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GCN5 enables HSP12 induction promoting chromatin remodeling not histone acetylation.

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ABSTRACT

Regulation of stress responsive genes represents one of the best examples of gene induction and the relevance and involvement of different regulators may change for a given gene depending on the challenging stimulus. *HSP12* gene is induced by very different stimuli, however the molecular response to the stress has been characterized in detail only for heat shock treatments. In this work we want to verify whether, the regulation of transcription induced by oxidative stress, utilizes the same epigenetic solutions relative to those employed in heat shock response. We also monitored *HSP12* induction employing spermidine, a known acetyltransferase inhibitor, and observed an oxidative stress that synergizes with spermidine treatment. Our data show that during transcriptional response to H_2O_2 , histone acetylation and chromatin remodeling occur. However, when the relevance of Gcn5p on these processes was studied, we observed that induction of transcription is *GCN5* dependent and this does not rely on histone acetylation by Gcn5p despite its HAT activity. Chromatin remodeling accompanying gene activation is rather *GCN5* dependent. Thus, *GCN5* controls *HSP12* transcription after H_2O_2 treatment by allowing chromatin remodeling and it is only partially involved in *HSP12* histone acetylation regardless its HAT activity.

Keywords: *HSP12*; chromatin remodeling; *GCN5*; nucleosome occupancy

INTRODUCTION

Oxidative stress, pH changes, alterations in nutrients amount, temperature or osmolarity changes, all these environmental stimuli induce an adaptive response in any cell type in order to achieve maximal cell survival. Thus, much attention has been paid to transcriptional behavior of stress-dependent genes, particularly on their regulation (Vihervaara et al., 2018).

In *Saccharomyces cerevisiae*, the expression of approximately 1000 genes is modulated by environmental stress, being 600 downregulated genes and 300-400 upregulated ones (Gasch et al., 2000; Causton et al., 2001). Among the proteins induced during the stress response, an important family consists of heat shock proteins (HSP) (Verghese et al., 2012). Many of these proteins play the conserved role of chaperones (Walter and Buchner, 2002) and contribute to the proteostasis maintenance (Gragerov et al., 1991).

S. cerevisiae Hsp12p is a 109 amino acid long protein which localizes at the plasma membrane (Sales et al., 2000) and is encoded by the *HSP12* gene. It belongs to a group of small proteins acting as chaperone involved in the folding of nascent proteins and in protection from protein misfolding (Hendrick and Hartl, 1993; Lindquist and Craig, 1988).

HSP12 gene transcription is finely regulated and its mRNA production is induced when yeast cells face extreme environmental conditions such as high temperatures, high osmolarity or oxidative stress (Gasch and Werner-Washburne, 2002). The *HSP12* promoter contains a heat shock element [HSE] whose sequence (nGAAn), lying about 800 bp upstream of the RNA start site, is recognized by the Hsf1p factor by which is constitutively bound (Gasch and Werner-Washburne, 2002) and becomes active after phosphorylation (Liu and Thiele, 1996), so the gene is induced to transcribe. Beside the HSE sequence, there are also seven stress-response elements (STRE) in the regulatory region of this gene, whose sequences (CCCCY) are located in different positions (from -874 to -190 bp upstream the transcriptional start site, TSS) (Kobayashi and McEntee, 1993; Ruis and Schüller,

1995). These sequences are recognized by the two stress associated factors Msn2 and Msn4p (Schmitt and McEntee, 1996), whose binding is necessary for *HSP12* transcriptional activation. It has been suggested that Msn2/4 activity is finely regulated by different partners to achieve an optimal stress response following different stress conditions (Sadeh et al., 2011). Heat shock dependent *HSP12* regulation has been extensively studied (Erkina and Erkin, 2006; Erkin et al., 2008; Erkin et al., 2010) and the involvement of transcription factors (Hsf1, Msn2/4) together with consistent chromatin changes occur (histone acetylation of promoter region and chromatin remodeling) (Erkin et al., 2008; Erkin et al., 2010).

Since different environmental stress stimuli may switch on different regulative stress responsive pathways (Sadeh et al., 2011), we wanted to investigate how *HSP12* chromatin is rearranged after oxidative stress when gene activation occurs. Our results show that the oxidative challenge of *HSP12* by treatment with H_2O_2 induces chromatin hyperacetylation by a *GCN5* independent mechanism, while a chromatin remodeling occurs in a *GCN5* dependent manner. These findings suggest that Gcn5p may be involved in the establishment of different chromatin states beyond its acetyltransferase activity.

MATERIALS AND METHODS

Culture media and conditions

Yeast cultures were grown and manipulated according to standard protocols (Sherman et al., 1983) YPD medium (1% bacto yeast extract, 2% bacto peptone, 2% glucose) was used for growth of all strains.

Hydrogen Peroxide (H_2O_2) treatment, Spermidine treatment and combined treatment were performed adding to the culture medium H_2O_2 0.4 mM (30°C for 20 min), or Spermidine 8 mM (30°C for 3h),

respectively. When both compounds were utilized in the combined treatment, cells were first incubated at 30°C for 3h with Spermidine, and 20 minutes before the end of Spermidine treatment H₂O₂ 0.4 mM was added.

***S. cerevisiae* strains:**

BY4742 (WT): Mat α : HIS 3- Δ 1; LEU- Δ 0; LYS2- Δ 0; URA3- Δ 0.

gcn5 Δ : Mat α : HIS 3- Δ 1; LEU- Δ 0; LYS2- Δ 0; URA3- Δ 0; GCN5::KAN (Chimenti et al., 2009)

paa1 Δ : Mat α : HIS 3- Δ 1; LEU- Δ 0; LYS2- Δ 0; URA3- Δ 0; PAA1::KAN (this work)

Oligonucleotides

UBC6 F: 5' - GATACTTGAATCCTGGCTGGTCT - 3'

UBC6 R: 5' - AAGGGTCTTCTGTTTCATCACCTG - 3'

HSP12 F: 5' - AGTCATACGCTGAACAAGGT - 3'

HSP12 R: 5' - CGTTATCCTTGCCTTTTTCG - 3'

NUC6 F: 5' - TCAGATGAAAGATGAATAGACATAGGA - 3'

NUC6 R: 5' - AAAGTAACATCCCAATGCGG - 3'

HSP12 UPSTREAM 1 F: 5' - GGGGCGGCACAAAATAACAT - 3'

HSP12 UPSTREAM 1 R: 5' - CAGGGGCTGTAGATCTAGTT - 3'

HSP12 UPSTREAM 2 F: 5' - CCCTCTTGATCTGAAAGGTC - 3'

HSP12 UPSTREAM 2 R: 5' - CTTTCTACCTGCGTCAGAC - 3'

HSP12 DOWNSTREAM 1 F: 5' - GTCTGACGCAGGTAGAAAAG - 3'

HSP12 DOWNSTREAM 1 R: 5' - GGAAGACACCCTTGTTGTCT - 3'

HSP12 DOWNSTREAM 2 F: 5' - AGACAACAAGGGTGTCTTCC - 3'

HSP12 DOWNSTREAM 2 R: 5' - CTTGGTTGGGTCTTCTCAC - 3'

HSP12 (Hinf I) F: 5'-ATCACTGACAAGGCCGACAA-3'

HSP12 (Hinf I) R: 5'-ACCTTCAGCGTTATCCTTGC-3'

RT qPCR

RNA was extracted from exponential growing cells (0.5 OD/ml) as previously described (Verdone et al., 1996). After DNase treatment 0.75 µg RNA were subjected to retro-transcription using 1 µl oligo-dT 10 µM, 1 µl dNTPs 10 mM.

50U Bioscript Reverse Transcriptase (Bioline) were added in each sample and incubated at 42°C for 1h. The enzyme was inactivated by heating samples at 85°C for 5 min. cDNA was amplified in triplicate for each sample, using Sso Advanced SYBR Green supermix (Bio-Rad) in a Mini Opticon Real-time PCR System (Bio-Rad). The values were normalized to those of UBC6 gene expression that has been reported to be invariant after different treatments (Teste et al., 2009).

Chromatin immunoprecipitation

ChIP assays were performed as previously described (D'Alfonso et al., 2016). Briefly: exponential growing cells were crosslinked with formaldehyde and the reaction was stopped with glycine after 20 minutes. After centrifugation, cells were resuspended in lysis buffer (140mM NaCl, 50mM Hepes/KOH pH 7.5, 1mM EDTA, 1% TritonX-100, 0.1% sodium deoxycholate, 0.1% SDS) with protease inhibitor (Complete mini EDTA-free Roche). Cells were then and then lysed with glass beads.

Lysates were sonicated to shear DNA into fragments (average length 500bp) and 300 µg of chromatin extracts were incubated with 3 µg of antibody against α-H3 C-Term (Abcam, ab1791) and α-H3 Panac (Millipore, 06–599). Additional 300 µg of chromatin extracts were used as negative control (Beads Only, BO no antibody) and INPUT (genomic sample). Chromatin-antibody complexes were recovered using Dynabeads protein A (Invitrogen) according to antibodies

manufacturer's indications. Subsequently, samples were first extensively washed and then eluted with 100 µl of elution buffer (50mM Tris-HCl pH 8, 10mM EDTA, 1% SDS). Overnight incubation at 65 °C reversed the crosslinking. Samples were treated with proteinase K and RNase A. DNA was purified in standard conditions. INPUT, IP and BO were then amplified by q-PCR using specific primers.

The fold increase (Rq) was relative to histone H3 [$Rq = 2^{-\Delta Ct}$, with $\Delta Ct = Ct(\text{H3 Panac}) - Ct(\text{H3})$]. The values of acetylation levels were obtained normalizing H3 acetylation values to total H3 values.

Micrococcal Nuclease (Mnase) PCR

200 ml culture were grown for each sample and cells were harvested in exponential phase. Cells resuspended in 10 ml of buffer containing 1 M sorbitol, 50 mM Tris-HCl pH 7.5 and 10 mM β -mercaptoethanol, were incubated with 0,05mg/OD Zymolase 100T for 15 min at 30°C to produce spheroplasts.

After mild centrifugation, spheroplasts were resuspended in nystatin buffer (50 mM NaCl, 1.5 mM CaCl_2 , 20 mM Tris-HCl pH 8.0, 1 M sorbitol, 100 µg/ml nystatin), split into 0.4 ml aliquots, and subjected to digestion with MNase (12 U/µl) for 30 min at 37°C. MNase reaction was stopped with 20% SDS and 50 mM EDTA and samples incubated with Proteinase K (0,5 mg/ml) at 56°C for 90 min. Purified DNA was then subjected to 1% agarose gel electrophoresis to isolate and excise mononucleosome-sized DNA fragments (200bp) . DNA was eluted by 0.6 M Na-Acetate overnight incubation and then purified.

DNA was amplified by q-PCR using *HSP12* gene specific primers.

Nuc6, a stable nucleosome within the ribosomal genes cluster, was also amplified to normalize the Ct values obtained by three independent experiments as previously reported (D'Alfonso et al., 2016).

Restriction Assay

In vivo digestion with Hinf I (40U/sample) was performed on spheroplasts for 30 minutes at 37°C. The reaction was stopped (20% SDS, 50 mM EDTA) and proteinase K treatment (0.5 mg/ml) was performed for 90 min at 56°C. DNA was purified and aliquots amplified by q-PCR using specific primers. The values were normalized to UBC6 expression (Teste et al., 2009).

Statistical analysis

All quantified data are shown as mean, standard deviation and standard error of relative enrichments calculated from at least three biological replicates.

p values were calculated using Student's T-Test (p-value $\leq$ 0,05 *; p-value $\leq$ 0,01 **; p-value $\leq$ 0,001 ***)

RESULTS

Transcriptional and chromatin response of *HSP12* gene after H₂O₂ treatment.

Stress response genes are activated by several stimuli and, as previously reported, the optimal response depends on a given stimulus followed by a physiological reaction triggering epigenetic regulations (Sadeh et al., 2011). *S. cerevisiae* response to a heat shock stress has been studied for

HSP12 gene (Erkina and Erkin, 2006; Erkina et al., 2008; Erkina et al., 2010). Here we studied *HSP12* transcription and histone acetylation in cells under oxidative stress conditions focusing on differences and similarities of the genetic response following the H_2O_2 treatment.

Yeast cells grown to exponential phase were treated with 0.4mM H_2O_2 for 20 minutes, RNA purified and analyzed. Transcriptional response of *HSP12* gene is reported in Figure 1A: at least five-fold induction is observed, thus confirming previous observations (Erkina and Erkin, 2006). Previous works (Erkina and Erkin, 2006) have shown that together with a robust transcriptional response to heat shock, also a substantial epigenetic regulation occurs at *HSP12* promoter that becomes hyperacetylated (Erkina and Erkin, 2006; Erkina et al., 2008; Erkina et al., 2010). In order to verify whether oxidative stress provokes a response similar or different to that triggered by heat shock, we measured histone H3 acetylation at *HSP12* promoter and coding region by ChIP analysis, using α -H3PanAc antibodies. Cells were grown and treated with H_2O_2 as described for transcriptional analysis (see above) and then subjected to the ChIP procedure. Cells treated with 0.4mM H_2O_2 increase histone acetylation both at the promoter (Figure 1B) and at the coding region (Figure 1C).

Taken together, data reported in Figure 1 show that an oxidative stress stimulus induces *HSP12* transcription associated to histone hyperacetylation of both its promoter and coding regions.

HAT inhibitor spermidine unexpectedly induces transcriptional activation of *HSP12* gene.

In order to clarify whether the *HSP12* histone hyperacetylation is functional to gene transcription, we decided to interfere with the histone acetylation process (supplementary material) by treating yeast cells with spermidine (SPD), a histone acetyltransferase inhibitor affecting also longevity (Eisenberg et al., 2009). After three hours exposure to 8mM SPD followed by 20 min of 0.4mM

H₂O₂ treatment, *HSP12* transcription was evaluated. As reported in Figure S1A, a very high induction of *HSP12* is observed after 0.4mM H₂O₂ combined with 8mM SPD treatment, compared to each single treatment.

These results suggest that the HAT inhibitor SPD does not reduce *HSP12* transcription after H₂O₂ treatment that is, conversely, greatly increased as shown in Figure S1A. We hypothesized that the spermidine metabolism leading to putrescine (see the scheme in Figure S1B), would generate a consistent amount of H₂O₂ thus representing an additional oxidative stress source. In order to verify this hypothesis, we prevented the putrescine production from spermidine producing the *paa1Δ* strain, lacking the enzyme transforming spermidine in acetylspermidine and excluding further transformations to putrescine as described in (Wallace et al., 2003).

When *paa1Δ* cells were treated with 8mM SPD, the transcriptional response was clearly less pronounced in comparison to that of the WT cells (Figure S1C) confirming a role of *PAA1* gene in this process.

Together, data reported in Figure S1 show that the 8mM SPD treatment does not inhibit *HSP12* transcription after 0.4mM H₂O₂ but rather reveals increased transcription; the reduced transcriptional response of *HSP12* in *paa1Δ* cells suggests that the impaired spermidine acetylation leads to putrescine reduction and H₂O₂ formation.

Role of *GCN5* in *HSP12* transcription efficiency and chromatin modification.

In *S. cerevisiae* histone acetylation is mainly provided by the activity of two enzymes: Esa1p and Gcn5p. While *esa1* mutation leads to an inviable phenotype (Allard et al., 1999), *gcn5* mutants are viable (Grant et al., 1997). The global effect of Esa1p on histone acetylation has been reported (Durant and Pugh, 2007) while Gcn5p acetylates a subset of genes particularly involved in stress

response (Huisinga and Pugh, 2004; Gaupel et al., 2015). We wanted to verify whether the oxidative stress-induced H3 histone hyperacetylation of the *HSP12* gene, is controlled by Gcn5p. Thus, we studied the *GCN5* role in *HSP12* transcription during oxidative stress by evaluating *HSP12* transcripts after H₂O₂ treatment in the *gcn5Δ* strain compared to WT. Results are reported in Figure 2. After RNA purification and quantification of *HSP12* mRNA, we observed a 5-6 fold induction in WT cells, while the *gcn5Δ* strain activates *HSP12* gene to a lower extent (Figure 2A). This finding suggest that *GCN5* contributes to *HSP12* transcriptional control. Considering that increase of histone H3 acetylation is observed together with *HSP12* gene activation (Erkina and Erkin, 2006) and given the transcriptional and epigenetic response of *HSP12* gene after H₂O₂ (reported in Figure 1), we decided to investigate whether the *GCN5* dependent *HSP12* activation after H₂O₂ treatment, depends on Gcn5p HAT activity. Therefore, we measured, by ChIP analysis, H3 histone acetylation of coding region and promoter of *HSP12* after its induction, both in WT and *gcn5Δ* mutant using α-H3Panac antibodies. Results are reported in Figure 2B and 2C respectively: surprisingly, *gcn5Δ* acetylation levels remain similar both within the ORF and the promoter region when compared to the WT. These observations indicate that *GCN5* mutation affects *HSP12* transcription upon H₂O₂ treatment through a mechanism that only partially involves histone acetylation.

***GCN5* deletion limits the chromatin remodeling of *HSP12* control region.**

The Gcn5p acetyltransferase belongs to the SAGA complex, promoting RNA polymerase II transcription. This protein holds a bromodomain allowing the protein to recognize and bind acetylated residues of proteins (Owen et al., 2000). Furthermore, it has been reported that *GCN5* and SWI/SNF remodeling complex cooperate during transcriptional activation (Qiu et al., 2016).

According to these considerations, we wanted to verify whether the absence of Gcn5p would cause a defect in chromatin remodeling. The impact of chromatin remodeling on *HSP12* gene control region has been reported during heat shock induction (Erkina and Erkin, 2006). Thus, we wanted to verify whether the process would change in *gcn5Δ* cells, providing an explanation for the strongly reduced transcriptional response observed in the *gcn5Δ* mutant, upon H₂O₂ exposure (Figure 2A).

We studied the *in vivo* accessibility of two regions partially covered by nucleosome+1, previously mapped (Jiang and Pugh, 2009), toward Hinf I restriction enzyme. +1 nucleosome is considered the prototypical regulative nucleosome in yeast (Jiang and Pugh, 2009).

Cells were treated or not with 0.4mM H₂O₂ for 20 min and subjected to Hinf I restriction (see M&M). After Hinf I digestion, DNA was purified and amplified by qPCR for AP1 or AP2 regions. These regions correspond to the DNA sequences covered by nucleosome +1 (Celona et al., 2011) (schemes below histograms in Figure 3). Thus, quantification of amplification products (AP1 and AP2) directly reflects protection to restriction enzyme in these regions. As reported in Figure 3, AP2 region becomes significantly more protected after 0.4mM H₂O₂ treatment, while accessibility of the AP1 region remains the same; this observation suggests a sliding of the protection (after H₂O₂ treatment) toward the region downstream the TSS region. A graphical representation with detailed mapping of the analyzed region is given in the lower part of Figure 3.

Results reported in Figure 2 show that Gcn5p affects *HSP12* transcription without a direct involvement in modification of histone acetylation after *HSP12* induction. We thus wanted to verify whether the remodeling process of *HSP12* +1 nucleosome would be altered in the *gcn5Δ* strain, thus explaining the transcriptional effects previously observed (Figure 2A). In order to verify this point we employed MNase accessibility assay. Cells treated or not with H₂O₂ were subjected

to MNase digestion. After DNA recovery and purification, amplification in qPCR conditions allowed us to verify regions that are resistant to MNase digestion. As observed for Hinf I protection (in Figure 3), also the accessibility profile to MNase changes after H₂O₂ treatment. In fact, as reported in Figure 4, MNase digestion after H₂O₂ treatment is less effective in the AP2 region for the WT strain, thus confirming that accessibility around the region occupied by +1 nucleosome is changed. When MNase-PCR assay was performed in the *gcn5Δ* strain, a different scenario emerged compared to that observed in WT strain (Figure 4). While WT cells increase the downstream protection of +1 nucleosome upon H₂O₂ treatment, *gcn5Δ* cells maintain at the region AP2 the same protection (Figure 4). This implies a failure of +1 nucleosome sliding in absence of Gcn5p compared to WT.

Thus, taken together, data reported in Figure 4 indicate that the +1 nucleosome remodeling upon H₂O₂ treatment occurs by moving the protection downstream the TSS in WT cells, while in absence of Gcn5p this remodeling does not significantly takes place.

DISCUSSION

Regulation of inducible genes such as *HSP12* is due to different interventions of regulator elements that allow the cell to respond to environmental challenges (Verghese et al., 2012).

Particularly, *HSP12* gene is activated by Msn2/4 factors and Hsf1p (Sadeh et al., 2011), that induce gene activation and chromatin rearrangements evidenced by an increase in chromatin acetylation and remodeling (Erkina and Erkin, 2006; Erkin et al., 2008; Erkin et al., 2010). The overlapping of different regulation levels, and the ability of different stimuli to induce this gene, prompted us

to analyze chromatin regulation when the cells are challenged by oxidative stress after H₂O₂ treatment since previous studies were performed by heat stimulation (Erkina and Erkin, 2006). H₂O₂ treatment provoked increased histone acetylation of both promoter and coding regions. This response was similar to that observed upon heat shock treatments. However, we showed that acetylation does not depend on the enzymes inhibited by SPD (Iki3p and Sas3p) (Eisenberg et al., 2009) and we unexpectedly found higher HSP12 transcription. In order to justify this enhanced transcriptional response we hypothesized that the SPD is converted to putrescine with H₂O₂ production after its acetylation by Paa1p. Interruption of this metabolic pathway, by *paa1Δ* mutation, reduced transcriptional response to SPD, thus suggesting the involvement of this pathway in H₂O₂ overproduction.

Since Gcn5p has been linked to oxidative stress response (Gaupel et al., 2015), we considered its possible involvement in the *HSP12* gene regulation. We hypothesized a *GCN5*-dependent *HSP12* regulation and studied *HSP12* induction in *gcn5Δ* cells upon H₂O₂ treatment. Although a reduced transcription was observed, acetylation levels measured in *gcn5* mutant cells remained high even after H₂O₂ exposure, thus suggesting a sharp distinction between *GCN5* involvement in regulating *HSP12* transcription and its HAT activity. Accordingly, recent work (Bruzzzone et al., 2018) has shown that yeast HATs play a profound redundant role on transcription. Gcn5p belongs to different regulatory transcriptional complexes (Grant et al., 1997) and its interactions with several chromatin remodeling complexes have been reported (Qiu et al., 2016). Chromatin remodeling is one of the most critical aspects of gene regulation and the role of +1 nucleosome (relative to TSS) in yeast is particularly relevant. Thus, we hypothesized that the role played by Gcn5p in *HSP12* activation could be not due to its HAT activity, but to the *GCN5*-dependent recruitment of chromatin remodeling complexes that facilitates transcription. In support of this hypothesis we

considered that: i) the Gcn5p bromo domain is able to bind acetylated chromatin (Owen et al., 2000) and ii) interactions between Gcn5p and chromatin remodeling complexes have been reported (Qui et al., 2016). The +1 nucleosome accessibility evaluation toward both HinfI restriction enzyme and MNase digestion showed changes in accessibility upon gene induction suggesting a sort of nucleosome-sliding far away from the TSS. The same assay performed in *gcn5Δ* mutant revealed failure of any sliding process. Thus only WT cells, but not those from the *gcn5Δ* mutant, respond to H₂O₂ treatment through a +1 nucleosome sliding thus implying some Gcn5p impact in the remodeling process.

In conclusion we confirm that H₂O₂ treatment induces *HSP12* transcription together with histone acetylation increase both at promoter and coding regions of *HSP12* and SPD treatment further stimulates transcription providing further oxidative stress. Moreover, we show for the first time that *GCN5* is necessary to reach the maximal *HSP12* gene induction. Unexpectedly the Gcn5p requirement is not due to its acetyltransferase activity, rather due to its ability to recruit chromatin remodeling complexes. Our observations also show the polyvalent regulatory nature of this evolutionarily conserved enzyme, whose activity and interlocutors require more detailed characterizations.

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Figure Legends

Figure 1 *HSP12* expression and histone acetylation after H_2O_2 treatment. A: *HSP12* mRNA produced by H_2O_2 treated (grey) or untreated (black) cells. Quantification is normalized to *UBC6* expression. Values with standard deviations from three different biological replicates. * $p < 0.05$; ** $p < 0.01$. B: ChIP analysis of H3 acetylation of *HSP12* promoter in H_2O_2 treated (grey) or untreated (black) cells. Enrichment values come from DNA recovered and amplified after immunoprecipitation against H3Ac compared to those of DNA recovered and amplified after immunoprecipitation against the unmodified H3 form (H3). C: as in B, ChIP analysis of H3 acetylation level in *HSP12* ORF region.

Figure 2 *HSP12* expression and histone acetylation after H_2O_2 treatment in WT and *gcn5Δ*. A:

HSP12 mRNA levels produced by $\pm H_2O_2$ treatment in WT or in *gcn5Δ*. Quantification is normalized to *UBC6* expression. Values with standard deviations from three different biological replicates.

* $p < 0.05$; ** $p < 0.01$. B: ChIP analysis of H3 acetylation of *HSP12* ORF regions in WT or *gcn5* cells

after $\pm H_2O_2$ treatment. Grey and black histograms refer to treated or untreated conditions

respectively. Enrichment values of DNA recovered and amplified after immunoprecipitation

against H3Ac are normalized to the values of DNA recovered and amplified after

immunoprecipitation against the unmodified H3 form (H3). C: as in C, analysis for promoter region

in WT and *gcn5* cells.

Figure 3 +1 nucleosome occupancy at *HSP12* gene. A: DNA resistant to in vivo Hinf1 restriction is

quantified after amplification at AP1 and AP2 regions (map positions relative to *HSP12* TSS are

reported). Histograms indicate amplification values of samples of treated (grey) or untreated

(black) cells. Schematic drawing under histograms indicated position and intensity of nucleosome

+1. Map of nucleosome occupancy according to (Celona et al., 2011) is also reported.

Figure 4: WT DNA resistant to in vivo extensive Mnase digestion is quantified after amplification at

AP2 region from WT (left) or *gcn5* cells(right). Histograms indicate amplification values of samples

treated (grey) or not (black) with H_2O_2 . Schematic drawing under histograms indicated position

and intensity of nucleosome +1.

Figure 1 HSP12 expression and histone acetylation after H₂O₂ treatment. A: HSP12 mRNA produced by H₂O₂ treated (grey) or untreated (black) cells. Quantification is normalized to UBC6 expression. Values with standard deviations from three different biological replicates. *p<0.05; **p<0.01. B: ChIP analysis of H3 acetylation of HSP12 promoter in H₂O₂ treated (grey) or untreated (black) cells. Enrichment values come from DNA recovered and amplified after immunoprecipitation against H3Ac compared to those of DNA recovered and amplified after immunoprecipitation against the unmodified H3 form (H3). C: as in B, ChIP analysis of H3 acetylation level in HSP12 ORF region.

457x257mm (236 x 236 DPI)

Figure 2 HSP12 expression and histone acetylation after H₂O₂ treatment in WT and *gcn5*Δ. A: HSP12 mRNA levels produced by ±H₂O₂ treatment in WT or in *gcn5*Δ. Quantification is normalized to UBC6 expression. Values with standard deviations from three different biological replicates. *p<0.05; **p<0.01. B: ChIP analysis of H3 acetylation of HSP12 ORF regions in WT or *gcn5* cells after ±H₂O₂ treatment. Grey and black histograms refer to treated or untreated conditions respectively. Enrichment values of DNA recovered and amplified after immunoprecipitation against H3Ac are normalized to the values of DNA recovered and amplified after immunoprecipitation against the unmodified H3 form (H3). C: as in C, analysis for promoter region in WT and *gcn5* cells.

457x257mm (236 x 236 DPI)

Figure 3 +1 nucleosome occupancy at HSP12 gene. A: DNA resistant to in vivo Hinf1 restriction is quantified after amplification at AP1 and AP2 regions (map positions relative to HSP12 TSS are reported). Histograms indicate amplification values of samples of treated (grey) or untreated (black) cells. Schematic drawing under histograms indicated position and intensity of nucleosome +1. Map of nucleosome occupancy according to (Celona et al., 2011) is also reported.

120x73mm (150 x 150 DPI)

Figure 4: WT DNA resistant to in vivo extensive Mnase digestion is quantified after amplification at AP2 region from WT (left) or *gcn5* cells(right). Histograms indicate amplification values of samples treated (grey) or not (black) with H₂O₂ . Schematic drawing under histograms indicated position and intensity of nucleosome +1.

254x143mm (236 x 236 DPI)