HAT–HDAC interplay modulates global histone H3K14 acetylation in gene-coding regions during stress

Anna Johnsson^{1,2}, Mickaël Durand-Dubief¹, Yongtao Xue-Franzén^{1,2}, Michelle Rönnerblad¹, Karl Ekwall^{1,2} & Anthony Wright^{1,2*}

¹Department of Biosciences and Medical Nutrition, Karolinska Institute, Huddinge, Sweden, and ²School of Life Sciences, Södertörn University, Huddinge, Sweden

Histone acetylation and deacetylation are important for gene regulation. The histone acetyltransferase, Gcn5, is an activator of transcriptional initiation that is recruited to gene promoters. Here, we map genome-wide Gcn5 occupancy and histone H3K14ac at high resolution. Gcn5 is predominantly localized to coding regions of highly transcribed genes, where it collaborates antagonistically with the class-II histone deacetylase, Clr3, to modulate H3K14ac levels and transcriptional elongation. An interplay between Gcn5 and Clr3 is crucial for the regulation of many stress-response genes. Our findings suggest a new role for Gcn5 during transcriptional elongation, in addition to its known role in transcriptional initiation.

Keywords: chromatin; class-II HDAC; Gcn5; histone acetylation; *Schizosaccharomyces pombe*

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INTRODUCTION

Post-translational modifications of histones are implicated in various processes that involve chromatin reorganization such as gene expression, silencing, chromatin condensation, DNA repair and replication. One of the most studied modifications is the acetylation of specific lysine residues within histones through the enzymatic activity of histone acetyltransferases (HATs). Acetylation is reversible due to the antagonistic action of histone deacetylases (HDACs). Several studies *in vitro* and *in vivo* have shown different substrate preferences for various HATs and HDACs (reviewed in Shahbazian & Grunstein, 2007), but the interplay between HATs and HDACs has been less extensively studied.

The Gcn5 HAT is evolutionarily conserved and is the catalytic subunit of several multi-subunit complexes such as the Spt-Ada-Gcn5 acetyltransferase (SAGA) co-activator complex (reviewed in Baker & Grant, 2007). Gcn5 acetylates many lysine residues of

histones H3 and H2B. In *Saccharomyces cerevisiae*, SAGA is recruited to promoter regions by transcription factors such as Gal4 and Gcn4. Genome-wide expression profiling suggests a role for SAGA in the regulation of stress-related genes (Huisinga & Pugh, 2004). In the fission yeast, *Schizosaccharomyces pombe*, mutants lacking Gcn5 show essentially normal growth and gene-expression levels (Johnsson *et al*, 2006). Gcn5 is, however, required for cellular adaptation under various environmental stress conditions, suggesting a crucial role for Gcn5 in chromatin reorganization during adaptation. A subset of stress-response genes require Gcn5 for their regulation (Johnsson *et al*, 2006), but it is not known how Gcn5 contributes to the regulation of these genes.

Genome-wide studies of Gcn5-dependent acetylation have been carried out in *S. cerevisiae* (Roh *et al*, 2004; Rosaleny *et al*, 2007). Here, we investigate the role of Gcn5 in *S. pombe* at a high-resolution, genome-wide level, by combining studies of Gcn5 occupancy and enzymatic specificity *in vivo*, as well as its functional interaction with HDACs. We show that Gcn5 is located preferentially on the coding regions of highly expressed genes and that it is required for efficient transcriptional elongation. Gcn5, Clr3 (a class-II HDAC) and lysine 14 on histone H3 (H3K14) are specifically important for a range of stress responses, in which Gcn5 and Clr3 have opposite roles. These opposite roles modulate the global levels of H3K14ac, most clearly on the coding regions of highly expressed genes.

RESULTS AND DISCUSSION

To investigate the role of Gcn5 in the *S. pombe* genome, we first determined its genome-wide occupancy by chromatin immunoprecipitation (ChIP) using high-density tiling arrays with 20 base-pair resolution. Surprisingly, we found that Gcn5 is most strongly associated with the coding regions of genes (Fig 1A), suggesting a role for Gcn5 in transcriptional elongation. Such a role might predict a higher level of Gcn5 association in the coding regions of more highly transcribed genes. Fig 1B shows that the relative level of Gcn5 binding increases progressively for sets of genes grouped in ascending order according to their estimated mean transcription level. Mapping the levels of Gcn5 binding onto a model gene of standard length shows that the main expression-level-correlated binding of Gcn5 is observed in the coding regions. The binding

¹Department of Biosciences and Medical Nutrition, Karolinska Institute, S-141 57 Huddinge, Sweden

²School of Life Sciences, Södertörn University, S-141 89 Huddinge, Sweden

*Corresponding author. Tel: +46 8608 4708; Fax: +46 8608 4510;

E-mail: anthony.wright@sh.se

Fig 1 | Gcn5 is located in the coding region of highly transcribed genes. (A) High-resolution tiling arrays show the enrichment of Gcn5-Myc (blue trace) in the ORFs (black boxes) of many genes within a representative region of chromosome II. (B) Average gene analysis shows that Gcn5-Myc is preferentially enriched in the coding regions of highly expressed genes. Genes are grouped according to their expression level (n indicates the number of genes in each subclass). (C) Cell-plating assay showing that *gcn5Δ* is sensitive to MPA (50 μ l/ml), in a manner similar to the deletion of elongation factor TFIIIS. The control plate shows growth on PMG. (D) Gcn5-Myc occupancy correlates with the occupancy of several HDACs and histone marks. The table shows significant overlaps between Gcn5-bound ORFs ($n = 835$, twofold enrichment) and groups of ORFs bound by various HDACs or containing different HDAC-dependent chromatin modifications (normalized for H3 occupancy). The P -value indicates the significance associated with the hypogeometrical distribution test. HDAC, histone deacetylase; IGR, intergenic region; MPA, mycophenolic acid; ORF, open reading frame; PMG, pombe glutamate medium; wt, wild type.

patterns can be independently verified by gene-specific ChIP (supplementary Fig S1 online). However, the 700 or so most highly expressed genes also show an elevated average level of Gcn5 binding upstream of the coding region. Thus, our results agree with previous reports of Gcn5 as an activator of transcriptional initiation, while providing new evidence for the involvement of Gcn5 in transcriptional elongation. Consistently, *gcn5Δ* mutants showed enhanced sensitivity to mycophenolic acid (MPA), similar to mutants lacking the elongation factor TFIIIS (Fig 1C; Williams & Kane, 1996). MPA inhibits both the rate and processivity of transcriptional elongation by reducing intracellular pools of GTP and UTP (Mason & Struhl, 2005). Furthermore, genetic interactions between *gcn5* and several subunits of the elongator complex (*elp2*, *elp4*, *elp6* and *iki3*) have been reported (Roguev et al, 2008). Overlapping functions between elongator and Gcn5-containing SAGA complexes have also been observed in *S. cerevisiae* (Wittschieben et al, 2000). We conclude that Gcn5 has a role in transcriptional elongation, in addition to its previously characterized role in transcriptional initiation.

To identify HDACs that might interact functionally with Gcn5, we compared the localization of Gcn5 in intergenic regions (IGRs) and coding regions (ORFs (open reading frames)) with previous genome-wide studies of HDAC binding and HDAC-dependent histone modification in *S. pombe* (Wiren et al, 2005; Sinha et al, 2006; Durand-Dubief et al, 2007). Interestingly, we found that Gcn5 is often co-localized with one or more HDACs in both ORFs and IGRs (Fig 1D, top; supplementary Fig S2 online). Furthermore,

Gcn5 binding also overlaps significantly with ORF regions that are hyperacetylated on histone H3K9 and H3K14, which are known substrates for Gcn5, in HDAC mutants (Fig 1D). These data support the possibility of a functional link between Gcn5 and one or more HDACs in regulating the balance of histone acetylation.

To investigate the significance of various HDACs for Gcn5 function, we tested whether their deletion could suppress the sensitivity of *gcn5Δ* to a range of environmental stress conditions. In *S. pombe*, there are six HDACs: Clr6 and Hos2 (class I), Clr3 (class II), and Sir2, Hst2 and Hst4 (class III; Ekwall, 2005). Surprisingly, *clr3Δ* was the only HDAC deletion that could suppress the stress sensitivity of the *gcn5Δ* mutant (Fig 2A). Clr3, the homologue of the class-II HDAC Hda1 in *S. cerevisiae*, is required for silencing in heterochromatin regions, such as centromeric regions, the ribosomal DNA, the mating-type region and the telomeric loci (Bjerling et al, 2002; Wiren et al, 2005). Interestingly, Clr3 has also been implicated in the regulation of stress-related genes (Wiren et al, 2005). In contrast to Clr3, mutants affecting the class-III HDACs, *sir2Δ*, *hst2Δ* and *hst4Δ*, were not able to suppress the *gcn5Δ* phenotypes (Fig 2A; supplementary Fig S3 online), even though they act on many of the Gcn5-associated genes (Fig 1C). By contrast, the *gcn5Δhos2Δ* double mutant showed a synthetic growth phenotype in all conditions tested (Fig 2A). This suggests that the class-I HDAC Hos2 supports Gcn5 function through a distinct route. Indeed, Hos2 has been implicated in gene expression through its ability to

Fig 2 | Genetic interaction between Gcn5 and the class-II histone deacetylase Clr3 links the stress response to acetylation of histone H3K14. Cell-plating assay showing that (A) *clr3Δ* specifically rescues growth of the *gcn5Δ* mutant on 1 M KCl and heat (36 °C), and (B) that mutation of histone H3 that mimics hypoacetylation of lysine 14 (H3K14R) is uniquely sensitive to 1 M KCl. Growth on rich medium (YES) at 25 °C is shown (control). wt, wild type; YES, yeast extract with supplements.

deacetylate H4K16ac in the ORF regions of highly expressed genes (Wiren *et al*, 2005).

The specific suppression of *gcn5Δ* stress phenotypes by the deletion of *clr3+* suggests that the two enzymes modulate acetylation levels of a common chromatin mark, leading to functional consequences that cannot be achieved by the interaction of Gcn5 with other HDACs. Interestingly, Gcn5 in *S. cerevisiae* preferentially acetylates H3K14 (Zhang *et al*, 1998; Grant *et al*, 1999) and Clr3 preferentially deacetylates H3K14ac (Bjerling *et al*, 2002; Wiren *et al*, 2005). H3K14 is thus a good candidate for a common target for Gcn5 and Clr3. To test whether the H3K14ac mark is important for Gcn5-mediated stress responses, we used strains carrying point mutations in the histone *h3.2* gene (Mellone *et al*, 2003). Briefly, in strains lacking two of the three *h3* genes, mutants were made that convert the lysines at residue 14 of the remaining H3 copy to either an alanine (mimics a non-charged, hyperacetylated state) or an arginine (mimics a charged, hypoacetylated state). As histone H3K9 is also a substrate of Gcn5-mediated acetylation (Zhang *et al*, 1998; Grant *et al*, 1999), we also tested mutations affecting this lysine. Interestingly, the H3K14R mutant specifically showed an elevated sensitivity to environmental stress (Fig 2B). Neither the H3K14A mutant nor the H3K9 mutant showed any detectable growth defect under the same conditions. As H3K14A is constitutively non-charged, we speculated that it might suppress the *gcn5Δ* phenotype. However, the H3K14A mutant is sensitive to stress in strains lacking Gcn5, whereas double mutants *gcn5ΔH3K9R* and *gcn5ΔH3K9A* are only mildly affected (supplementary Fig S4 online). Thus, although these results further support H3K14 as an important Gcn5 target during stress, they also suggest that H3K14 and Gcn5 have some non-overlapping roles. The significance of H3K14 is not general to all lysines in the H3 tail because the nearby H3K9 residue, which is acetylated by Gcn5, has little, if any, role in the stress response.

To identify genomic regions where Clr3 has the capacity to reverse defects in Gcn5-dependent acetylation of H3K14, we investigated the roles of Gcn5 and Clr3 in genome-wide H3K14 acetylation. We carried out high-resolution genome-wide mapping of H3K14ac in wild-type, *gcn5Δ* and *gcn5Δclr3Δ* strains under KCl-induced stress. Even a cursory analysis of the data revealed marked differences among the three strains (Fig 3A). The *gcn5Δ* strain showed a strong global decrease in H3K14ac compared with the wild type. Interestingly, the wild-type H3K14ac pattern was partly restored in the *gcn5Δclr3Δ* mutant. The use of a control antibody specific for the C-terminus of histone H3 (H3Cter) to monitor the density of nucleosomes did not change the overall conclusions. Compared with wild type, the *gcn5Δ* mutant showed no obvious difference in nucleosome occupancy, whereas *gcn5Δclr3Δ* showed a slightly increased H3Cter density. The global nature of the effects was further analysed by mapping data for all genes onto an average gene (Fig 3B). In the wild type, we found more enrichment of H3K14ac in transcribed ORF regions than in intergenic regions with a peak at the 5' end of the coding regions (Fig 3B, upper panel). The generally higher level of H3K14ac in the coding regions can be explained by a higher histone density compared with intergenic regions, but the 5'-end peak of H3K14ac in expressed genes is still apparent after normalization for histone density (Fig 3C, upper panel). In *gcn5Δ*, there was a general decrease in H3K14ac (Fig 3B, middle panel), resulting in loss of the 5' peak, which was apparent after normalization for histone density (Fig 3C, middle panel). This shows that in *S. pombe*, H3K14 is predominantly acetylated by Gcn5 *in vivo*. This conclusion significantly extends the results of previous studies in *S. cerevisiae* that have shown a positive correlation between Gcn5 binding, H3K14 acetylation and gene expression (Pokholok *et al*, 2005). Interestingly, there was also a slight increase in nucleosome density within highly expressed genes in the *gcn5Δ* mutant (supplementary Fig S5 online), which is consistent with a recent report suggesting a role for Gcn5 in nucleosome eviction in the *S. cerevisiae gal1* gene (Govind *et al*, 2007).

The *gcn5Δclr3Δ* double mutant showed a significant restoration of the H3K14ac mark compared with *gcn5Δ* (Fig 3B, lower panel), which is consistent with H3K14ac being a primary substrate for Clr3-mediated deacetylation *in vivo* (Bjerling *et al*, 2002; Wiren *et al*, 2005). Normalization for histone density showed that the *gcn5Δclr3Δ* mutant has partly restored H3K14ac levels for highly expressed genes, but that global H3K14ac levels are still lower than in the wild type (Fig 3C, lower panel). Thus, under these conditions other HATs are able to acetylate H3K14, but with a much lower efficiency than Gcn5. Taken together, our data show that Gcn5 and Clr3 modulate levels of H3K14ac most clearly in the coding region of actively transcribed genes over the whole genome, and that the role of Gcn5 in H3K14 acetylation can be partly replaced by that of another HAT under conditions of reduced deacetylation.

We postulated that the specific role of Clr3, which cannot be played by other HDACs known to deacetylate H3K14ac, might be focused on genes in which Gcn5 has a role in transcriptional elongation. Consistent with this view, the MPA sensitivity of *gcn5Δ* was completely rescued in the *gcn5Δclr3Δ* double mutant (Fig 4A). Deletion of *sir2+*—the HDAC that is most closely related to Clr3 in terms of function—in the *gcn5Δ* mutant had no effect on MPA sensitivity (Fig 4A). Thus, our results suggest

Fig 3 | Gcn5 and Clr3 have opposing effects on global histone H3K14 acetylation in gene coding regions. (A) High-resolution tiling arrays showing occupancy of H3K14ac (corrected for nucleosome density) and histone H3 in wild-type, *gcn5*Δ and *gcn5*Δ*clr3*Δ strains. A representative region of chromosome III is shown. (B,C) Average gene analysis of H3K14ac levels in wild-type, *gcn5*Δ and *gcn5*Δ*clr3*Δ strains, (B) before and (C) after correction for nucleosome density. Genes were divided into subclasses with various expression levels as in Fig 1B. IGR, intergenic region; WT, wild type.

that Clr3 might have a specific role in the regulation of genes that require Gcn5 for efficient elongation. An analogous role for HDAC has been reported previously for Sir2, which has a specific role in the repression of several low-expressed genes (Durand-Dubief *et al*, 2007).

A further prediction is that Gcn5-dependent and Clr3-dependent acetylation would result in changes in gene expression. Therefore, we carried out transcription profiling under KCl-induced stress conditions to find genes with altered transcript levels in the

*gcn5*Δ*clr3*Δ mutant compared with the *gcn5*Δ single mutant. More than 10% of genes (*n* = 527) showed a significant (*P* < 0.05) difference in their transcript levels between the strains. To determine whether these *clr3*Δ-mediated changes reverse gene-expression changes resulting from the deletion of *gcn5*+, we compared the transcript-level ratios of *gcn5*Δ*clr3*Δ versus *gcn5*Δ with the transcript-level ratios of *gcn5*Δ versus wild type (Johnsson *et al*, 2006). The scatter plot in Fig 4B shows that these changes are negatively correlated (correlation coefficient, −0.48),

Fig 4 | Deletion of *clr3* suppresses the elongation phenotype and restores the expression of genes affected by the deletion of *gcn5*. (A) Cell-plating assay showing suppression of MPA sensitivity in *gcn5Δ* by deletion of *clr3* + but not *sir2* +. Data for wild type, *TFIISΔ* and *gcn5Δ* (upper panel) and annotations are as in Fig 1C. (B) Scatter plot showing that genes with decreased expression in *gcn5Δ* compared with wild type had increased expression in *gcn5Δclr3Δ* compared with *gcn5Δ*, and vice versa, during KCl-induced stress. Expression ratios of the 216 genes shown were significantly altered in both data sets ($P < 0.05$). MPA, mycophenolic acid; wt, wild type.

as would be expected if the differences manifest the ability of *clr3Δ* to suppress defects resulting from *gcn5Δ*. However, we could not formally exclude the possibility that Clr3 affects the expression of these genes independently of Gcn5. To address this, we used microarrays to identify Clr3-regulated genes. Only a few genes were affected by *clr3Δ* independently of Gcn5 and none of the identified genes showed clear coupling to transcriptional elongation. It therefore seems unlikely that the suppression of *gcn5Δ* by *clr3Δ* is mediated by a Gcn5-independent mechanism. We conclude that the interplay between Gcn5 and Clr3, which has clear consequences for the global levels of H3K14ac, also affects the expression of many genes.

The observation that deletion of *clr3* reversed the effect of the *gcn5* deletion on genes that are upregulated and downregulated in *gcn5Δ* cells was somewhat surprising, as Clr3 has so far been associated with silencing and heterochromatin maintenance (Bjerling *et al*, 2002; Sugiyama *et al*, 2007), and H3K14ac is a chromatin mark previously associated with gene activation (Pokholok *et al*, 2005). Moreover, the genetic interaction between *gcn5* and *hos2* observed in this study suggests an activating role for Gcn5 that would act in parallel with the deacetylation of H4K16ac in coding regions by Hos2, which is known to stimulate gene expression (Wiren *et al*, 2005). However, the repression of gene expression by Gcn5 and its reversal by Clr3

could, in principle, result from the opposite response to H3K14 acetylation in a subset of genes. Alternatively, Gcn5 and Clr3 could modulate the acetylation status of an alternative residue on a histone or non-histone protein where acetylation leads to gene repression. Indeed, Gcn5 has been showed to repress the expression of *ste11* and *mei2* by a mechanism unrelated to histone acetylation (Helmlinger *et al*, 2008). A repressive function for Gcn5 has also recently been described in *S. cerevisiae*, such that Gcn5-mediated acetylation of the RSC chromatin-remodelling complex inhibits its role as an activator of transcription (VanDe-mark *et al*, 2007). An important conclusion of this study is that Gcn5 and Clr3 are also predicted to have antagonistic roles at such non-histone targets.

In summary, Gcn5 is preferentially associated with the coding regions of highly expressed genes, suggesting a role for Gcn5 during transcriptional elongation. The sensitivity of *gcn5Δ* to MPA strongly supports this conclusion. The antagonistic interaction between Gcn5 and Clr3, and the importance of H3K14 for stress responses suggest an important role for H3K14 acetylation during stress. The specific suppression of the *gcn5Δ*-elongation defect by deletion of *clr3* + suggests that the interplay among Gcn5, Clr3 and H3K14ac affects the transcriptional elongation efficiency of important stress genes. The reciprocal interplay between Gcn5 and Clr3 affects the expression of many genes during stress conditions, including genes repressed by Gcn5 where acetylated targets might be non-histone proteins.

METHODS

Strains and media. See supplementary information online for strain-related details.

Chromatin immunoprecipitation. ChIP was carried out as described by Robyr & Grunstein (2003) with modifications; see supplementary information online.

ChIP-on-chip analysis. GeneChip *S. pombe* Tiling 1.0FR Arrays (Affymetrix, Santa Clara, CA, USA) were used for ChIP-on-chip analysis of at least two biological replicates. For normalization, the Tiling Analysis Software version 1.1 was used. Data are available at Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo>, accession number GSE13790).

Average gene analysis. Data sets from ChIP-on-chip and transcription profiling experiments were used to map chip data from all genes onto a model gene of average length, using a procedure modified from Pokholok *et al* (2005) and Li *et al* (2007). Briefly, the coding region of each gene in *S. pombe* was divided into 20 equally sized bins, while a maximum of 450 bp upstream of the start codon (5'IGR) and downstream of the stop codon (3'IGR) was divided into a maximum of 9 bins, each equivalent to 50 bases. Values for each probe were assigned to the closest bin. Expression data from Wilhelm *et al* (2008) were used to divide the genes into five expression-level categories.

Expression profiling microarray. For expression profiling, the Eurogentech (Liege, Belgium) platform was used (Xue *et al*, 2004). Preparation of RNA, normalization and data analysis were carried out as described by Johnsson *et al* (2006). Data are available at Gene Expression Omnibus (accession number GSE13817).

See supplementary information online for details on further materials and methods.

Supplementary information is available at *EMBO reports* online (<http://www.emboareports.org>).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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