

# Gcn5 facilitates Pol II progression, rather than recruitment to nucleosome-depleted stress promoters, in *Schizosaccharomyces pombe*

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## ABSTRACT

In the fission yeast, the MAP kinase Sty1 and the transcription factor Atf1 regulate up to 400 genes in response to environmental signals, and both proteins have been shown to bind to their promoters in a stress-dependent manner. In a genetic search, we have isolated the histone H3 acetyltransferase Gcn5, a component of the SAGA complex, as being essential for oxidative stress survival and activation of those genes. Upon stress, Gcn5 is recruited to promoters and coding sequences of stress genes in a Sty1- and Atf1-dependent manner, causing both an enhanced acetylation of histone H3 and nucleosome eviction. Unexpectedly, recruitment of RNA polymerase II (Pol II) is not impaired in  $\Delta gcn5$  cells. We show here that stress genes display a 400-bp long nucleosome depleted region upstream of the transcription start site even prior to activation. Stress treatment does not alter promoter nucleosome architecture, but induces eviction of the downstream nucleosomes at stress genes, which is not observed in  $\Delta gcn5$  cells. We conclude that, while Pol II is recruited to nucleosome-free stress promoters in a transcription factor dependent manner, Gcn5 mediates eviction of nucleosomes positioned downstream of promoters, allowing efficient Pol II progression along the genes.

## INTRODUCTION

Cells have the capacity to adapt to external and harsh conditions. Microorganisms, which are more exposed to

the environment than animals, are particularly prone to induce massive changes on their gene expression patterns in an attempt to allow adaptation and/or survival. *Schizosaccharomyces pombe* has gene expression networks that show remarkable capability to adapt to a whole range of environmental changes [for reviews, see (1–3)]. In particular, it displays a large transcriptional response that is common to all or most stress conditions, and that is ruled by the MAP kinase Sty1/Spcl (4,5). Upon stress, Sty1 is phosphorylated and translocates to the nucleus, where it regulates transcription of many genes through the b-ZIP transcription factor Atf1 (4,6–8). The core environmental stress response (CESR) includes 140 genes upregulated by at least 2-fold by four out of five types of stresses, many of which depend on Sty1 and, to a lesser extent, on Atf1 (9,10). However, the effectors of Sty1/Atf1 in transcriptional activation of the CESR genes are unknown. Post-translational modification of the histone protein components of eukaryotic chromatin is a key player in the regulation of gene expression, both through remodeling of the chromatin structure and by sequential recruitment of the numerous components of transcription initiation and elongation [for a review, see (11)].

One of the main complexes displaying histone acetyltransferase (HAT) activity in *S. pombe*, *S. cerevisiae* and mammalian cells is the structurally conserved SAGA complex, which contains up to 20 subunits, including the HAT Gcn5 (12). SAGA and related complexes can regulate an integrated set of multiple histone modifications, counteracting repressive effects that alter chromatin and regulate gene expression, since it contains at least Ubp8, which deubiquitinates H2B as a triggering step to trimethylate lysine 4 of H3 (counteracting the repressive

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dimethylation mark) and acetylation of histone H3 (mediated by Gcn5) (13,14). The functions of SAGA in transcriptional activation (both at initiation and elongation) have been widely studied in *S. cerevisiae* (15,16). In this yeast, SAGA was originally characterized as a co-activator which favored recruitment of TATA binding protein (TBP) and Pol II for transcriptional activation by Gal4 (17–20). Not all SAGA subunits are essential for transcription initiation in all genes assayed. Thus, among the SAGA dependent genes (21), some require Gcn5 for induction (i.e. *HO*, *VTC3*, *PHO84*) (22,23) but some do not (i.e. *GAL1*, *AHD1*, *BDF2*, *PDR5*) (18,23–25); in the first set of genes, Gcn5 is absolutely required for the recruitment of TBP to promoters (18,19). In *S. pombe*, Gcn5 has already been related to the transcriptional stress program (12,26,27), but its precise role in transcription regulation of the CESR genes has not been established.

The relationship between transcriptional regulators, histone modifiers, transcriptional activity and nucleosome positioning is starting to be unravelled with genome-wide studies on several eukaryotes. Recent genome-wide studies have revealed different nucleosome footprints over eukaryotic genomes (28–31). Taking all genes on average, each gene shows a nucleosome depleted region (NDR) of around 150 bp upstream of the transcriptional start site (TSS), with nucleosomes evenly positioned downstream. Even though a trend of gene averages may be useful for some general features of nucleosome positioning, it does not necessarily provide reliable predictions on nucleosome density and gene expression, or on particular gene-by-gene features. Several of these nucleosome positioning studies have been performed in *S. cerevisiae*, where genome-wide expression data are already available. These studies in yeast indicate that nucleosome occupancy, especially at promoters, inversely correlates with gene activity: genes with NDRs upstream of the TSS display high gene expression at the experimental conditions, while repressed genes have nucleosomes positioned at their promoters as well as in their open reading frames (ORFs) (32). For instance, stress genes in yeast display on average uniform nucleosome positioning along promoter and ORF, while ribosomal and organelle organization (housekeeping) genes, which are being expressed during normal mitotic growth, display promoter NDRs (29). Furthermore, a connection between nucleosome positioning patterns and transcriptional plasticity (i.e. capacity to modulate gene expression upon environmental changes) has been established, so that high plasticity genes display evenly distributed and dynamic nucleosomes close to the TSS (33). Lastly, comparative nucleosome maps between two experimental conditions with clear changes in the global transcriptional program have been performed, with the general conclusion that there are NDRs present at promoters that become active under one of the conditions assayed (34). However, these studies also have revealed that gene expression and nucleosome density relationship does not apply to all genes in the yeast genome, but to a vast majority of them (35,36).

We aimed to investigate the role of Styl and Atf1 on CESR activation. We screened for deletion mutants with

altered sensitivity to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Cells lacking Gcn5 display strong sensitivity to stress conditions and severely impaired transcriptional activation of CESR genes. Lack of Gcn5 abolishes the H<sub>2</sub>O<sub>2</sub>-dependent acetylation of histone H3 at CESR genes, as well as nucleosome eviction. Surprisingly, stress-dependent Pol II recruitment at promoters is not affected by the lack of Gcn5, but promoter escape is. Concomitantly, all CESR promoters we have analyzed are devoid of nucleosomes under basal conditions, which explains why Pol II recruitment is independent of histone modifiers. The +1 nucleosome, downstream of the TSS, is evicted upon inducing conditions in a Gcn5-dependent manner. In conclusion, the deficient acetylation of histones in cells lacking Gcn5 does not affect Pol II entry but rather Pol II progression along genes.

## MATERIALS AND METHODS

### Yeast strains and growth conditions

We used the wild-type *S. pombe* strains 972 (*h*<sup>-</sup>) and 975 (*h*<sup>+</sup>) (37) and mutants thereof. The origins and genotypes of strains used in this study are outlined in Table 1. Cells were grown in rich medium or in synthetic minimal medium as described previously (37).

### RNA analysis

Total RNA from *S. pombe* rich media cultures was obtained, processed and transferred to membranes as described previously (38). Membranes were hybridized with the [ $\alpha$ -<sup>32</sup>P]dATP-labelled *gpd1*, *ctt1*, *hsp9* or *srx1* probes, containing the complete ORFs, of the glycerol-3-phosphate dehydrogenase-, catalase-, heat shock protein 9- and sulfiredoxin-coding genes.

**Table 1.** Strains used in this study

Strain	Genotype	Origin
972	<i>h</i> <sup>-</sup>	(54)
AV18	<i>sty1::kanMX6 h</i> <sup>-</sup>	(55)
HMP47	<i>gcn5-HA::ura4<sup>+</sup> leu1-32 ura4-D18 ade6<sup>-</sup> h</i> <sup>-</sup>	(56)
393	<i>tbp1-HA::kanMX6 leu1-32 ura4-D18 ade6<sup>-</sup> h</i> <sup>-</sup>	(57)
$\Delta$ hfi1	<i>hfi1::kanMX6 leu1-32 ura4-D18 ade6<sup>-</sup> h</i> <sup>+</sup>	(58)
$\Delta$ spt20	<i>spt20::kanMX6 leu1-32 ura4-D18 ade6<sup>-</sup> h</i> <sup>+</sup>	(58)
$\Delta$ spt17	<i>spt17::kanMX6 leu1-32 ura4-D18 ade6<sup>-</sup> h</i> <sup>+</sup>	(58)
HU799	<i>gcn5::KanMX6 leu1-32 ura4-D18 ade6-M216 h</i> <sup>-</sup>	(26)
CN011	<i>rpb1-HA::kanMX6 h</i> <sup>+</sup>	This work
CN009	<i>rpb1-HA::kanMX6 sty1-1 leu1-32 ura4-D18 h</i> <sup>-</sup>	This work
MS52	<i>rpb1::HA::natR gcn5::kanMX4 leu1-32 ura4-D18 h</i> <sup>-</sup>	This work
MS112	<i>gcn5::kanMX6 h</i> <sup>-</sup>	This work
MS161	<i>gcn5::natMX6 h</i> <sup>+</sup>	This work
MS176	<i>gcn5-HA::ura4 atf1::natMX6 leu1-32 ura4-D18 ade6<sup>-</sup> h</i> <sup>-</sup>	This work
MS183	<i>ada2::kanMX6 h</i> <sup>-</sup>	This work
MS184	<i>spt8::kanMX6 h</i> <sup>-</sup>	This work
MS194	<i>tbp1-HA::kanMX6 gcn5::natMX6 h</i> <sup>-</sup>	This work
MS208	<i>gcn5-HA::ura4 sty1::natMX6 leu1-32 ura4-D18 ade6<sup>-</sup> h</i> <sup>-</sup>	This work

### H<sub>2</sub>O<sub>2</sub> sensitivity assay

For survival on solid plates, *S. pombe* strains were grown, diluted and spotted in YE5S media agar plates as described previously (39), containing or not 3 or 5 mM H<sub>2</sub>O<sub>2</sub>.

### Preparation of *S. pombe* TCA extracts and immunoblot analysis

To analyze the *in vivo* acetylation state of total histone H3, modified trichloroacetic acid (TCA) extracts were prepared as previously described (39). Immunoblotting was performed using commercial polyclonal anti-H3AcK9/14 (Upstate, ref. 06-599) or anti-H3 (Ab1791 Abcam) antibodies.

### Chromatin immunoprecipitation

Fifty milliliters of cells at an OD<sub>600</sub> of 0.5 per sample were cross-linked adding 1% formaldehyde for 20 min at 25°C; cross-linking was stopped with 125 mM glycine. Cell pellets, washed twice with PBS, were resuspended in 0.25 ml of breaking buffer (0.1 M Tris pH 8.0; 2% glycerol and 1 mM PMSF) and lysed with a bead beater. Pellets were washed twice with lysis buffer (50 mM HEPES-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 1 mM PMSF) and resuspended in 0.25 ml lysis buffer. Lysates were sonicated in a Bioruptor (Diagenode), yielding chromatin fragments of 500-bp average size. Lysis buffer was added up to 1 ml, and samples were centrifuged at 16 000g for 30 min at 4°C. Fifty microliter of the soluble chromatin were kept as input, while the rest was immunoprecipitated with specific antibodies [5 µl of anti-HA antiserum (12CA5), 1 µl of anti-phospho Ser5 CTD of Pol II (Ab5131 Abcam), 1 µl of anti-phospho Ser2 CTD of Pol II (Ab5395 Abcam), 1 µl of anti-H3 polyclonal antibody (Ab1791 Abcam) or 1 µl of anti-acetylated H3K9/14 (06-599; Upstate)]. At the same time, 10 µl of protein G-Sepharose beads (Amersham) were added and incubation proceeded rotating overnight at 4°C. Beads were washed once in lysis buffer, twice in lysis buffer containing 0.5 M NaCl, twice in washing buffer (10 mM Tris pH 8.0, 0.25 M LiCl, 0.5% NP-40, 0.5% sodium deoxycholate, 1 mM EDTA and 1 mM PMSF) and once in TE (10 mM Tris pH 8.0, 1 mM EDTA). DNA was eluted by incubation of the beads for 20 min at 65°C with 0.1 ml elution buffer (50 mM Tris pH 7.5, 10 mM EDTA pH 8.0, 1% SDS), and further incubation with 0.15 ml of TE with 0.67% SDS. Elution supernatants were pooled, and formaldehyde cross-linking was reversed by overnight incubation at 65°C. Proteins were digested by incubation for 2 h at 37°C with 0.3 mg/ml proteinase K and 0.04 mg/ml glycogen. One hundred and twenty-five millimolar NaCl was added, chromatin was purified by phenol/chloroform extraction and precipitated with 1 ml of ethanol for 30 min at -20°C. DNA was ethanol precipitated, air-dried and resuspended in 0.1 ml of TE buffer. Recovered DNA was amplified by quantitative real time PCR using SYBR Green kit and Light cycler 480 (Roche). The specific primers, amplifying promoter, ORF or terminal region, corresponded to the

following positions with respect to the translation initiation sites: -420 to -316, +483 to +580 and +1455 to +1555 of the *gpd1* gene; -506 to -403, +832 to +930 and +1804 to +1906 of the *ctt1* gene; -291 to -190 of the *hsp9* gene; -359 to -258 of the *srx1* gene. Control primers, spanning an intergenic region of *S. pombe* chromosome I (position 465226 to 465326) were also used. Results were expressed as a percentage of the input. The error bars (SEM) were calculated from biological triplicates or duplicates, unless indicated.

### Identification of nucleosome depleted regions (NDR) of CESR genes

Raw data of microarray hybridization with mononucleosome DNA reported by Lantermann *et al.* (40) was taken from the Gene Expression Omnibus (GSE16040). Affymetrix GeneChip *S. pombe* Tiling 1.0FR array probes were mapped to the Sanger Centre genome sequence annotation of 17 July 2009, and probes matching more than one genomic location were removed (41). Nucleosome occupancy was calculated as the log<sub>2</sub> ratio of mononucleosome to the naked genomic DNA signal after quantile normalization (42). The resulting output was denoised by Multilevel 1-D discrete Meyer wavelet decomposition using the Matlab Wavelet Toolbox.

### Purification of mononucleosomal DNA

Wild-type (972) and *Agcn5* (HU799) strains were cultured in 500 ml of YE5S medium to an OD<sub>600</sub> of 0.5, and were treated or not with 1 mM H<sub>2</sub>O<sub>2</sub> for 2 min. Mononucleosomes were obtained as described before (40) with some modifications. Cell wall digestion was performed with 37.5 mg of zymolyase 20T (ICN Biochemicals), and incubation time for *Agcn5* strain was reduced to 20 min at 30°C.

### Nucleosome-scanning analysis

Three hundred nanograms of purified mononucleosomal and genomic DNA (obtained from the same protocol as above but without MNase added) were analyzed by qPCR. Two sets of 15 (for *ctt1*) or 12 (for *gpd1*) overlapping primer pairs were designed using Primer3 which tiled 1.15 kb of the *ctt1* gene or 1.0 kb of the *gpd1* gene. Each primer pair amplified a 100 ± 32 bp PCR product that overlapped with the neighbouring primer pair 11–46 bp. The specific primers amplifying the *ctt1* gene corresponded to the following sequences with respect to the TSS (+1): -495 to -395, -426 to -328, -349 to -247, -277 to -181, -199 to -105, -131 to -39, -106 to +5, -17 to +92, +67 to +191, +180 to +281, +259 to +370, +352 to +462, +444 to +536, +491 to +590 and +548 to +650. Regarding *gpd1*, the specific primers corresponded to the following sequences: -700 to -601, -636 to -536, -556 to -421, -437 to -337, -356 to -225, -292 to -194, -209 to 105, -129 to -31, -52 to +45, +10 to +105, +92 to +206, +199 to +297. PCR efficiency for each primer pair was calculated with serial dilutions of template DNA (from 10<sup>-1</sup> to 10<sup>-4</sup>).



## RESULTS

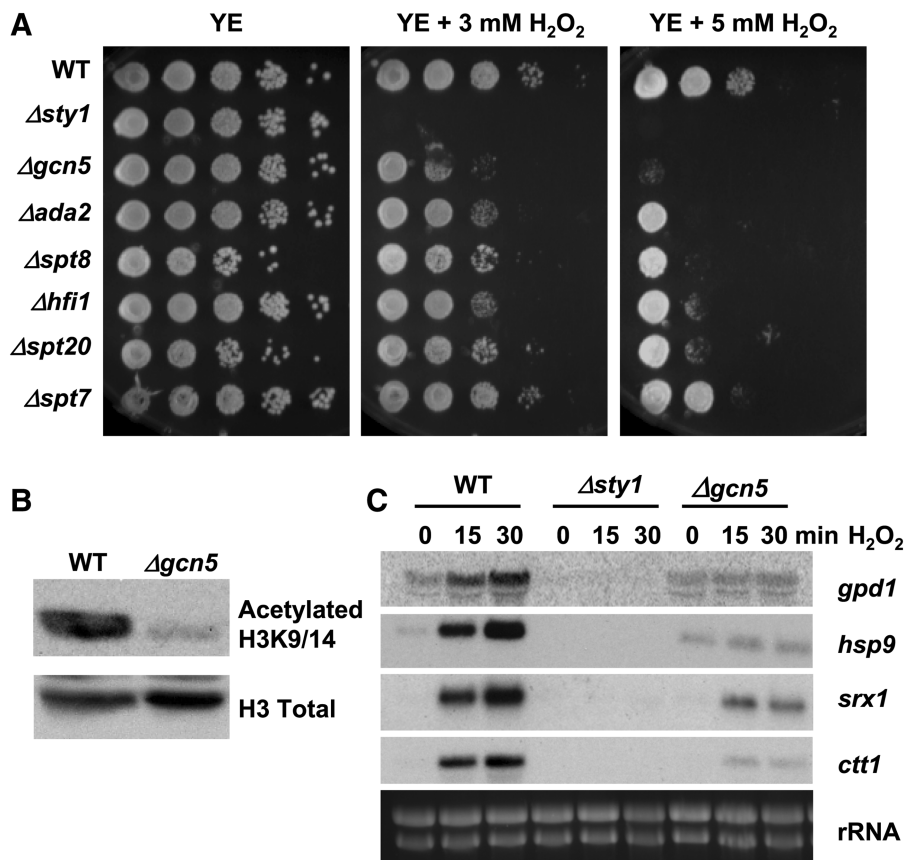
### Gcn5 is required for the transcriptional induction of CESR genes

To test whether the Sty1 MAPK pathway regulates transcription by affecting chromatin organization, we screened for mutants sensitive to environmental stresses on the basis that mutations in chromatin-modifying activities required to mediate massive changes in gene expression should result in cells displaying stress sensitivity. We analyzed a collection of *S. pombe* deletion strains, paying close attention to those mutants with altered histone acetylation activities, and consistently isolated *Δgcn5* strain as impaired in survival against H<sub>2</sub>O<sub>2</sub> on solid plates (Figure 1A). Gcn5 is one of the main components of the SAGA complex. The integrity of the complex is required for normal sensitivity to oxidative stress, since cells lacking the SAGA components Ada2, Spt8, Ada1/Hfi1, Spt20 or Spt7 also displayed sensitivity to peroxides (Figure 1A). In budding yeast, Spt7, Hfi1 and Spt20 are essential for complex assembly. *Schizosaccharomyces pombe* cells lacking Spt7 displayed the weakest sensitivity

phenotype, but the mutant expresses a 160 amino acids long NTD of the protein. The phenotype of strain *Δspt8* was also weak, confirming the opposing role of Spt8 to that of Gcn5 (12). We confirmed that cells lacking Gcn5 had reduced total levels of H3 acetylation at K9 and K14 (Figure 1B), as recently reported (43). As shown in Figure 1C, strain *Δgcn5* is severely defective in CESR gene induction. Therefore, histone acetylation seemed to strongly regulate tolerance to environmental threats, such as oxidative stress, by regulating transcription of CESR genes.

### Gcn5 promotes histone acetylation and nucleosome eviction at stress genes upon treatment

To test whether Gcn5 has to be physically associated to CESR genes to modulate its induction, we performed chromatin immuno-precipitation (ChIP) experiments and found Gcn5 to be located at promoters (Figure 2A, left panel) and also, although to a lesser extent, at ORFs of stress genes (Figure 2A, right panel and Supplementary Figure S1A and B). The protein was present at CESR genes under basal conditions but it further accumulated



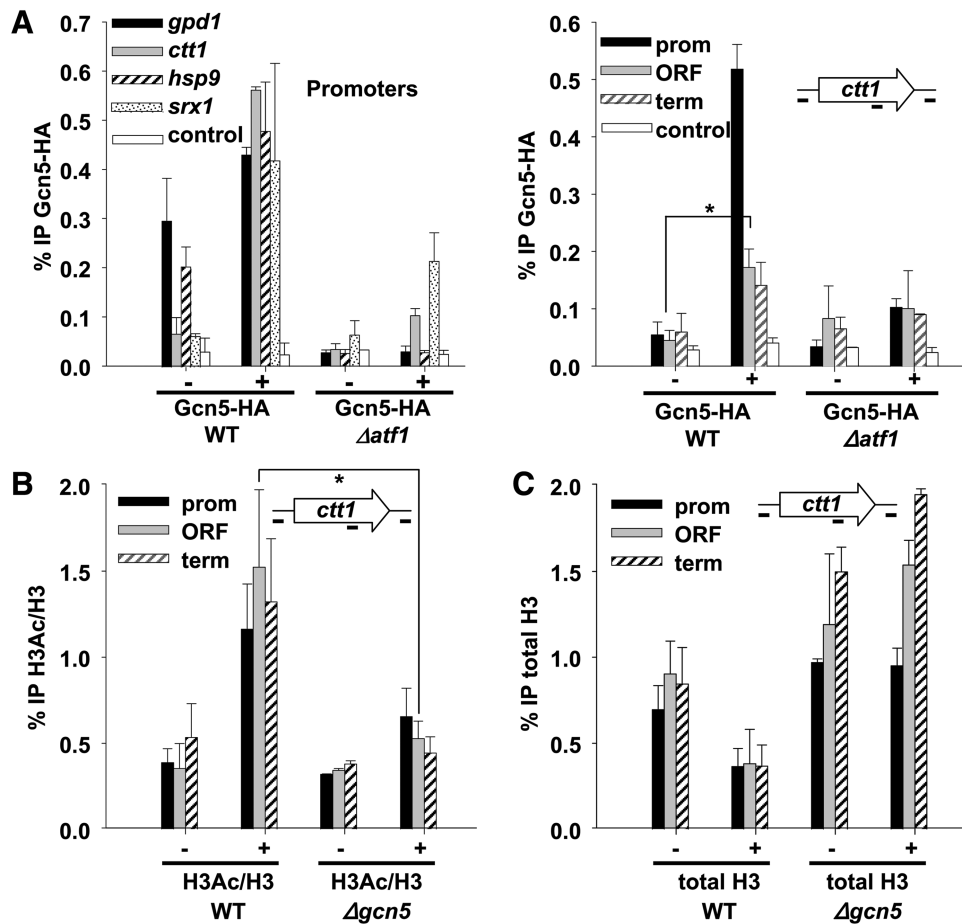
**Figure 1.** Gcn5 is essential for the activation of the Sty1-, Atf1-dependent stress response. (A) *Δgcn5* strain and other mutants of the SAGA complex are sensitive to oxidative stress. Serial dilutions from cultures of strains 972 (WT), AV18 (*Δsty1*), MS161 (*Δgcn5*), MS183 (*Δada2*), MS184 (*Δspt8*), *Δhfi1*, *Δspt20* and *Δspt7* were spotted onto rich plates with (YE + 3 mM H<sub>2</sub>O<sub>2</sub>; YE + 5 mM H<sub>2</sub>O<sub>2</sub>) or without (YE) the indicated concentrations of H<sub>2</sub>O<sub>2</sub>. (B) Total levels of histone H3 acetylation at lysines 9 and 14 depend on Gcn5. Protein extracts from strains 972 (WT) and MS112 (*Δgcn5*) were analyzed by western blot with antibodies against acetylated H3K9/14 or total H3, as a loading control. (C) Stress-dependent transcriptional analysis of wild-type and *Δgcn5* cells. Cultures of strains 972 (WT), AV18 (*Δsty1*) and MS112 (*Δgcn5*) were treated with 1 mM H<sub>2</sub>O<sub>2</sub> for the indicated times. Total RNA was analyzed by northern blot with probes for *gpd1*, *ctt1*, *hsp9*, or *srx1*. rRNA is shown as a loading control.

upon H<sub>2</sub>O<sub>2</sub> stress, both in an Atf1- (Figure 2A and Supplementary Figure S1B) and Sty1- (Supplementary Figure S1A) depending manner. We then tested whether the pro-activating effects of Gcn5 over CESR gene transcription were concomitant to its HAT activity. To do so, we performed ChIP analysis of total and acetylated histone H3. Since the size of sheared chromatin in ChIP experiments is around 500-bp long, only relative zonal changes (i.e. before and after stress) can be detected with this experimental approach (NDRs smaller than 500 bp would not be evidenced). Indeed, the levels of acetylated H3, as determined by the ratio of acetylated H3 per total histone H3, were significantly enhanced upon stress in a Gcn5-dependent manner (Figure 2B for *ctt1* and Supplementary Figure S1C for *gpd1*). As a result, a

decrease in total histone H3 levels was detected after stress, which was also totally dependent on the presence of Gcn5 (Figure 2C for *ctt1* and Supplementary Figure S1D for *gpd1*). Therefore, histone acetylation by Gcn5 promotes chromatin remodeling at CESR genes, favoring nucleosome eviction.

**Pol II progression along stress genes is impaired in cells lacking Gcn5**

An obvious consequence of the absence of chromatin remodeling in cells lacking Gcn5 could be an impaired recruitment of Pol II at promoters. Unexpectedly, Pol II binding to CESR promoters, which is Sty1 dependent (Supplementary Figure S2A), was barely affected by



**Figure 2.** Gcn5 is recruited to stress genes and promotes histone H3 acetylation and nucleosome eviction. (A) Gcn5 physically binds to stress promoters in an Atf1-dependent manner. Cultures of strains HMP47 (*gcn5-HA* WT) and MS176 (*gcn5-HA Δatf1*) were treated (+) or not (-) with 1mM H<sub>2</sub>O<sub>2</sub> for 15 min. ChIP experiments using anti-HA antibodies, coupled to quantification by real-time PCR, were performed using primers covering only promoter regions (*gpd1*, *ctt1*, *hsp9* and *srx1* promoters; left panel), or promoter (prom), coding (ORF) and termination (term) sequences of the *ctt1* gene (right panel). Primers of an intergenic region were used as a negative control (control). Error bars (SEM) for all ChIP experiments were calculated from biological triplicates. Significant difference between Gcn5-HA binding to ORF before and after stress in wild-type cells was determined by the Student's *t*-test (\**P* < 0.05). (B) Gcn5 promotes histone H3 acetylation upon stress imposition in promoters and coding region of stress-dependent genes. Cultures of strains 972 (WT) and MS112 (*Δgcn5*) were treated (+) or not (-) with 1 mM H<sub>2</sub>O<sub>2</sub> for 15 min. ChIP assays were performed using antibodies specific for acetylated lysines 9 and 14 of histone H3 (H3Ac) or against unmodified C-terminal domain of H3 (H3), as described in A. The percentage of immunoprecipitation of acetylated H3 versus total H3 is indicated (% IP H3Ac/H3). Significant difference between wild-type versus *Δgcn5* cells at ORF upon stress was determined by the Student's *t*-test (\**P* < 0.05). (C) Gcn5 promotes a decrease in H3 levels as an indicator of nucleosome eviction at stress genes. The same experiment as in Figure 2B is represented here as the percentage of immunoprecipitation of total H3 (% IP total H3).

the lack of Gcn5, as determined by ChIP of the TATA binding protein Tbp1-HA (Figure 3A) or the large Pol II subunit, Rpb1-HA (Supplementary Figure S2B). We then analyzed whether Pol II positioning along the CESR genes, which is also Sty1 dependent (Supplementary Figure S2C), was affected by the absence of Gcn5, both in *ctt1* (Figure 3B) and in *gpd1* (Supplementary Figure S2D) genes. Contrary to what we observed at promoters, we detected a significant defect on Pol II binding at ORFs and termination sites, which could be indicative of defects on Pol II progression along the genes. To further detect problems in transcription dynamics, we measured phosphorylation of Pol II CTD at Ser5 and Ser2. Indeed, we could detect accumulation of Ser5 phosphorylation mainly at promoters upon stress both in wild-type and a *gcn5* deletion (Figure 3E for promoters, Figure 3C for *ctt1* and Supplementary Figure S2E for *gpd1*). However, the presence of phosphorylated Ser2 at ORFs and termination sites was impaired in cells lacking Gcn5 (Figure 3D for *ctt1* and Supplementary Figure S2F for *gpd1*). Our results suggest that promoter escape, but not pre-initiation complex formation, is the main transcriptional event regulated by Gcn5.

In order to pinpoint the defects on Pol II progression of  $\Delta gcn5$  cells, we performed time course experiments coupled with total Pol II occupancy at promoter and coding regions. Our goal was to test whether Pol II recruitment and progression occurred with similar kinetics and to a similar extent in wild-type and  $\Delta gcn5$  cells, analyzing *ctt1* and *gpd1* gene occupancy at shorter time points, and obtaining a snap-shot of the first waves of transcription after H<sub>2</sub>O<sub>2</sub> stress (0–900 s, Figure 4, and 0–75 s, Supplementary Figure S3). Confirming our hypothesis, Pol II recruitment at promoters had a very similar pattern of induction in both strains ('prom' bars, Figure 4 and Supplementary Figure S3). However, detection of Pol II in coding and termination sequences ('ORF' and 'ter' bars) was dramatically delayed in cells lacking Gcn5, and confirmed the impaired progression of Pol II into the *ctt1* and *gpd1* transcribed regions in  $\Delta gcn5$  cells.

#### The promoters of stress genes are depleted of nucleosomes prior to stress

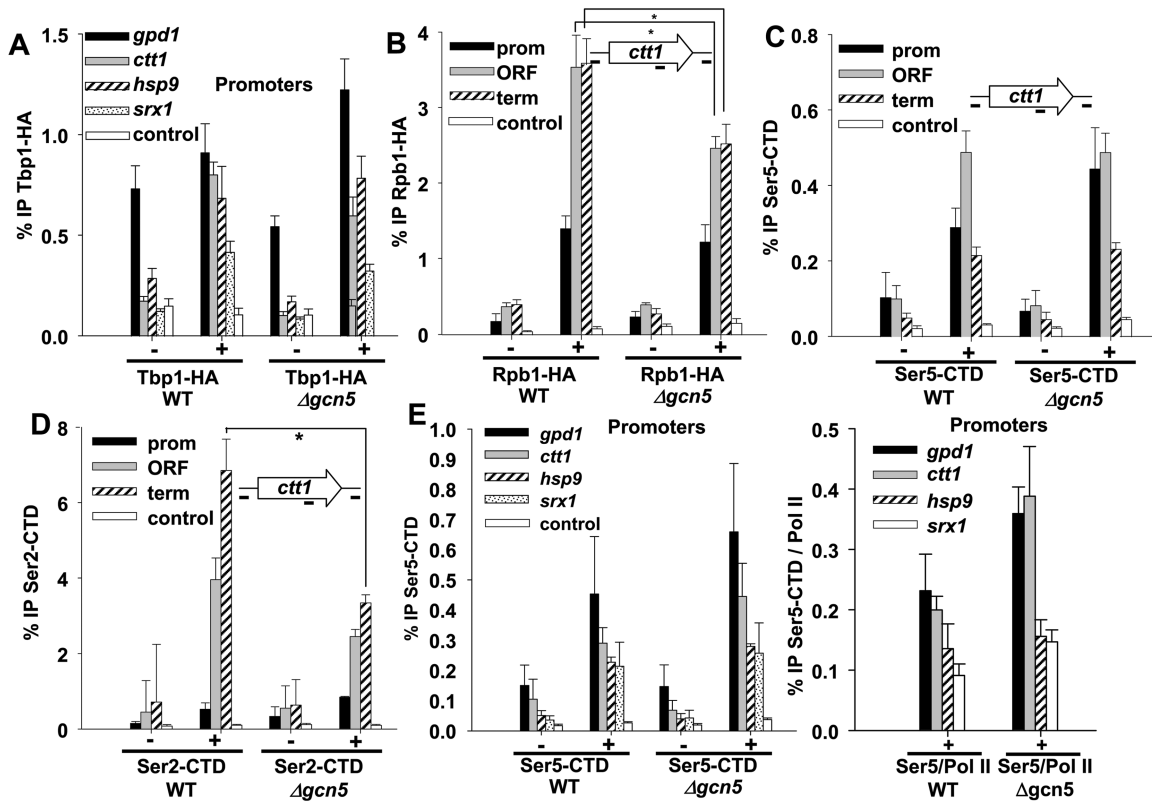
The genome-wide nucleosome map of *S. pombe* has been recently reported (40). As previously described for promoters in *S. cerevisiae*, *Drosophila* and human cells, the average nucleosome occupancy displays a pronounced NDR of 150–200 bp upstream of the TSS, with a standard nucleosomal distribution downstream of the TSS. However, this average distribution includes both genes that are actively transcribed and those which are silent. In general, high expression correlates with open promoters, that is, with lower promoter nucleosome occupancy (40). Under the experimental conditions used in the nucleosome mapping report, the CESR genes are not actively transcribed (see Figure 1C, time 0 of WT). Unexpectedly, all the Sty1-, Atf1-dependent genes, we have analyzed have a pronounced and large NDR (290–490 bp) upstream of the TSS, even under un-induced

conditions (Figure 5A). To investigate whether this was a general situation of other stress response genes, we analyzed the nucleosome profile prior to the addition of H<sub>2</sub>O<sub>2</sub> of the 50 genes showing the highest overexpression under oxidative stress (10,41) using microarray data (40). We found that 40 out of the 50 genes analyzed showed a large NDR immediately upstream from their ORFs. The average nucleosome profile of 35 out of these 40 genes for which the TSS had been determined, reveals the presence of a NDR ~400 bp long (Figure 5B) very similar to that of the five individual genes shown in Figure 5A.

We decided to determine the position of nucleosomes at the *ctt1* and *gpd1* genes before and after stress, isolating mono-nucleosomes from fission yeast cultures and PCR-amplifying them with overlapping pairs of primers covering a 1.15 and 1.0 kb regions of the promoters and coding sequences of the *ctt1* and *gpd1* genes, respectively, as described elsewhere (35). This technique, based on PCR-amplification of previously isolated nucleosomal DNA, would help us circumvent the resolution limitations of the ChIP technique, which does not allow differentiating changes in histone modifications at near-by positions. As shown in Figure 6, we confirmed the presence of a 340-bp and 490-bp long NDRs just upstream of the *ctt1* and *gpd1* TSS prior to stress imposition, confirming the data from the genome-wide nucleosome mapping (40). A first nucleosome was positioned just downstream of the TSS of both genes, which we called +1. Upon stress, this +1 nucleosome, as well as the +2 and +3 nucleosomes, displayed diminished amplitude—reflection of a weaker DNA-histone core association—than under resting conditions (Figure 6A and B, compare WT with WT + H<sub>2</sub>O<sub>2</sub>), an indication of their transient dissociation due to Gcn5-dependent acetylation of histones and polymerase passage. The –1 nucleosome, upstream of the promoter, also displayed partial eviction, which may be due to Gcn5 and Pol II acting bidirectionally. This decreased amplitude—weaker DNA-nucleosome association—was not observed in cells lacking Gcn5 (Figure 6A and B, compare WT + H<sub>2</sub>O<sub>2</sub> with  $\Delta gcn5$  + H<sub>2</sub>O<sub>2</sub>). On the contrary, stronger association of DNA to the +1 nucleosome was detected after stress in  $\Delta gcn5$  cells at both genes; histone deacetylase Clr3, which is also recruited to stress genes upon induction, could be responsible for this effect. Furthermore, cells lacking Gcn5 have a displaced +1 nucleosome prior to stress compared to wild-type cells, what brought closer the +1 and +2 nucleosomes; the significance of this shift is unknown. In conclusion, the lack of nucleosomes at the promoter regions of stress genes prior to induction explains the dispensability for Gcn5 function to recruit Pol II, whereas the absence of +1 nucleosome–DNA dissociation in cells lacking Gcn5 justifies the detected Pol II progression problems of this strain.

#### DISCUSSION

Gene induction is the main strategy followed by unicellular eukaryotes to adapt to changing environments. We have shown here that upon oxidative stress, the MAP



**Figure 3.** Lack of Gcn5 barely affects pre-initiation complex formation at stress promoters, but severely impairs Pol II progression. (A) Pol II recruitment to promoters is not defective in cells lacking Gcn5. Strains 393 (*tbp1-HA* WT) and MS194 (*tbp1-HA Δgcn5*), expressing Tbp1-HA, were cultured and treated (+) or not (-) with 1 mM H<sub>2</sub>O<sub>2</sub> for 15 min. ChIP experiments were performed as in Figure 2A using primers of promoter regions. (B) Pol II recruitment to coding sequences is significantly affected in cells lacking Gcn5. Strains CN011 (*rpb1-HA* WT) and MS52 (*rpb1-HA Δgcn5*) were cultured and treated (+) or not (-) with 1 mM H<sub>2</sub>O<sub>2</sub> for 15 min. ChIP experiments were performed as described in Figure 2A. Significant differences between wild-type versus *Δgcn5* cells upon stress at ORF and termination (term) sites were determined from six biological replicates by the Student's *t*-test (\**P* < 0.05). (C) Phosphorylation at Ser5 of the CTD of the large Pol II subunit Rpb1. Strains 972 (WT) and MS161 (*Δgcn5*) were cultured and treated (+) or not (-) with 1 mM H<sub>2</sub>O<sub>2</sub> for 15 min. ChIP experiments with specific antibodies for phosphorylated Ser5 (Ser5-CTD) were performed as described in Figure 2A. (D) Phosphorylation at Ser2 of the CTD of the large Pol II subunit Rpb1. Strains 972 (WT) and MS112 (*Δgcn5*) were cultured and treated (+) or not (-) with 1 mM H<sub>2</sub>O<sub>2</sub> for 15 min. ChIP experiments with specific antibodies for phosphorylated Ser2 (Ser2-CTD) were performed as described in Figure 2A. Significant difference between wild-type versus *Δgcn5* cells at terminator (term) sites upon stress was determined by the Student's *t*-test (\**P* < 0.05). (E) Phosphorylation at Ser5 of the CTD of the large Pol II subunit Rpb1. Strains and conditions as in (C), but analysing Ser5 occupancy at promoters (left panel). Right panel: The same experiment as in left panel is represented here as the percentage of immuno-precipitation of phosphorylated Rpb1-HA at Ser5 versus total Rpb1-HA (% IP Ser5-CTD/Pol II).

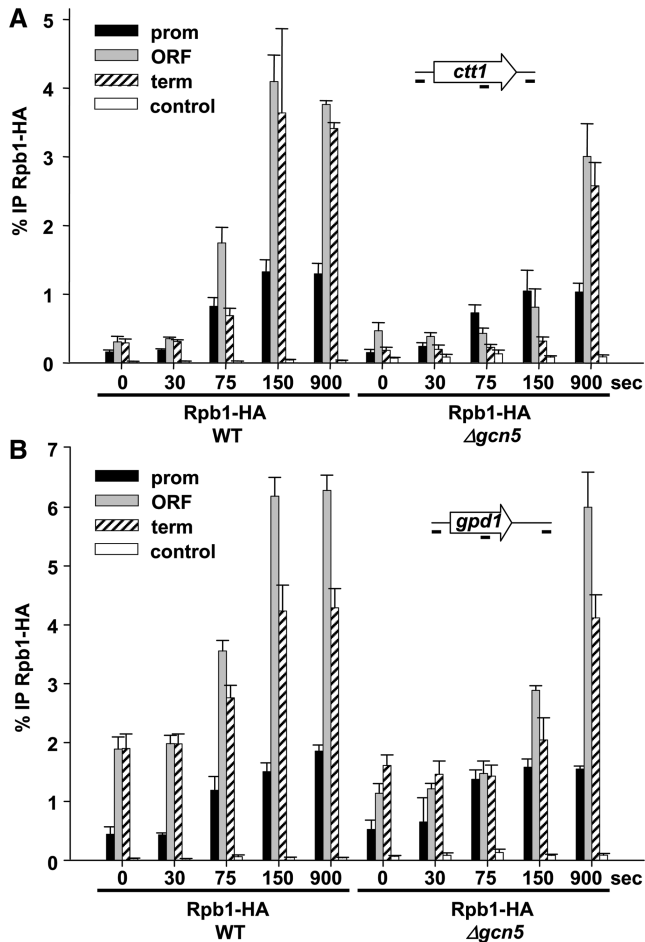
kinase Sty1 and its transcription factor Atf1 trigger the binding of Gcn5 and Pol II to stress promoters. We have also demonstrated that Gcn5 has a role in histone H3 acetylation and nucleosome eviction at stress genes. As previously suggested before for other environmental stresses (26,27,43,44), Gcn5 is essential for wild-type tolerance to oxidative stress.

Upon stress, two clear events occur at CESR genes: Gcn5 recruitment (Figure 2A) and Pol II loading (Figure 3A and B). Gcn5 is probably recruited to promoters by Atf1, and migration of the HAT to the ORFs could be linked to Pol II elongation. However, the polymerase binds to DNA in cells lacking Gcn5 in a stress-dependent manner (Figure 3A). The fact that the stress promoters are free of nucleosomes (Figure 5) pushes us to hypothesize that the absence of Pol II binding to promoters under basal conditions is not a consequence of chromatin barriers, but rather of the low

affinity for its binding site(s); the presence of the Sty1-Atf1 couple would be required to promote further stabilization of the transcriptional machinery.

In *S. cerevisiae*, up to 10% of the genome depend on the SAGA complex for expression, as determined by analysis of the transcriptome of strains lacking Spt20, a SAGA subunit essential for the stability of the complex (21). Many of these genes, however, do not require Gcn5 for expression (21), including *GALI*, one of the most studied SAGA-dependent genes; accordingly, recruitment of TBP or Pol II to *GALI* promoter is unaffected by the absence of Gcn5 (18). Regarding the Gcn5-dependent genes, Pol II access to promoters is impaired in the absence of the HAT, contrary to what we have observed in *S. pombe* CESR genes. The completely different promoter nucleosome architecture of Gcn5-dependent genes of budding and fission yeast explains this apparently contradictory observation (Figure 7) (45): only *S. pombe*

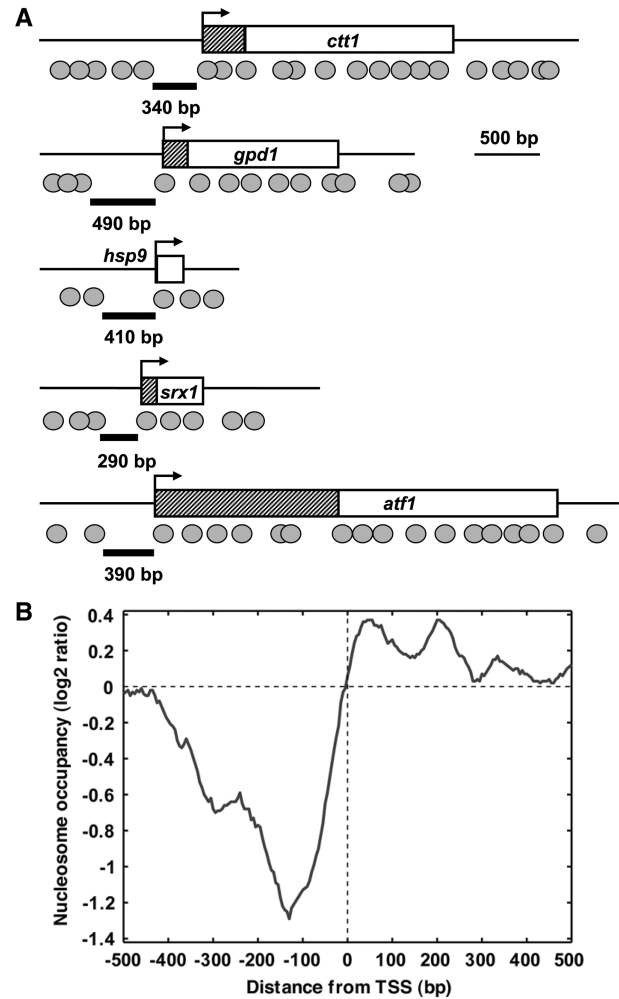




**Figure 4.** Short-time kinetics of Pol II progression along the *ctt1* (A) and *gpd1* (B) genes: Gcn5 mediates promoter escape. Strains CN011 (*rpb1-HA*) and MS52 (*rpb1-HA*  $\Delta gcn5$ ), expressing the large Pol II subunit Rpb1-HA, were cultured and treated with 1mM  $H_2O_2$  for the time points in seconds indicated in the figure. ChIP experiments with anti-HA antibodies were performed as described in Figure 2A.

Gcn5-dependent genes are devoid of nucleosomes upstream of the TSS under basal conditions.

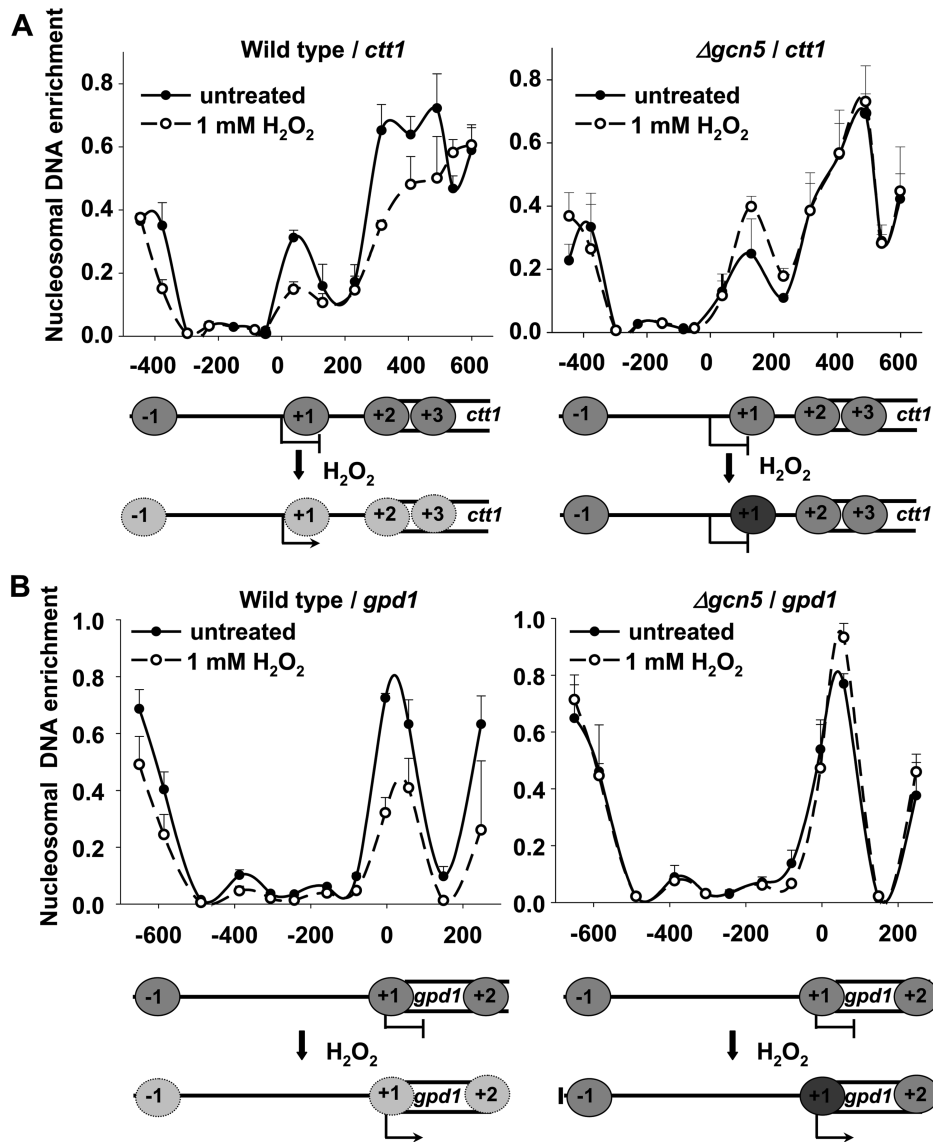
Several genome-wide studies in eukaryotes have revealed different nucleosome scenarios for promoters, ORFs and intergenic regions (see 'Introduction' section). In those reports, gene averages are often displayed, showing differences among eukaryotic organisms. However, these gene averages may lead to confusion when tried to be applied to a particular subset of genes. Yeast constitutes an excellent model to study at a gene-to-gene basis the relationship between nucleosome architecture, RNA polymerase II activity and the role of histone modifiers. With our studies, we have evidenced the presence in fission yeast of a group of high plasticity genes, whose expression dramatically change upon environmental stresses, and which display a wide and profound NDR under non-induced conditions. Pol II requires the presence of the transcription factor, Atf1, to gain access to the promoters of these genes. However, histone acetylation is not required for this purpose, because there is no competition between



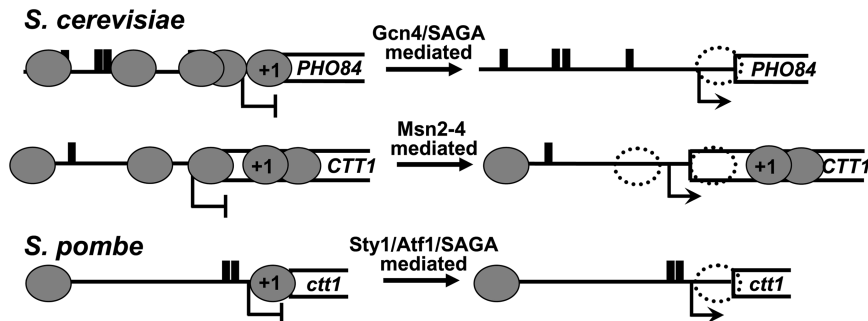
**Figure 5.** Promoter nucleosome signature at CESR genes. (A) Large nucleosome depleted regions in five *S. pombe* CESR promoters prior to gene activation. Nucleosome localization across genomic regions encompassing CESR genes *gpd1*, *ctt1*, *hsp9*, *srx1* and *atf1*. Stripped and white rectangles represent untranslated and ORFs, respectively. Transcription start sites (black arrows) and the size of NDRs (thick bars) are indicated. Nucleosomes are represented by gray ovals. Data have been taken from a genome-wide nucleosome positioning analysis (40), and from published transcription start sites (50). (B) Overlay of nucleosome occupancy profiles for 35 out of the 50 genes showing the highest overexpression under oxidative stress (Supplementary Table S1). Nucleosome profiles for individual genes were determined in the absence of  $H_2O_2$ . Tiling microarray signals along the genomic region  $-500$  to  $+500$  bp relative to the TSS for each gene (40) were interpolated prior to averaging each position of the 35 genes. Nucleosome occupancy indicates the log<sub>2</sub> ratio of mononucleosome to naked genomic DNA signal.

nucleosomes and the transcriptional machinery to gain access to DNA. We suggest that this mode of regulation may be used for other eukaryotic model systems with a much lower ratio of coding versus non-coding DNA, such as *Drosophila* and human genomes. On one hand, it may be useful to have open NDRs preceding each gene to facilitate Pol II recognition of entry sites, since only a small percentage of the genome has to be transcribed. On the other hand, there are increasing evidences of post-recruitment mechanisms, such as the modulation of





**Figure 6.** Nucleosome-scanning analysis of CCSR genes shows Gcn5 dependent +1 nucleosome eviction after oxidative stress. Mononucleosomes were isolated from cultures of strains 972 (wild type, left panel) and HU799 (*Δgcn5*, right panel) before (closed circles, continuous lines) and after (open circles, dashed lines) 2 min with 1 mM H<sub>2</sub>O<sub>2</sub>. qPCR was performed using 15 overlapping primer pairs along 1.15 or 1.0 kb, covering the promoter, the TSS (black arrow) and coding region (white rectangle) of the *ctt1* (A) or *gpd1* (B) genes, respectively. Error bars (SEM) were calculated from biological triplicates (A) or duplicates (B). Nucleosomes are represented as gray ovals, where colour intensities are proportional to occupancy levels.



**Figure 7.** Scheme depicting the role of Gcn5 in transcription promotion in both yeasts. In *S. cerevisiae*, Gcn5 is required to promote signal-dependent nucleosome eviction at the *PHO84* promoter (40). SAGA is also recruited to the *CTT1* promoter upon heat shock, and changes in its nucleosomal architecture also occur (51–53). On the contrary, the *S. pombe* Gcn5-dependent CCSR genes (such as *ctt1*) are devoid of nucleosomes under basal conditions, and therefore, only eviction of downstream nucleosomes is required upon signal activation.

paused Pol II at promoters, as largely contributing to eukaryotic regulation of gene expression [for reviews, see (46–48)]. In fact, it has been reported that up to 20% of all *Drosophila* genes displayed signatures of paused Pol II (46), and that a large fraction of human genes, estimated to be around 50%, showed paused Pol II at inactive genes using genome-wide ChIP analyses (49).

## SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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