Yeast *HAT1* and *HAT2* deletions have different life-span and transcriptome phenotypes

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Abstract HAT-B is a yeast histone acetyltransferase composed of Hat1, Hat2 and Hif1 proteins. We demonstrate that a *hat2* mutant or a *hat1hat2* double mutant, but not a *hat1* mutant, have an extended life-span. Transcriptome analysis shows that the single *hat* mutants are not very different from wild type. However, the comparison of the *hat1* and *hat2* transcriptomes shows that they are different. The *hat1hat2* double mutant shows a transcriptional phenotype similar to that of the *hat1* mutant but strongly enhanced. These results indicate that Hat2p could have additional functions in the cell to those of Hat1p.

Keywords: Hat1; Hat2; HAT-B; Life-span; DNA-chip

1. Introduction

Acetylation of lysine residues in nucleosome core histones is a reversible process that occurs in all eukaryotic organisms studied and which depends on two different sets of enzymatic activities, histone acetyltransferases (HAT) and histone deacetylases (HD). This post-translational modification is involved in processes such as activation or repression of gene transcription, nucleosome assembly during replication, DNA repair and recombination or cell cycle and growth control (reviewed in [1–3]). These and other processes may be activated or repressed depending on the specific acetylation pattern of the chromatin. Thus, acetylation is thought to act as a signalling system [4–7], actively participating in the interaction of regulatory proteins. One of the most interesting processes to which acetylation has been related is that of senescence. In yeast, replicative senescence refers to the budding capacity as a function of cell generations. This concept is based on the fact that individual yeast cells undergo a finite number of cell divisions [8]. The link between histone acetylation and replicative senescence comes from the observation that deletion of *SIR2*, a gene encoding an HD [9,10], produces a shorter life-span [11,12]. Moreover, HD activity of Sir2p is necessary to maintain the wild type life-span [10]. Deletion of other yeast HDs differentially affects life-span phenotypes: *rpd3* mutant has extended life-spans whereas *hda1* mutant has a normal life-span [11]. There are no life-span studies on mutants in HAT genes. Aging and histone acetylation have also been related to telomeric silencing. Thus, silencing of telomeric regions declines with increased replicative age in yeast (reviewed in [13]). Whereas Sir2p [14], and its HD activity [10], are required for silencing at telomeres, Rpd3p, and its subunits Sin3p and Sap30p, function to counteract telomeric and *HM* silencing [15]. Other HDs have also been implicated in silencing [16]. Among the HAT enzymes, deletion of *SAS3*, *SAS2* or *GCN5* genes have different consequences on silencing [15,17–20]. Hence, taken together, these observations strongly support the hypothesis that there is a relationship, although quite complex, between life-span, silencing and histone acetylation.

The yeast *HAT1* was the first HAT gene described [21]. This gene encodes the catalytic subunit of the HAT-B complex [22]. This enzyme specifically modifies Lys 12 of free histone H4 and has been implicated in the acetylation of cytoplasmic histone molecules required for post-replicative nucleosome assembly. However, it has been recently demonstrated that yeast HAT-B complex is mainly localized in the nucleus and that is composed of three proteins, Hat1, Hat2 and Hif1 [23,24]. Deletion of *HAT1*, in combination with specific histone H3 amino terminal tail mutations, results in a significant defect in telomeric silencing [25]. Hat2p acts as a bridge between Hat1 and Hif1 proteins [23] and is required for high affinity binding of Hat1p to histone H4 [22]. It seems to be essential for all the functions carried out by Hat1p because deletion of *HAT2* always produces similar defects to those of *HAT1* [22,25,26]. Deletion of *HIF1* displays similar defects in telomeric silencing [23,24] and DNA double-strand break repair to those of *HAT1* [24]. At present, no unique phenotype has been specifically and directly associated to a particular HAT-B subunit.

In this study, we analyse the effect of *HAT1* and/or *HAT2* gene deletions on the life-span and the transcriptome of the yeast cells. Surprisingly, using these two independent approaches, we have observed that *hat1* and *hat2* mutants display different phenotypes. We show here that deletion of *HAT2* but not of *HAT1* provokes extended life-span in yeast cells. This is the first unique phenotype described for a *HAT* gene. Furthermore, transcriptomes of *hat1* and *hat2* mutants behave inversely. All these results indicate that the functions of Hat2p and Hat1p are not completely overlapping in the cell.

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Abbreviations: HAT, histone acetyltransferase; HD, histone deacetylase; GO, gene ontology

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2. Materials and Methods

2.1. Yeast strains

For life-span and transcriptome studies we used the *Saccharomyces cerevisiae* wild type (S288c background) yeast strain YPH250 (*MAT*a, *ura3*-52, *lys2*-801, *ade2*-101, *trp1*-Δ1, *his3*-Δ200, *leu2*-Δ1) [22] and its derived deletion strains, BQS1200 (*hat1*::*KanMX4*), BQS1205 (*hat2*::*KanMX4*), BQS1341 (BQS1205 *hat1*::*NatMX4*), BQS1431 (*sir2*::*NatMX4*) and BQS1433 (BQS1205 *sir2*::*NatMX4*). Deletion mutants were constructed by integrative disruption following the protocols described in [27,28].

Yeast strains derivatives from W303-1a (*MATa, ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3, can1-100*), with appropriate deletions or six-hemagglutinin HA epitope tags were used for chromatin immunoprecipitation assays. BQS1154 (HAT1-HA6-TRP1 in a W303-1a genetic background) was used as parental strain for generating BQS1172 (*hat2*::*KanMX4*) and BQS1184 (*hif1*::*NatMX4*). BQS1164 (*SIR2-HA6-TRP1*) was parental strain for BQS1173 (*hat1*::*KanMX4*) and BQS1439 (*hat2*::*KanMX4*). JRY4470 (*sir2*::*LEU2*) [29], and BQS298 (JRY4470 *hat1*::*KanMX4*), have been used as well. Homologue recombination [30] was used for integrating the HA epitope tags at their actual chromosomal loci.

2.2. Life-span determination

Life-span of yeast strains was determined as described by Kennedy et al. [31] and Kim et al. [11]. Briefly, cells were grown at 28 °C in solid YPD plates (1% yeast extract, 2% peptone, 2% glucose, and 1.5% agar). Individual virgin cells were selected microscopically and aligned in row in different areas with a micromanipulator. Life-spans were determined by scoring the total number of daughter cells produced and removed. At least 60 cells were counted for each strain and at least two independent experiments were done for each strain. Survival curves were considered to be significantly different when they were positive using the Wilconxon (Gehan) test, as implemented in SPSS for Windows release 9.0 (SPSS 1999).

2.3. Chromatin immunoprecipitation assays

Chromatin immunoprecipitation PCR assays were performed as described [32], with modifications [33]. For cross-linking, yeast cells were treated with 1% formaldehyde for 20 min at room temperature. Primer mixes were empirically adjusted for balanced signals. The exact primer sequences are available on request.

2.4. Total RNA extraction, radioactive sample labelling and macroarray hybridisation

Cells were grown overnight at 30 °C in 50 mL of YPD medium (2% glucose, 2% peptone, 1% yeast extract) up to exponential growth phase (OD_{600} = 0.5). RNA extraction cDNA labelling and hybridisation on homemade nylon membrane macroarrays were done as described [34].

2.5. Quantification of hybridisation signals and normalisation procedures

Image acquisition was performed in a Fuji Film FLA3000 Phosphorimager and quantified by using ArrayVision 7.0 software (Imaging Research, Inc.) taking the sARM density (with the corresponding subtracted background) as a signal. Transcript levels 1.45 times over background were considered as valid data and normalised.

The normalisation process and the measure of the significance level for each ORF were done by using ArrayStat software (Imaging Research, Inc.). cDNA hybridisations were subjected to double normalisation, between two conditions (mutant vs. wild type) and between replicates. Experiments were done in triplicate. Reproducibility of the replicates was tested considering the data as independent and allowing the program to take a minimum number of valid replicates of two in order to calculate the mean values for every gene. Data were normalised between different strains by iterative median and corrected by the False Discovery Rate test to estimate the statistical errors associated to each gene.

Gene Ontology (GO) search was done in the Saccharomyces Genome Database (SGD) web interface (http://db.yeastgenome.org/cgi-bin/GO/goTermFinder). We considered significant categories when P values were below 10^{-4}.

2.6. Accession numbers

GEO accession number for the series of 15 individual macroarray hybridisations is GSE2434.

3. Results and discussion

3.1. The hat2 mutant displays longer life-span

There are multiple observations that relate the acetylation profile of the core histones in heterochromatin with yeast life-span. Kim et al. [11] have shown that deletion of several HDs has a profound influence on the life-span of yeast cells, although the effects of particular mutations are very variable. The conclusion is that no simple relationship exists between the acetylation state of histones and the life expectancy. A study of the effects on life-span of *HAT* gene deletions would help to understand this process. However, no study has been reported to date with those genes. We selected HAT-B for our study because of its relationship with telomeric silencing [25]. We disrupted the *HAT1* and *HAT2* genes in an S288c background and measured the life-span of the resulting strains. The average life-span of the *hat2* mutant was extended by 30%, as compared to the wild type control, from 13 to 17 generations, whereas the *hat1* mutation had no significant effect on life-span (Fig. 1A). Next, we examined the life-span of the double mutant *hat1hat2*. The average life-span of this strain was extended by four generations, a behaviour similar to that of single *hat2* mutant (Fig. 1B). To discard that a difference in the growth of these strains was affecting the life-span, we performed several studies on the growth rate of *hat1*, *hat2* and *hat1hat2* mutants in different conditions and we could not find any significant difference between them (data not shown). The presence of both Hat1p and Hat2p in the cell is necessary for the normal level of K12 acetylated H4 in free histones (A. Poveda and R. Sendra, personal communication). Consistent with the observation that Hat2p, but not Hat1p, extends life-span, we could not find any difference in the life-span of yeast cells bearing K12R mutation – a non-acetylatable form of histone H4 – compared to the wild type strain (not shown). Taken together, these results suggest that the function of Hat2p is not limited to enhancing the HAT activity of Hat1p. Deletion of *HIF1* gene, the third component of the complex, does not show any increment of life-span (A. Poveda and R. Sendra, personal communication). This is the first time that a unique phenotype has been observed for a subunit of the HAT-B complex, except for the obvious lack of the HAT activity.

On the other hand, HD activity of Sir2p is required for the redistribution of Sir proteins from telomeres to the rDNA that occurs in old cells to prevent premature aging [35]. Therefore, *sir2* mutants show reduced replicative life expectancies [11,12]. In spite of the fact that a *sir2* mutation and a *hat2* mutation (when combined with certain histone H3 mutations) have similar defects in telomere silencing, their aging-related phenotypes are opposite. We wondered if the opposite effects of Hat2p and Sir2p on life-span were somehow related. To test this we made a double *sir2hat2* mutant and its life-span was analysed and compared to a single *sir2* mutant. As seen in Fig. 1C, *sir2hat2* behaved like the single *sir2* mutant, indicating that *SIR2* has a dominant epistatic effect over *HAT2*.

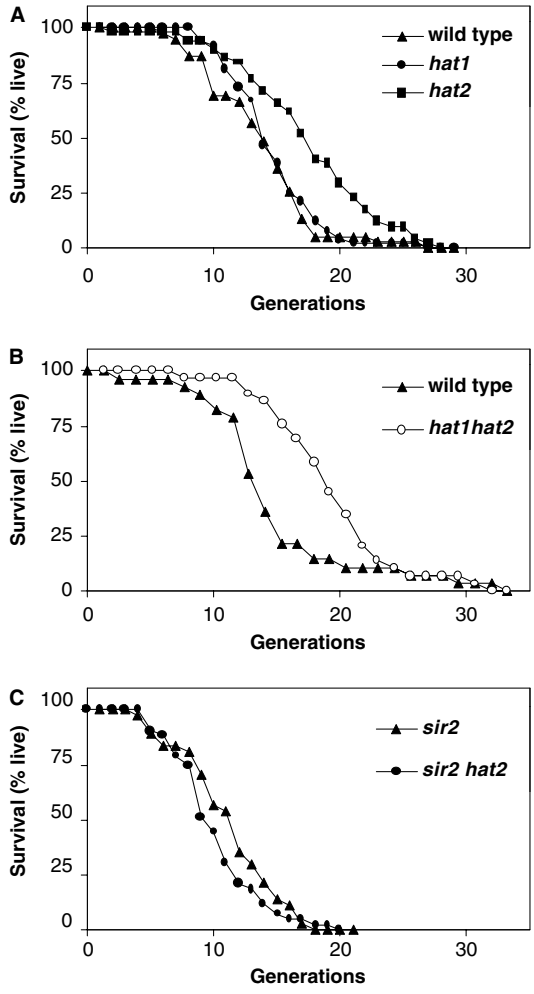

Fig. 1. Effect of *HAT* genes deletion on yeast life-span. Life-span of single *hat1* and *hat2* mutants (A) *hat1hat2* (B) and *sir2hat2* double mutants (C) strains were analysed and compared to the parental (YPH250) or the *sir2* strains. The total number of daughter cells produced by 60 cells was scored. The percentage of living cells is plotted as a function of age in generations. There was no significant difference between the life-spans of strains YPH250 and its *hat1*Δ derivative or between *sir2* and *sir2hat2*. The life-span differences between YPH250 and the *hat2* and *hat1hat2* deletions are significant ($P < 0.001$).

3.2. Sir2p is not retained at telomeres by HAT-B complex proteins

Sir2p has been described as a limiting factor that promotes silencing at subtelomeric regions and rDNA and to be required to prevent premature aging in yeast (reviewed in [36,37]). In this model, the amount of Sir2p available in the nucleolus for silencing would be controlled by the sequestration of Sir2p at telomeres [38]. While the general state of telomeric heterochromatin is hypoacetylated [39], this does not exclude the possibility that HAT-B proteins may play a role in Sir2p telomeric retention. Sir2p has been described as an NAD-dependent HD that deacetylates Lys 9 and 14 of histone H3 and Lys 16 of histone H4 [10]. However, other authors have shown that Sir2p can deacetylate histones previously acetylated by Hat1p or Esa1p [9]. In any case, deacetylation of Lys 16 of H4 is necessary for telomeric silencing and, interestingly, it seems to be a pre-requisite for the ability of HAT-B to acetylate Lys 12 and 5 of histone H4 [40]. In order to know if the targets of HAT-B and Sir2p are related we performed chromatin immunoprecipitation experiments using antibodies raised against specific acetylation sites in histones. In particular, we comparatively analysed the acetylation state of Lys 14 of H3, the major target of Sir2p HD activity, and Lys 12 of H4, the major substrate of HAT-B activity, in different mutant strains. The Ty5 retrotransposon, which is integrated in a subtelomeric location, was used as heterochromatic probe and the promoters of several non-silenced genes as non-heterochromatic controls. As expected, deletion of *SIR2* gene produced an increment of histone H3 Lys 14 acetylation at telomeres whereas deletion of *HAT1* gene had no effect on acetylation at this position (results not shown). The results obtained using antibodies against acetylated Lys 12 of histone H4 were surprising. As can be seen in Fig. 2A, deletion of the *SIR2* gene notably increases the acetylation state of this position, which had not been described to date as a target of this enzyme. Hence, Sir2p participates in the active maintenance of the in vivo hypoacetylation of histone H4 Lys 12. Deletion of genes of known components of HAT-B complex, *HAT1*, *HAT2* or *HIF1,* had no effect on the acetylation state of Lys 12 of H4 at subtelomeric locations. We also analysed the *hat1sir2* double mutant and the results were identical to the *sir2* single mutant (Fig. 2B), demonstrating that a HAT activity other than HAT-B is responsible for this acetylation.

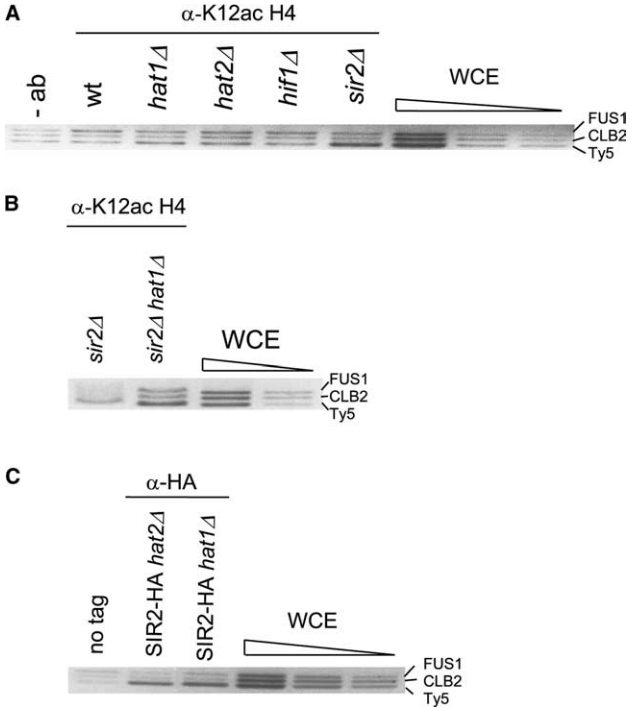

Fig. 2. Chromatin immunoprecipitations. (A) and (B) Chromatin immunoprecipitation assays were done in different yeast strains using an antibody against Lys 12 acetylated histone H4. Yeast Ty5 retrotransposon is on the left subtelomere of chromosome III. *FUS1* and *CLB2* are controls of non-silenced regions. As a control, PCR was done using extracts without antibody (-ab) and prior to immunoprecipitation (whole-cell extract, WCE). (C) Mutant strains *hat2* and *hat1* containing an HA epitope-tagged *SIR2* were immunoprecipitated with an anti-HA antibody. As a control, PCR was done using extracts from a strain without tagged *SIR2* (no-tag) and prior to immunoprecipitation (WCE).

Finally, we investigated whether HAT-B could retain Sir2p at telomeric loci through a mechanism independent of its HAT activity. To study this, we analysed the subtelomeric position of Sir2p in strains bearing mutations in the components of HAT-B complex. Fig. 2C shows that deletion of the *HAT1* or *HAT2* gene does not modify the typical subtelomeric position of Sir2p. We have obtained similar results when deleting *HIF1* (results not shown). Taken together, these results suggest that the increased life-span observed in *hat2* mutants is not due to a telomeric retention of Sir2p.

3.3. Transcriptional profile of the hat mutants

If Hat2p has additional roles to those of Hat1p, then it seems likely that this fact would be reflected in the transcriptional phenotype. With the aim of casting some light on this, we analysed the transcriptome of *hat1, hat2* and *hat1hat2* mutants. The experiments were done in triplicate by using three different exponentially growing YPD cultures for each mutant. We also checked that independent deletion mutants generated the same results (not shown).

Although single *hat1* or *hat2* mutants transcriptomes are not very different from the wild type (Fig. 3A and B), we noticed a difference between both mutant strains when we plotted *hat2* vs. *hat1* (Fig. 3C). It becomes clear that both mutants are more distinct between them than each one with respect to the wild type. This result is highlighted when analysing the number of genes up- or downregulated (Fig. 3). There are many more genes differentially represented between *hat1* and *hat2* mutants. In order to find out the significance of this difference between *hat1* and *hat2* transcriptomes, we performed a GO search. Many genes differentially represented in *hat1* and *hat2* mutants corresponded to specific GO classes (Table 1). A detailed inspection of the comparisons of the mutants with regard to wild type revealed that many of these genes behaved similarly in the single mutants when compared with wild type (see http://scsie.uv.es/chipsdna). However, because the fold changes were lower, and less genes of a particular GO were considered differentially expressed, the GO finder software did not detect many statistically-enriched GO categories in the *hat1* mutant and no enriched categories were detected in the *hat2* mutant (Table 1). In summary, despite the fact that both are subunits of the same HAT-B complex, *hat1* and *hat2* mutants display different transcriptome phenotypes when exponentially growing in a glucose- rich medium.

The analysis of the double mutant showed large differences with regard to the wild type (Fig. 3D). In this case 30% of the genes were considered differentially expressed after statistical analysis. The study of the GO categories revealed that the transcriptome of the *hat1hat2* mutant is more similar to that observed in the *hat1* mutant, but that the number of genes and the fold changes observed are higher in the double mutant. It seems that the *hat2* deletion not only does not compensate but also even enhances the transcriptional phenotype of a *hat1* mutant.

3.4. Does Hat2p have functions in addition to those of the HAT-B complex?

Although Hat2p has been considered to date just a non-catalytic subunit of Hat1p-dependent HAT complexes, the direct conclusion from life-span analyses is that Hat1p and Hat2p do not perform a single and identical function inside the cell. Thus, Hat2p would have additional functions to those of Hat1p. The fact that *HAT2* but not *HAT1* deletion extends life-span indicates that these other functions are not related to the HAT activity of HAT-B complex. Another possibility is

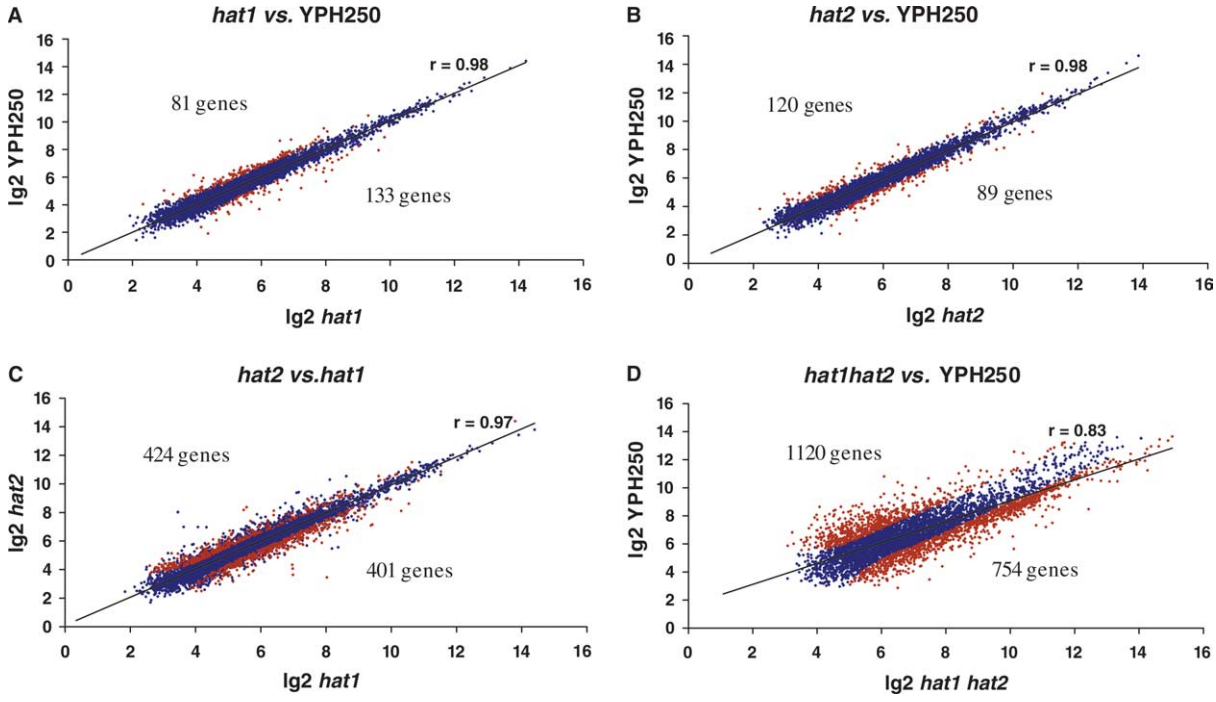

Fig. 3. Comparison of transcriptomes for different hat mutants. The transcriptomes of *hat1* (A), *hat2* (B), and *hat1hat2* (D) mutants were analysed and compared to the wild type transcriptome. *hat1* and *hat2* transcriptomes are compared in (C). Each panel represents the plot of all significant gene expression data of two yeast strains. Graphs are in logarithmic scale and the Pearson correlation of linear regression is shown. Genes in red are those considered as differentially expressed between the two strains. The numbers on both sides of the cloud represent the genes upregulated (below the cloud) or downregulated (above the cloud) in the strain plotted on abscises regarding the strain plotted on ordinates.

Table 1
Summary of comparative transcriptomic profiles

Genes upregulated			Genes downregulated		
GO category	*P*-value	#Genes	GO category	*P*-value	#Genes
hat1/YPH250					
Protein folding	9.46×10^{-6}	6	Ribosome biogenesis	6.39×10^{-15}	27
Polyamine transport	7.18×10^{-5}	3			
hat1hat2/YPH250					
Amino acid metabolism	2.93×10^{-10}	64	Ribosome biogenesis	1.37×10^{-20}	80
Alcohol metabolism	5.27×10^{-7}	35	Cell growth	9.61×10^{-9}	300
Glycolysis	1.12×10^{-6}	14			
Transport	2.79×10^{-6}	179			
hat2/hat1					
Ribosome biogenesis	2.92×10^{-49}	93	Protein folding	2.1×10^{-8}	15
Cell growth	3.25×10^{-8}	181	Energy pathways	7.1×10^{-8}	31

Only GO significant categories (*P*-value $<10^{-4}$) and the number of genes for each category are shown. The comparison of *hat2* vs. wt (YPH250) did not show any statistically significant categories.

that the increased life-span observed in a *hat2* mutant is related to heterochromatin silencing. However, deletion of *HAT2* or *HAT1* genes, combined with mutations of specific lysine residues of the N-terminal tail of histone H3, provokes an identical severe silencing defect [25], suggesting that Hat2p and Hat1p act in the same way. We have also discarded a relationship between the increased life-span of *hat2* mutant and a redistribution of Sir2p between telomeres and rDNA. Moreover, the fact that transcriptomic effects are not related with the subtelomeric location of genes (our unpublished results) suggests that the postulated new roles for Hat2p are not directly related to chromatin silencing. Nevertheless, the epistatic effect of *SIR2* on *HAT2* suggests that Hat2p affects life-span upstream Sir2p within the same pathway.

It has been reported that the transcriptome of a *sir2* mutant is the opposite to a caloric restriction (CR) phenotype [13]. Although the molecular mechanism is controversial [10,13,41], it seems that CR causes changes in Sir2p activity which, in turn, affects life-span. However, the *hat2* transcriptome has no similarity to a CR phenotype, which would be expected if *hat2* were just the reverse of a *sir2* mutant. Thus, our results indicate that Hat2p would act on Sir2p through a pathway other than CR. Perhaps *hat2* deletion causes a mild endogenous stress that is sensed by Sir2p.

The existence of additional roles for Hat2p is also supported by the study of *hat* mutant transcriptomes. If Hat2p were only a co-activator of Hat1p in the HAT complexes, its absence would not modify the transcriptome of a *hat1* mutant. However, the double *hat1hat2* mutant has a distinct transcriptome implying that Hat2p has additional functions in the cell. Moreover, the differences observed between *hat2* and the double *hat1hat2* mutant transcriptomes not only support the additional functions of Hat2p, but also point to a negative modulating role of Hat2p on Hat1p activity (Fig. 4). The comparison of transcriptomes observed in *hat1* and *hat2* mutants would support this last hypothesis.

To summarize, the proteins Hat1 and Hat2 undoubtedly participate in common processes, such as Lys12 acetylation of free H4 histone. However, our data suggest that they also have other roles in cell physiology. It is not clear what those roles could be. Nevertheless, this is not the first case of a HAT complex composed of subunits that participate in different functions. In fact, this is a general feature of HATs. For instance, the yeast HAT complex NuA3 has, in addition to Sas3p (the catalytic subunit), other components such as Anc1p (TAF14) (reviewed in [1]). This protein is present in five additional complexes: two chromatin-remodelling complexes (INO80 and SWI/SNF), the mediator and two basal transcription factors (TFIID and TFIIF). With the results presented here, HAT-B becomes, as other HATs, a complex composed by subunits implicated in more than one function.

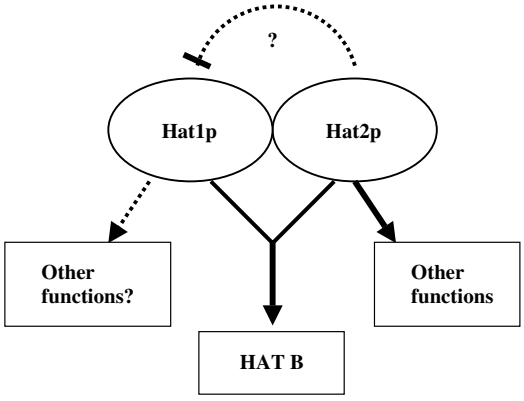

Fig. 4. Proposed model for the functional relationships between Hat1p and Hat2p. Hat1, Hat2 and Hif1 proteins interact physically and functionally, forming the HAT-B complex. The fact that Hat2p, but not Hat1p, modulates yeast life-span does suggest that Hat2p would have additional functions in the cell. However, the transcriptome analyses of the *hat* mutants also suggest a negative modulating effect of Hat2p on Hat1p.

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References

[1] Carrozza, M.J., Utley, R.T., Workman, J.L. and Côté, J. (2003) The diverse functions of histone acetyltransferase complexes. Trends Genet. 19, 321–329.

[2] Peterson, C.L. and Laniel, M.A. (2004) Histones and histone modifications. Curr. Biol. 14, R546–R551.
[3] Kurdistani, S.K. and Grunstein, M. (2003) Histone acetylation and deacetylation in yeast. Nat. Rev. Mol. Cell Biol. 4, 276–284.
[4] Tordera, V., Sendra, R. and Pérez-Ortín, J.E. (1993) The role of histones and their modifications in the informative content of chromatin. Experientia 49, 780–788.
[5] Turner, B.M. (1993) Decoding the nucleosome. Cell 75, 5–8.
[6] Strahl, B.D. and Allis, C.D. (2000) The language of covalent histone modifications. Nature 403, 41–45.
[7] Jenuwein, T. and Allis, C.D. (2001) Translating the histone code. Science 293, 1074–1080.
[8] Mortimer, R.K. and Jonhston, J.R. (1959) Life-span of individual yeast cells. Nature 183, 1751–1752.
[9] Landry, J., Sutton, A., Tafrov, S.T., Heller, R.C., Stebbins, J., Pillus, L. and Sternglanz, R. (2000) The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. Proc. Natl. Acad. Sci. USA 97, 5807–5811.
[10] Imai, S., Armstrong, C.M., Kaeberlein, M. and Guarente, L. (2000) Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 403, 795–800.
[11] Kim, S., Benguria, A., Lai, C.Y. and Jazwinski, S.M. (1999) Modulation of life-span by histone deacetylase genes in *Saccharomyces cerevisiae*. Mol. Biol. Cell 10, 3125–3136.
[12] Kaeberlein, M., McVey, M. and Guarente, L. (1999) The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. Genes Dev. 13, 2570–2580.
[13] Sinclair, D.A., Mills, K. and Guarente, L. (1998) Molecular mechanisms of yeast aging. Trends Biochem. Sci. 23, 131–134.
[14] Gottschling, D.E., Aparicio, O.M., Billington, B.L. and Zakian, V.A. (1990) Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription. Cell 63, 751–762.
[15] Sun, Z.W. and Hampsey, M. (1999) A general requirement for the Sin3-Rpd3 histone deacetylase complex in regulating silencing in *Saccharomyces cerevisiae*. Genetics 152, 921–932.
[16] Brachmann, C.B., Sherman, J.M., Devine, S.E., Cameron, E.E., Pillus, L. and Boeke, J.D. (1995) The *SIR2* gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression, and chromosome stability. Genes Dev. 9, 2888–2902.
[17] Reifsnyder, C., Lowell, J., Clarke, A. and Pillus, L. (1996) Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. Nat. Genet. 14, 42–49.
[18] Osada, S., Sutton, A., Muster, N., Brown, C.E., Yates, J.R., Sternglanz, R. and Workman, J.L. (2001) The yeast SAS (something about silencing) protein complex contains a MYST-type putative acetyltransferase and functions with chromatin assembly factor ASF1. Genes Dev. 15, 3155–3168.
[19] Meijsing, S.H. and Ehrenhofer-Murray, A.E. (2001) The silencing complex SAS-I links histone acetylation to the assembly of repressed chromatin by CAF-I and Asf1 in *Saccharomyces cerevisiae*. Genes Dev. 15, 3169–3182.
[20] Ehrenhofer-Murray, A.E., Rivier, D.H. and Rine, J. (1997) The role of Sas2, an acetyltransferase homologue of *Saccharomyces cerevisiae*, in silencing and ORC function. Genetics 145, 923–934.
[21] Kleff, S., Andrulis, E.D., Anderson, C.W. and Sternglanz, R. (1995) Identification of a gene encoding a yeast histone H4 acetyltransferase. J. Biol. Chem. 270, 24674–24677.
[22] Parthun, M.R., Widom, J. and Gottschling, D.E. (1996) The major cytoplasmic histone acetyltransferase in yeast: links to chromatin replication and histone metabolism. Cell 87, 85–94.
[23] Poveda, A., Pamblanco, M., Tafrov, S., Tordera, V., Sternglanz, R. and Sendra, R. (2004) Hif1 is a component of yeast histone acetyltransferase B, a complex mainly localized in the nucleus. J. Biol. Chem. 279, 16033–16043.
[24] Ai, X. and Parthun, M.R. (2004) The nuclear Hat1p/Hat2p complex: a molecular link between type B histone acetyltransferases and chromatin assembly. Mol. Cell 14, 195–205.
[25] Kelly, T.J., Qin, S., Gottschling, D.E. and Parthun, M.R. (2000) Type B histone acetyltransferase Hat1p participates in telomeric silencing. Mol. Cell. Biol. 20, 7051–7058.
[26] Ruiz-García, A.B., Sendra, R., Galiana, M., Pamblanco, M., Pérez-Ortín, J.E. and Tordera, V. (1998) HAT1 and HAT2 proteins are components of a yeast nuclear histone acetyltransferase enzyme specific for free histone H4. J. Biol. Chem. 273, 12599–12605.
[27] Goldstein, A.L. and McCusker, J.H. (1999) Three dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. Yeast 15, 1541–1553.
[28] Wach, A., Brachat, A., Pohlmann, R. and Philippsen, P. (1994) New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. Yeast 10, 1793–1808.
[29] Fox, C.A., Ehrenhofer-Murray, A.E., Loo, S. and Rine, J. (1997) The origin recognition complex, SIR1, and the S phase requirement for silencing. Science 276, 1541–1547.
[30] Knop, M., Siegers, K., Pereira, G., Zachariae, W., Winsor, B., Nasmyth, K.Y. and Schiebel, E. (1999) Epitope tagging of yeast genes using a PCR-based strategy: more tags and improved practical routines. Yeast 15, 963–972.
[31] Kennedy, B.K., Austriaco, N.R., Zhang, J. and Guarente, L. (1995) Mutation in the silencing gene *SIR4* can delay aging in *S. cerevisiae*. Cell 80, 485–496.
[32] Hecht, A., Strahl-Bolsinger, S. and Grunstein, M. (1999) Mapping DNA interaction sites of chromosomal proteins. Crosslinking studies in yeast. Meth. Mol. Biol. 119, 469–479.
[33] Tanaka, T., Knapp, D. and Nasmyth, K. (1997) Loading of an Mcm protein onto DNA replication origins is regulated by Cdc6p and CDKs. Cell 90, 649–660.
[34] Alberola, T.M., García-Martínez, J., Antúnez, O., Viladevall, L., Barceló, A., Ariño, J. and Pérez-Ortín, J.E. (2004) A new set of DNA macrochips for the yeast *Saccharomyces cerevisiae*: features and uses. Int. Microbiol. 7, 199–206.
[35] Kennedy, B.K., Gotta, M., Sinclair, D.A., Mills, K., McNabb, D.S., Murthy, M., Pak, S.M., Laroche, T., Gasser, S.M. and Guarente, L. (1997) Redistribution of silencing proteins from telomeres to the nucleolus is associated with extension of life-span in *S. cerevisiae*. Cell 89, 381–391.
[36] Cockell, M., Gotta, M., Palladino, F., Martin, S.G. and Gasser, S.M. (1998) Targeting Sir proteins to sites of action: a general mechanism for regulated repression. Cold Spring Harb. Symp. Quant. Biol. 63, 401–412.
[37] Moazed, D. (2001) Enzymatic activities of Sir2 and chromatin silencing. Curr. Opin. Cell Biol. 13, 232–238.
[38] Smith, J.S., Brachmann, C.B., Pillus, L. and Boeke, J.D. (1998) Distribution of a limited Sir2 protein pool regulates the strength of yeast rDNA silencing and is modulated by Sir4p. Genetics 149, 1205–1219.
[39] Suka, N., Suka, Y., Carmen, A.A., Wu, J. and Grunstein, M. (2001) Highly specific antibodies determine histone acetylation site usage in yeast heterochromatin and euchromatin. Mol. Cell 8, 473–479.
[40] Makowski, A.M., Dutnall, R.N. and Annunziato, A.T. (2001) Effects of acetylation of histone H4 at lysines 8 and 16 on activity of the Hat1 histone acetyltransferase. J. Biol. Chem. 276, 43499–43502.
[41] Anderson, R.M., Latorre-Esteves, M., Neves, A.R., Lavu, S., Medvedik, O., Taylor, C., Howitz, K.T., Santos, H. and Sinclair, D.A. (2003) Yeast life-span extension by calorie restriction is independent of NAD fluctuation. Science 302, 2124–2126.