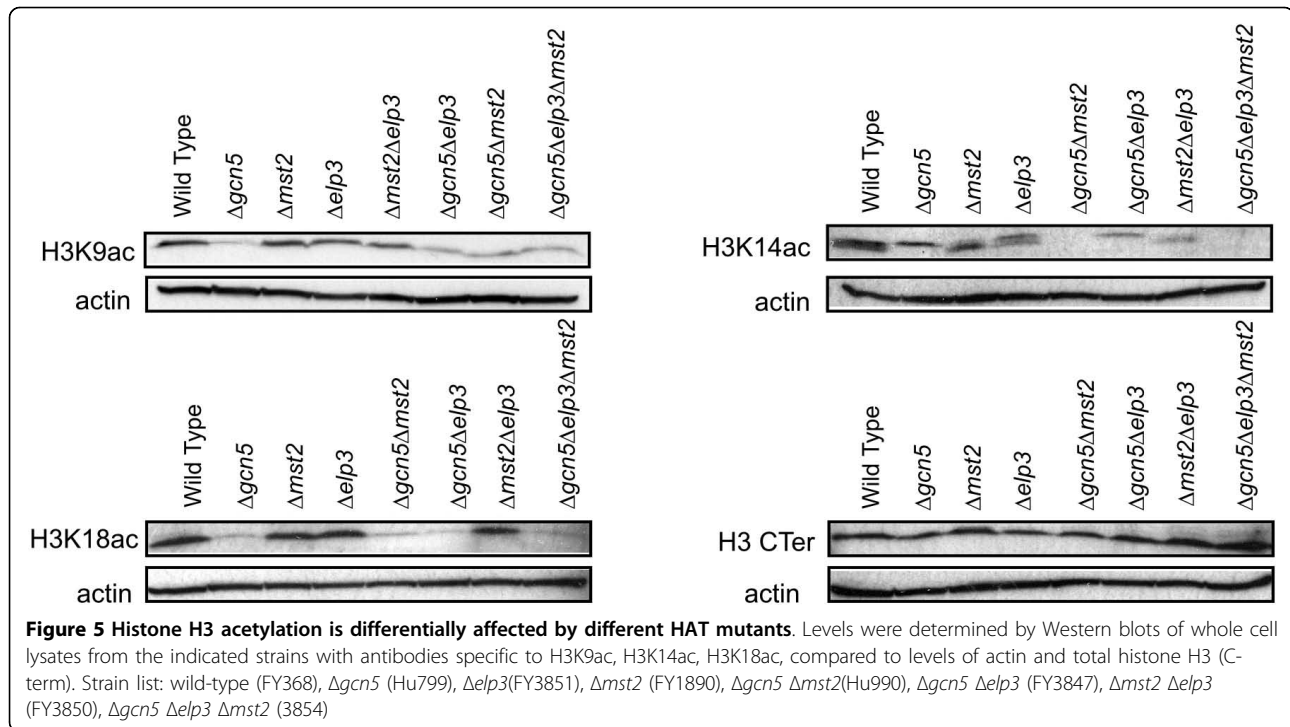


Figure 4 Salt response is redundantly regulated by multiple HAT families. Venn diagram of differentially down and up-regulated genes after salt stress in $\Delta gcn5$, $\Delta mst2$ and $\Delta gcn5\Delta mst2$ HAT mutants as determined by the Eurogenetech array platform (Additional File 3). Strains $\Delta gcn5$ (Hu799), $\Delta mst2$ (1890) $\Delta gcn5\Delta mst2$ (Hu990).



with this there were no GO categories that were significantly enriched in the down-regulated genes, however there were many that were enriched in the up-regulated genes (Additional File 1). These GO groups include metal and salt transporters, and other metabolic processes. When we compared these GO categories with those observed for the other mutants, we found that there was some overlap with the double mutant $\Delta gcn5 \Delta mst2$ and the triple mutant $\Delta gcn5 \Delta elp3 \Delta mst2$, suggesting general stress responses in these mutants are down-regulated (Fig 6).

Discussion

Fission yeast shares several conserved histone acetyltransferases with other eukaryotes. In the GNAT family, the Elp3 (KAT9) and Gcn5 (KAT2) enzymes are highly conserved [7]. In the MYST family, the Kat5 HAT Esal/Tip60/Mst1 protein is also highly conserved. However, the relationships between the other MYST family members are less clear-cut. In budding yeast, two additional MYST proteins *ScSas2* and *ScSas3* have been characterized. *ScSas2* antagonizes *ScSir2* in telomere silencing as a component of the SAS complex. *ScSas3*, a component of NuA3 [16], overlaps with Gcn5 such that a double mutant *Sc gcn5 sas3* is lethal [26]. By contrast, there is only one additional MYST protein in *S. pombe*, Mst2, which resembles both *S. cerevisiae* proteins in primary sequence. Like *ScSas2*, *SpMst2* antagonizes *SpSir2* in telomere

silencing [11], while work from this study suggests that like *ScSas3*, *SpMst2* acetylates histone H3K14. This work shows that *SpMst2* functions similarly to both *ScSas2* and *ScSas3*. To dissect out the relationship between these potentially overlapping HAT enzymes Mst2, Elp3 and Gcn5, we compared their phenotypic and transcriptional interactions in *S. pombe*.

First, we performed the initial characterization of the catalytic HAT, Elp3, which is the likely catalytic subunit of the Elongator complex in *S. pombe*. The strain *elp3* is viable, although it suffers defects in overall cell growth. It shows a delay in entry into the cell cycle, and premature cell cycle exit (Fig. 1B). It should be noted that some of the phenotypes seen in Elp3 defective strains could result from defects in other functions that have been reported for the Elongator complex in addition to its role as a HAT [39,40]. We next investigated whether $\Delta elp3$ shows genetic interactions with mutations in other non-essential HAT genes encoded by *gcn5*⁺ and *mst2*⁺. Double and triple mutants are all viable, although they show increasingly severe growth defects as more HAT genes are mutated. The viability of *Sp* $\Delta mst2 \Delta gcn5$ contrasts with the lethality of *Sc* $\Delta sas3 \Delta gcn5$ and suggests that Mst2 is not a simple equivalent of *ScSas3*. Alternatively, there may be another enzyme in fission yeast, which provides an additional degree of redundancy. We suggest that Mst2 fulfills many of the functions associated with both *ScSas2* and *ScSas3* (see below).

Table 2 Differentially expressed genes in *mst1^{ts}*

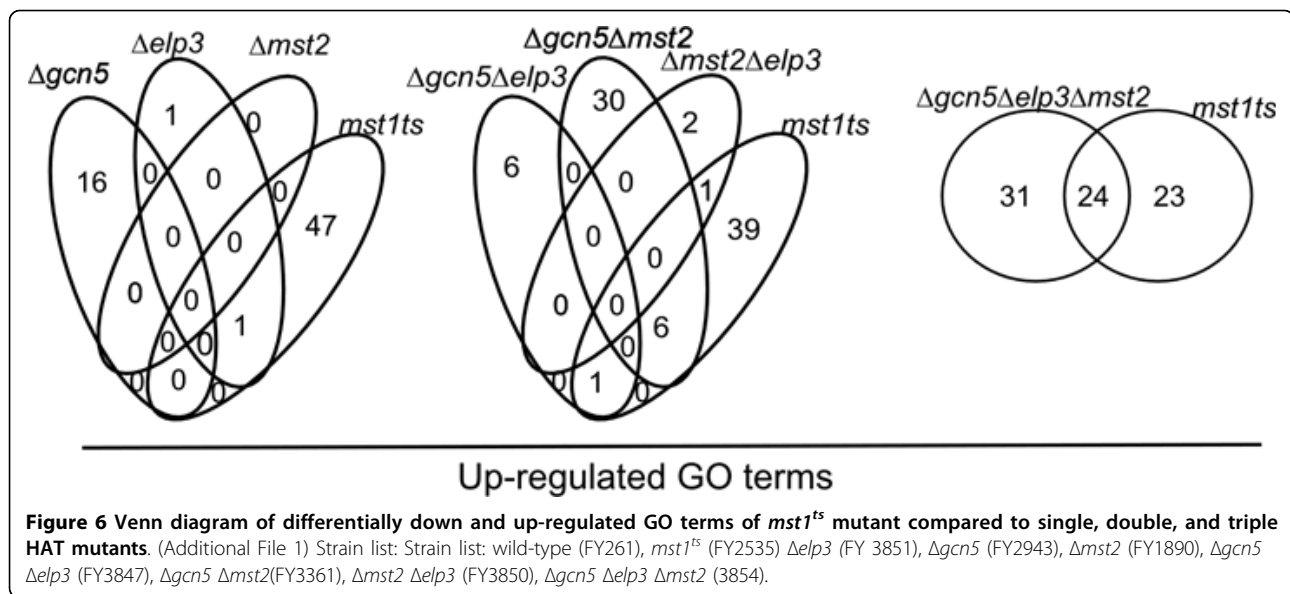
Down-regulated genes

| mst ^{1ts} | | |
|--------------------|-------------|-------------|
| Gene | Log2 change | p-value |
| SPAC1039.02 | -2.66 | 0.000635869 |
| SPBC29A3.05 | -2.18 | 5.55E-05 |
| SPCC757.06 | -2.08 | 1.87E-07 |

Up-regulated genes

| mst ^{1ts} | | | mst ^{1ts} | | | mst ^{1ts} | | |
|--------------------|--------------|-------------|--------------------|--------------|-------------|--------------------|-------------|-------------|
| Gene | Log 2 change | p-value | Gene | Log 2 change | p-value | Gene | Log2 change | p-value |
| SPBC1348.09 | 1.89 | 3.69E-11 | SPAC977.17 | 2.40 | 1.80E-08 | map2 | 3.52 | 2.50E-12 |
| SPAC22F8.05 | 1.90 | 0.001560473 | SPBC1348.14c | 2.41 | 2.14E-15 | SPAPJ695.01c | 3.52 | 2.00E-09 |
| SPAC3C7.13c | 1.92 | 3.23E-10 | SPAC5H10.02c | 2.46 | 3.89E-11 | SPBC1198.01 | 3.55 | 7.19E-16 |
| SPBPB2B2.06c | 1.93 | 0.000987078 | SPCC663.08c | 2.54 | 0.009807792 | SPAC22A12.17c | 3.57 | 1.96E-11 |
| SPBPB2B2.07c | 1.93 | 9.41E-10 | SPBC725.10 | 2.57 | 9.52E-05 | SPAPB1A11.03 | 3.59 | 1.50E-13 |
| nta1 | 1.94 | 3.20E-06 | SPBC83.19c | 2.58 | 3.49E-12 | SPAC1F8.04c | 3.59 | 5.41E-07 |
| SPCC191.01 | 1.94 | 5.61E-06 | SPCC1393.12 | 2.58 | 4.35E-11 | SPAC11D3.01c | 3.61 | 1.72E-07 |
| SPCC70.04c | 1.95 | 2.09E-06 | SPBPB2B2.08 | 2.60 | 0.007868315 | SPBPB8B6.03 | 3.64 | 2.07E-12 |
| meu7 | 1.96 | 2.81E-05 | SPCC777.04 | 2.63 | 3.77E-05 | SPBP4G3.03 | 3.65 | 2.74E-13 |
| SPAC9.10 | 1.98 | 8.57E-09 | SPAC26F1.05 | 2.64 | 1.00E-07 | SPCC663.06c | 3.66 | 3.28E-05 |
| map1 | 1.98 | 0.000618909 | pma2 | 2.64 | 6.19E-07 | SPBPB21E7.04c | 3.67 | 3.08E-06 |
| SPBC8E4.05c | 1.99 | 1.54E-08 | SPAC32A11.02c | 2.65 | 4.69E-09 | frp1 | 3.69 | 3.37E-05 |
| SPAC637.03 | 2.03 | 0.000132799 | SPBC2G2.17c | 2.66 | 2.11E-07 | SPCC737.04 | 3.85 | 3.10E-07 |
| SPBC4C3.08 | 2.04 | 6.23E-08 | SPAC56F8.15 | 2.68 | 0.001276426 | SPBC359.06 | 3.91 | 0.000499938 |
| meu10 | 2.05 | 4.69E-07 | SPAPJ691.02 | 2.72 | 1.52E-05 | apc10 | 3.96 | 4.49E-12 |
| SPCC1840.12 | 2.05 | 4.17E-06 | SPBC1773.06c | 2.76 | 0.000469242 | SPAPB8E5.10 | 3.98 | 1.31E-12 |
| SPBPB2B2.02 | 2.06 | 0.000305555 | SPBC1685.05 | 2.86 | 1.62E-11 | SPBC16E9.16c | 4.00 | 6.07E-13 |
| SPBC19C7.04c | 2.09 | 0.001283597 | SPAC4F10.17 | 2.88 | 6.57E-09 | SPAC29A4.12c | 4.06 | 1.45E-09 |
| SPAC5H10.04 | 2.10 | 1.33E-06 | SPCC16A11.15c | 2.94 | 5.09E-11 | SPAC869.08 | 4.08 | 1.01E-15 |
| SPBC947.05c | 2.11 | 5.62E-08 | SPCC1739.08c | 2.96 | 0.021512753 | SPBC3H7.08c | 4.09 | 9.33E-14 |
| SPAC3H8.09c | 2.11 | 1.77E-10 | SPAC23C11.06c | 2.99 | 1.15E-07 | SPBCPT2R1.08c | 4.16 | 0.000258571 |
| ish1 | 2.11 | 9.95E-06 | SPAC15E1.02c | 3.00 | 8.68E-09 | fbp1 | 4.32 | 3.57E-14 |
| SPAC513.02 | 2.12 | 1.94E-06 | SPBC1105.13c | 3.02 | 1.10E-07 | SPBC56F2.06 | 4.36 | 2.99E-07 |
| SPAC4H3.03c | 2.14 | 0.000486057 | grt1 | 3.04 | 7.61E-08 | mel1 | 4.43 | 2.62E-15 |
| SPAPB15E9.02c | 2.17 | 3.00E-08 | SPCC338.18 | 3.04 | 7.26E-07 | SPAC139.05 | 4.49 | 1.53E-09 |
| SPCC794.01c | 2.18 | 0.042343035 | SPBPB2B2.18 | 3.05 | 1.46E-05 | SPCPB16A4.06c | 4.53 | 1.21E-11 |
| SPAC1F8.02c | 2.18 | 3.57E-07 | SPCC1322.07c | 3.10 | 9.51E-09 | SPBC1289.14 | 4.61 | 8.12E-16 |
| SPBC4.01 | 2.20 | 9.35E-05 | SPCC757.03c | 3.11 | 2.10E-09 | ght3 | 4.72 | 0.001973142 |
| SPBPB8B6.02c | 2.23 | 1.43E-12 | SPAC4H3.08 | 3.16 | 4.98E-12 | zym1 | 4.85 | 1.83E-08 |
| SPBC36.02c | 2.23 | 0.000135759 | SPBPB2B2.12c | 3.16 | 0.00706426 | SPBC24C6.09c | 4.94 | 1.55E-13 |
| SPCC1393.07c | 2.23 | 4.88E-10 | spk1 | 3.17 | 0.000216514 | dak2 | 5.02 | 4.45E-12 |
| SPAC513.06c | 2.24 | 1.52E-09 | mei2 | 3.25 | 0.000888516 | SPAC186.02c | 5.11 | 6.43E-15 |
| SPCPB1C11.02 | 2.25 | 7.04E-07 | SPAC977.05c | 3.29 | 2.46E-05 | eno102 | 5.11 | 3.01E-13 |
| ste11 | 2.27 | 0.000335259 | SPAC1F8.08 | 3.32 | 2.20E-09 | SPAC3G9.11c | 5.13 | 5.88E-11 |
| wtf20 | 2.28 | 4.37E-06 | SPAC3G6.07 | 3.33 | 1.53E-05 | SPAC22G7.11c | 5.22 | 4.34E-14 |
| ppk33 | 2.28 | 0.000119009 | SPACUNK4.17 | 3.33 | 3.64E-07 | SPBC23G7.10c | 5.25 | 4.94E-06 |
| SPAC1F7.06 | 2.33 | 5.44E-12 | SPAC23H3.15c | 3.42 | 1.03E-05 | isp3 | 5.36 | 1.87E-11 |
| ssa1 | 2.34 | 0.000834021 | caf5 | 3.44 | 4.99E-07 | SPAC869.06c | 6.61 | 2.79E-13 |
| cta3 | 2.36 | 6.88E-11 | str3 | 3.48 | 2.47E-05 | SPAC869.09 | 6.68 | 2.91E-12 |
| agl1 | 2.37 | 0.026304809 | | | | | | |

This table lists the down and up-regulated genes of *mst1^{ts}* cells compared to wild-type cells using an Affymetrix microarray.



Phenotypic analysis suggests distinct effects of each HAT. For example, as shown previously *Δgcn5* mutants are de-repressed for mating and meiosis functions, and are sensitive to certain salt-stress conditions [27,28]. *Δmst2* mutants show modest sensitivity to DNA damaging agents such as HU and MMS. *Δgcn5 Δmst2* double mutants show significantly increased salt sensitivity, and a modest increase in DNA damage sensitivity relative to single mutants. Thus, we conclude that in response to salt stress, Gcn5 and Mst2 have a substantial functional overlap. In contrast, *Δgcn5 Δelp3* and *Δmst2 Δelp3* showed less evidence for synthetic phenotypes, with only a modest increase in TBZ sensitivity in *Δgcn5 Δelp3* relative to the parents (Fig. 1C & 1D). In fact, *Elp3* appears to be antagonistic to *Mst2*, because *Δelp3* suppresses *Δmst2* mutant sensitivity to high salt concentrations and HU. We speculate this could be due to slowing down the growth of *Δmst2* cells, allowing time for the cells to repair any damage that may have occurred. Together, these data suggest that Gcn5 affects multiple pathways, some overlapping with *Elp3*, and others overlapping with *Mst2*. However, since the double mutants and even the triple mutants are viable, either additional HAT enzymes play a role, or the acetylation modifications controlled by these enzymes are not strictly essential for viability.

To determine whether the functional redundancy between HATs is at the level of gene expression, we performed transcriptional profiling using DNA microarrays of *Δelp3*, *Δgcn5*, and *Δmst2*. Similar to previous results with *Δgcn5* [27,28] we found that few genes were affected by deletion of any single HAT gene under favorable growth conditions. Although HATs are presumed to be gene activators that facilitate transcriptional

initiation or elongation [5], we found that gene expression was as likely to be increased as reduced in our mutant strains. Up-regulated genes could represent indirect targets, although many Gcn5-repressed genes are known to be bound by Gcn5 [37]. This suggests that Gcn5 may play a direct role in gene repression, which has been described previously [27]. It is thus possible that the other HATs studied here also play direct roles in transcriptional repression.

When we examined the double mutants, we found that their gene expression profiles were not simply the combination of the profiles from the two single mutants, but affected additional genes. This supports the hypothesis that the HATs play partly redundant roles in gene regulation, with multiple enzymes contributing to expression of common targets. Importantly, although they may affect common genes, the regulatory mechanisms may be distinct. In budding yeast, although *ScGcn5* and *ScElp3* affect transcription of the *ScHsp70* genes *ScSSA3* and *ScSSA4*, this occurs by different mechanisms because Gcn5 is required for transcription factor binding while *Elp3* is required for proper Pol II elongation [41]. However, we have shown that in fission yeast *SpGcn5* also has a role in transcriptional elongation [37] which could suggest a more direct mechanistic overlap for *SpGcn5* and *SpElp3* in this species.

Only one gene was significantly increased in all strains: the RecQ-type DNA helicase SPBCPT2R1.08c, located in the sub-telomere domain (Tlh2; [32,33]). We showed previously that *Δmst2* increases silencing at the telomere and in telomere associated regions, consistent with a role for *Mst2* in antagonizing Sir2. Loss of *Mst2* results in a loss of overall H4 acetylation [11] and H3K9 acetylation in *Δmst2* in a very limited region adjacent to

the telomere 18 kb from the end. Interestingly, this region is telomere-distal to the *tlh2* helicase gene located 13 kb from the end of the telomere chromosome II, which is highly up-regulated in the mutant. In contrast, genes at 27.5, 29 and 31 kb away from the telomere are down-regulated in $\Delta mst2$ (SPAC186.06, SPAC186.05 and SPBCPT2R1.02 respectively), while at 47 kb telomere distal (SPAC869.01), there is no change in expression levels. This suggests a gradient effect on transcription of genes in these regions.

The few genes that are down-regulated in $\Delta gcn5$ are localized near the ends of chromosome I and II which suggest that the repression might be due to spreading of heterochromatin by loss of H3K9ac. The combined effect of the double mutant may indicate a combination of effects associated with loss of both histone H3K9 and H3K14 acetylation. Clearly, there are specific regions at the telomeres that are regulated by specific HATs but more work needs to be done to fully understand the boundaries of the telomeres and the regulation between the highly heterochromatic region and the rest of the chromosome.

We used gene ontology (GO) classification to define the functional roles of the few genes that were differentially expressed in single mutants. We found that those in $\Delta elp3$ and $\Delta gcn5$, but not $\Delta mst2$ associated with a few distinct classes. Consistent with previous results [27], $\Delta gcn5$ mutants showed increased expression of specific sexual differentiation genes as well as enrichment for GO terms [34] related to mating and meiosis.

Gcn5 represses transcription of *ste11*⁺ [27], which is an important regulator of the mating pathway in response to nutrient limitation. Although we find very similar functional groups that are positively regulated in the absence of $\Delta gcn5$, we did not see evidence for induction of *ste11* above our threshold level (Table 1).

Consistent with prior work, our study also found that *mei2*⁺ was up-regulated in $\Delta gcn5$ [27]; this was also seen for *mst1*^{ts} and to a lesser extent $\Delta mst2$ (Fig 3), indicating multiple inputs into this essential regulator of sexual development. The $\Delta gcn5 \Delta mst2$ mutant increased *mei2*⁺ and *ste11*⁺ mRNA levels above those observed in the single mutants, suggesting these two HATs function redundantly in repression. It is possible that they might also have non-histone targets in common since the repression of *mei2*⁺ expression by Gcn5 was suggested to be mediated by a histone independent mechanism [27].

In contrast, deletion of $\Delta elp3$ results in loss of *mei2*⁺ transcription and in the $\Delta gcn5 \Delta elp3$ and $\Delta mst2 \Delta elp3$ double mutants there is also a reduction of *mei2*⁺ transcription. Even the triple mutant shows lower levels of *mei2*⁺ than the $\Delta gcn5 \Delta mst2$ double mutant, suggesting that $\Delta elp3$ has an opposite effect on *mei2*⁺ transcription

than $\Delta gcn5$. Interestingly transcription of SPAC1039.02, a putative phosphoesterase, shows a similar, but opposite, regulation pattern as it is down-regulated in $\Delta gcn5$, *mst1*^{ts} and $\Delta mst2$ while being up-regulated in $\Delta elp3$. Thus, for some targets, Gcn5 and Mst2 appear to overlap, while Elp3 appears to be antagonistic. This finding suggests that the common model linking HAT enzymes with gene activation is too simplistic. Dissecting the contribution of each HAT to gene expression or other effects will require future molecular studies mapping the histone acetylation and physical binding of HATs at different gene regions or their association with the transcription machinery.

To determine the molecular basis for the overlap of HAT targets, we examined histone H3 acetylation. Three acetylation sites on histone H3 are associated with gene expression: K9, K14, and K18 [42]. H3K9 is acetylated in euchromatin, but methylated in heterochromatin; consistent with other species, our data indicate that Gcn5 is the primary contributor to H3K9Ac because acetylation of this residue is strongly reduced in $\Delta gcn5$ but relatively unperturbed by the other HAT mutations.

H3K14 acetylation is also associated with gene expression [42]. Mutations that change the H3K14 residue display hypersensitivity to KCl and CaCl₂ induced stress in $\Delta gcn5$ cells [37]. Little or no H3K14 acetylation is observed in the $\Delta gcn5 \Delta mst2$ double mutant. Consistent with this, we observed that $\Delta gcn5 \Delta mst2$ is significantly more salt-sensitive than either single mutation. We conclude that these two HATs both contribute to H3K14 acetylation in response to stress. Importantly, this acetylation is not essential for viability in *S. pombe*. Data from budding yeast also indicates an overlap between ScSas3 and ScGcn5 in H3K14 acetylation [26]. Interestingly, the budding yeast data also suggest that $\Delta sas3$ is not lethal in combination with disruption of other SAGA subunits that are essential for Gcn5 activity against histones, and deletion of histone N-terminal H3 tails entirely is not lethal in budding yeast [26]. Thus, the essential redundant function of these enzymes may not be in histone modification. It is now well-established that HAT proteins acetylate substrates other than histones [43]. Perhaps it is one such substrate that relies on Gcn5 or Sas3 in budding yeast, but on a different enzyme in fission yeast. These results indicate that Mst2 has functions in common with both budding yeast enzymes.

Finally, we found that as expected there was little overlap between expression profiles in the non-essential HAT mutant strains, and cells with a temperature sensitive mutation of the essential HAT *mst1*⁺. Mst1 is known to be required for DNA damage response and chromosome segregation [38]. However, we did not see

a significant increase in GO terms related to these functions, which is consistent with data suggesting its effects are not mediated through the transcriptional program [44]. There was a significant overlap of regulated genes between Mst1 and the triple mutant $\Delta gcn5 \Delta mst2 \Delta elp3$, as half of the genes regulated in the triple mutant were also regulated in $mst1^{ts}$. When we compared the regulated GO terms from $mst1^{ts}$ and $\Delta gcn5 \Delta elp3 \Delta mst2$ we found that these mutants shared terms related to metal and ion homeostasis, metal transports as well as regulation of meiosis. Since both strains show severe growth defects under the conditions employed, we suggest that these overlaps may represent the effects of general cellular stress rather than a particular transcriptional program.

Conclusions

We isolated and characterized the putative Elongator HAT Elp3, showing $\Delta elp3$ cells are delayed in the cell cycle relative to wild-type cells, and then compared it to other HAT mutants. Our study suggests, through analysis of single, double and triple mutants of MYST and GNAT family HATs, that histone acetyltransferases play partially redundant role in gene regulation, and that their role can be positive, or negative for the transcription of genes. We found evidence for significant redundancy between Mst2 and Gcn5 in regulation of stress response, and response to DNA damage. We found that different HAT families can share specificity for a common lysine residue in histone H3, thereby contributing to the modulation of gene expression. For example, we found that Gcn5 and Mst2 both acetylate histone H3 lysine 14. This work highlights the functional redundancies of different HAT families on transcription, salt response, and DNA damage repair. Future work will be

required to investigate the direct contributions of these HATs to individual genes, as opposed to selected regulation of other transcriptional master regulators.

Methods

Strains, media, and manipulations

Strains used in this study are listed in Table 3. Strains were grown and maintained on yeast extract plus supplements (YES) or Edinburgh minimal media (EMM) with appropriate supplements, using standard techniques [45]. Matings were performed on synthetic sporulation agar (SPAS) plates for 2-3 days at 25°C. Double mutants were constructed by standard tetrad analysis or random spore analysis. G418 plates were YES supplemented with 100 µg/ml G418 (Sigma).

Deletion of *elp3*⁺

The *elp3* deletion strain was created from a diploid strain purchased from Bioneer <http://www.bioneer.com>. The strain (*h+/h+*, *elp3+/elp3::KanMX6*, *leu1-32/leu1-32*, *ura4-D18/ura4-D18*, *ade6-M210/ade6-M216*) was haploidized by TBZ treatment to induce mitotic instability and chromosome loss. The cells were plated on YE media without adenine to select for red and pink colonies followed by selection for G418 resistant clones. The isolates were checked with PCR to assure the deletion and KanMX6 cassette was in the right place then back-crossed into a *wt* strain (*h-*, *leu1-32*, *ura4-D18*, *ade6-M216*).

Growth Assays

Wild type and $\Delta elp3$ cells were grown to stationary phase $>1 \times 10^8$ cells/ml and then diluted in YES media to four different concentration 1×10^4 , 1×10^5 , 4×10^5 and 1×10^6 cells/ml respectively and grown at 30°C. Cells were counted regularly for 48 hours generating growth curves to cover the beginning and end of the exponential growth.

Table 3 Strains used in this study

| Strain | Genotype | |
|--------|--|-----------------|
| FY261 | <i>h+</i> <i>can1-1 leu1-32 ade6-M216 ura4-D18</i> | SLF stock |
| FY368 | <i>h-</i> , <i>leu1-32, ura4-D18, ade6-M210</i> | AW stock |
| FY1104 | <i>h-</i> $\Delta rad3::ura4+$ <i>ura4 leu1-32 ade6-M210 (?)</i> | SLF stock |
| FY1584 | <i>h+</i> $\Delta swi6::his1+$ <i>ade6-M210 leu1-32 ura4-DS/E his1-102</i> | R. Allshire |
| FY1890 | <i>h+</i> $\Delta mst2::ura4+$ <i>ura4-D18 ade6-M210 leu1-32</i> | Gomez EB 2005 |
| FY2535 | <i>h+</i> $\Delta mst1::kanMX6 leu1::nmt-mst1L-S-leu1+$ <i>ade6-M210 ura4-D18</i> | Gomez EB 2008 |
| FY2943 | <i>h-</i> $\Delta gcn5::kanMX ura4-D18 leu1-32 ade6-M210?$ <i>his3-D1</i> | SLF stock |
| FY3361 | <i>h+</i> $\Delta gcn5::Kanmx6 \Delta mst2::ura4+$ <i>Ura4D18 ade6 m21? leu1-32 his3D1</i> | this work |
| FY3847 | <i>h+</i> $\Delta gcn5::KanMX4 \Delta elp3::KanMX6 leu1-32 ura4-D18 ade6-M210$ | this work |
| FY3850 | <i>h+</i> $\Delta elp3::KanMX6 \Delta mst2::URA4+$ <i>leu1-32 ura4-D18 ade6-M216</i> | this work |
| FY3851 | <i>h+</i> $\Delta elp3::KanMX6 leu1-32 ura4-D18 ade6-M210?$ | this work |
| FY3854 | <i>h+</i> $\Delta gcn5::KanMX4 mst2::URA4+$ <i>elp3::KanMX6 leu1-32 ura4-D18 ade6-M210</i> | this work |
| Hu799 | <i>h-</i> $\Delta gcn5::KanMX4, leu1-32, ura4-D18, ade6-M210$ | Johnsson A 2006 |
| Hu990 | <i>h-</i> $\Delta gcn5::KanMX4, mst2::ura4+, leu1-32, ura4-D18, ade6-M210$ | this work |

This table lists the genotypes and origins for the strains used in this study.

Damage assays

Cells were grown to mid-log phase, serially diluted five-fold and spotted onto YES plates or YES plates were irradiated with the indicated doses of UV irradiation using a Stratalinker (Stratagene) or YES plates containing 10 or 12 $\mu\text{g/ml}$ thiabendazole (TBZ), 5 mM hydroxyurea (HU), 7 μM camptothecin (CPT), 0.005% methyl methanesulfonate (MMS) and incubated for 2-3 days at 32°C. For the salt sensitivity assays cells were plated out in five-fold dilution series on rich media (YES) plates with or without KCl (1 M or 1.5 M) or CaCl₂ (0.1 M or 0.25 M) at either 30°C or 36°C for 2 to 4 days.

RNA prep and Microarray protocols for Affymetrix and Eurogenetech

RNA was isolated from three independent isolates growing at 30-32 degrees Celsius using phenol/chloroform extraction [45], phase lock gels (Eppendorf) and resuspended into TE after ethanol precipitation. Biotinylated cRNA was prepared for Affymetrix array analysis from 5 μg Total RNA using the standard Affymetrix one-cycle target labeling protocol. Samples were assayed for gene expression using Affymetrix GeneChip Yeast Genome 2.0 arrays consisting of probe sets representing 5,031 genes in *S. pombe* based on Sanger (June 2004). Data was analyzed using the R statistical environment. Affymetrix CEL files were processed and normalized using RMA [46]. The linear modeling package Limma [47] was used to calculate p-values and derive gene expression coefficients and to identify differentially expressed genes. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus [48] and are accessible through GEO Series accession numbers GSE17259 and GSE17262 <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=dzmlrwoyekgmyp-k&acc=GSE17298>. For microarrays using the Eurogenetech platform (salt sensitivity analysis), sample collection, processing and normalization was performed as in [28]. Genes were considered differentially expressed if the mutant/wt ratio was greater than 3.25 log₂ fold, with a p-value of 0.05 or less. Additional File 5 lists genes differentially expressed with a mutant/wt ratio of 1.7 log₂ fold, with a p-value of 0.05 or less.

qPCR

Primers for the entire *S. pombe* genome were created by Chris Seidel using uPrimer. Total RNA in the amount of 2-4 μg was treated with DNase (Turbo DNase Treatment Ambion #AM1907) and 200-600 ng of the treated RNA was converted to cDNA by RT-PCR with the use of two primers. The concentration and sequence for the primers are 2 ng/ μL final for the random primer pdN-15 and 20 ng/ μL final for the anchored oligo-dT OdT-19N. A Corbett robot was used to load PCR reactions in 384-well plate format for analysis on an ABI-7900. Standard curves for the 9 primer pairs were made with 5

ten-fold dilutions of gDNA starting with 1000 ng/15 μL qPCR reaction and ending with 0.1 ng/15 μL qPCR reaction. The qPCR experiment consisted of measuring 9 loci in 24 samples three times each. 8 ng of the cDNA was used in each qPCR reaction without purification of the RT reaction. The final primer concentration was 500 nM each.

Venn diagram program, GO term analysis

Venn diagrams were made using VENNY (Oliveros, J.C. (2007) VENNY. An interactive tool for comparing lists with Venn Diagrams <http://bioinfogp.cnb.csic.es/tools/venny/index.html>). Gene ontology classifications were classified at <http://amigo.geneontology.org> with the database filter set as GeneDB *S. pombe* with a threshold of maximum p value of 0.05 and minimum number of gene products of 2. We selected for publication only the biological process classifications.

Histone acetylation

Cell lysates was obtained as described by [49] with the following modifications; the volume of the cell cultures were increased 2.5 times to get enough material for 10 or more gels. Briefly cells were inoculated in YES and grown overnight (o/n) to 1×10^7 cells/ml in a total volume of 25 ml and harvested in room temperature (RT) by centrifugation, washed once in H₂O then resuspended in 0.75 ml H₂O and an equal volume of 0.6 M NaOH, incubated 10 minutes at RT. Cells were removed from the NaOH by centrifugation and dissolved in 175 μL of modified SDS-sample buffer (60 mM Tris-HCl, pH 6.8, 4% β -mercaptoethanol, 4% SDS, 0.01% BPB, and 5% glycerol). Approximately 15 μL were used to run on precast gradient gels (Bio-Rad, 161-1105). Transfer was performed using the iBlot™ Dry Blotting system (Invitrogen). After transfer the membranes were blocked in in PBS containing 3% milk for 30 min at RT followed by o/n incubation at 4°C with primary antibody (anti-histones); or blocked o/n at 4°C followed by incubation with anti-actin antibody, at a 1:12 000 dilution (ms ab8224, Abcam) 1 hour at RT. For detection of histones antibodies the following dilutions were used: H3 Cter 1:200 (ab1791, Abcam); H3K9Ac 1:500 (07-352, Upstate); H3K14Ac 1:200 (07-353, Upstate); H3K18 Ac 1:200 (07-354, Upstate). For detection IgG horseradish peroxidase (GE Healthcare) against mouse and rabbit respectively was used along with ECL Plus (GE Healthcare) and exposed to film for 20 seconds to 3 minutes.

Additional file 1: GO terms differentially regulated in HAT mutants.

This table lists the down and up-regulated gene ontology (GO) terms of mutant HATs.

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Additional file 2: Differentially regulated genes (3.25 fold) in the triple HAT mutant. This table lists the down and up-regulated genes of the triple HAT mutant compared to wild-type using an Affymetrix microarray.

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Additional file 3: Differentially expressed genes (3.25 fold) in double HAT mutants. This table lists the differentially changed genes in the double HAT mutants compared to wild-type using an Affymetrix microarray.

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Additional file 4: Genes with 2 fold change in gene expression after exposure to salt. This table lists the down and up-regulated genes after addition of salt using of mutant HATs using an Eurogentech microarray.

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Additional file 5: Differentially expressed gene (1.7 fold) in HAT mutants. This table lists the differentially changed genes in the HAT mutants compared to wild-type using an Affymetrix microarray.

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Authors' contributions

RLN performed, designed and analyzed experiments and wrote the manuscript. AJ performed, designed and analyzed experiments and wrote the manuscript. BF performed and analyzed experiments. MG performed and analyzed experiments. YXF performed and analyzed experiments. CS designed and analyzed experiments and provided reagents. APW performed, designed and analyzed experiments, and provided reagents. SLF designed and analyzed experiments, wrote the manuscript and provided reagents.

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