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Roles for Gcn5p and Ada2p in transcription and nucleotide excision repair at the *Saccharomyces cerevisiae MET16* gene

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ABSTRACT

Chromatin structure, transcription and repair of cyclobutane pyrimidine dimers at the *MET16* gene of wild type, *gcn5Δ* and *ada2Δ Saccharomyces cerevisiae* cells were studied under repressing or derepressing conditions. These two components of the SAGA/ADA chromatin remodelling complexes are expendable for the basal transcription of *MET16* but are mandatory for its full transcription induction. Despite their influence on transcription neither protein induces major changes in *MET16* chromatin structure, but some minor ones occur. Repair at the coding region of the transcribed strand is faster than repair at non-transcribed regions in all strains and either growth condition. Moreover, the more *MET16* is transcribed the faster the repair. The data show that by changing the transcription extent the rate of repair at each DNA strand is altered in a different way, confirming that repair at this locus is strongly modulated by its chromatin structure and transcription level. Deletion of *GCN5* or *ADA2* reduces repair at *MET16*. The results are discussed in light of the current understanding of Gcn5p and Ada2p functions, and they are the first to report a role for Ada2p in the nucleotide excision repair of the regulatory and transcribed regions of a gene.

INTRODUCTION

The genomic DNA in eukaryotes is wrapped around octamers of histone proteins forming the nucleosomes, the first order of chromatin organization. Additionally, the arrays of nucleosomes are further organized into higher-order chromatin structures with increasing levels of complexity (1). This compact state makes the DNA less accessible, hence the activity of important cellular processes including transcription, replication and DNA repair is influenced (2–4).

Eukaryotic nucleotide excision repair (NER) is a complex mechanism that requires over 30 proteins to remove DNA damage from naked DNA, let alone chromatin. Two pathways for NER exist. It is well documented that the transcribed strand (TS) of transcriptionally active genes is repaired more rapidly than non-transcribed sequences; this is termed transcription coupled repair (TCR) [reviewed in (5)]. In the yeast *Saccharomyces cerevisiae*, TCR has been shown to operate via two alternative sub-pathways mediated by Rad26p and the RNA polymerase-II Rpb9 subunit, respectively (6). On the other hand, global genome repair operates on non-transcribed regions and strands and is slower than TCR.

More recently there is evidence from a number of laboratories that the non-transcribed regions, especially those of the upstream regulatory sequences, are also faster repaired when genes are transcriptionally active. Here at the *MET17* gene chromatin structure was thought to be an influential factor (7). Concordantly, the structure of the nucleosome has been shown to modulate the rate of NER in the non-transcribed strand (NTS) of the *URA3* gene of *S.cerevisiae* (8–9). Furthermore, the wrapping of DNA around a nucleosome has been shown to inhibit the efficiency of NER *in vitro* (10–11).

Eukaryotic cells regulate the accessibility to nucleosomal DNA by using an intricate group of ATP-dependent remodelling complexes and DNA-binding proteins as well as several factors that covalently modify the histone proteins, including histone acetyltransferases (HATs), deacetylases, phosphorylases or methyltransferases (12–13). Several studies have shown that some proteins belonging to these groups, like Swi/Snf, Gcn5p or Cbf1p, influence the rate of repair *in vivo* and *in vitro* (7,14–17).

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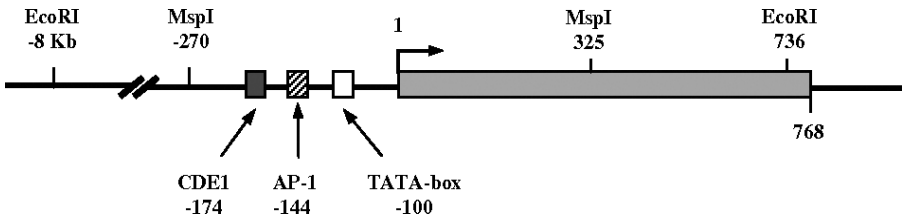

Figure 1. The *MET16* gene structure. The three regulatory elements CDE1, AP-1 and TATA-box, and relevant restriction enzymes sites are shown. Positions are indicated in relation to the first codon of the protein.

Among the different HATs present in *S.cerevisiae* Gcn5p is one of the best documented (18). This protein was initially identified as a transcriptional activator required to promote maximum transcription levels of genes dependent on the general transcription factor Gcn4p (19). In yeast, Gcn5p forms part of at least three chromatin acetylating complexes, the ADA, SAGA and SLIK complexes (20–21). Another component of the ADA and SAGA complexes, the transcriptional adaptor Ada2p, interacts with Gcn5p (22–23) and the acidic activation domain of Gcn4p (24). Ada2p is required to recruit the TATA-box-binding protein to Gcn5p-dependent promoters (25).

The *MET16* gene of *S.cerevisiae* encodes the enzyme 3′-phospho 5′-adenylylsulfate reductase of the methionine biosynthetic pathway (26). Its level of transcription is low at 0.3–0.7 transcripts per cell when methionine is available [(27); Mark Gerstein's Lab website, bioinfo.mbb.yale.edu]. *MET16* is mainly regulated by a methionine specific pathway (28) which depends on the binding of a complex of Cbf1p, Met28p and Met4p to the CDE1 site (Figure 1). We have shown that repair of cyclobutane pyrimidine dimers (CPDs) by NER at *MET16* is affected by both its chromatin structure and its transcription level (16). In that report we focused on how the Cbf1p chromatin-binding factor influenced transcription, chromatin structure and repair in the upstream regulatory region and the beginning of the coding region of *MET16*.

MET16 transcription is also regulated by the general control of amino acids (29) that relies on the binding of Gcn4p to the AP-1 site (Figure 1), although it requires Cbf1p to be fully functional (28). Here we have taken advantage of the dependence on Gcn4p for full transcription of the *MET16* gene to further study how transcription, nucleosome positions and the NER of CPDs are influenced by two proteins involved in chromatin remodelling, namely Gcn5p and Ada2p, that interact to promote transcription as described above. Events were studied in both strands of the *MET16* promoter and transcribed regions in relation to the transcriptional activity of *MET16* (repressed and derepressed). This has facilitated comparisons between the modulation of chromatin structure and how they impinge on NER.

MATERIALS AND METHODS

Yeast strains, growing conditions and UV irradiation

Cells from the haploid isogenic strains of *S.cerevisiae*, **PSY316**: (MATα, leu2-3, leu2-112, ura3-52, ade2-101, Δhis3-200, lys2, trp1), **PSY316-gcn5Δ**: (MATα, leu2-3, leu2-112, ura3-52, ade2-101, Δhis3-200, lys2, trp1, gcn5Δ) and PSY316-ada2Δ: (MATα, leu2-3, leu2-112, ura3-52, ade2-101, Δhis3-200, lys2, trp1, ada2Δ) were grown at 30°C in complete (YPD) medium to early logarithmic phase. *MET16* repression and derepression were achieved by growing the cells for 2 h in minimal medium supplemented with either 1 mM or 10 μM methionine, respectively, plus the other required amino acids. Cells were treated with 150 J/m^2 of UVC-light and aliquots were allowed to repair the damage for a period of 1–4 h in the same conditioning medium (17). The determination of the UV sensitivity of the three strains was undertaken as described previously (30).

DNA isolation and NER quantification

The genomic DNA was isolated from untreated cells, and from cells treated with UV-light and allowed to repair or not as described previously (17,30). The rate of CPD removal by NER at the MspI restriction fragment of *MET16* (Figure 1) was determined at nucleotide resolution. MspI digestion, *Micrococcus luteus* CPD-endonuclease treatment, single strand DNA isolation and 3′ end [^{32}P]dATP labelling were carried out as described previously (17). The individual DNA fragments corresponding to strands cut with the CPD endonuclease were resolved by electrophoresis in denaturing 6% polyacrylamide gels and the signal was quantified using ImageQuant 5.0 software after scanning in a Storm 860 Phosphorimager (Molecular Dynamics). Pyrimidine tracts and groups of bands too close to be individually determined were quantified as a single band. The rate of repair at each CPD position was calculated as the $T_{50\%}$ value; i.e. the time required to repair 50% of the lesions present immediately after treatment (31). $T_{50\%}$ values higher than the 4 h repair interval used in these experiments were extrapolated up to 10 h. The initial level of damage induced by the UV-treatment was estimated as the percentage of radioactivity present in the bands corresponding to damaged DNA in relation to the sum of all the fragments, damaged plus undamaged, in the 0-sample.

The repair data shown in Results correspond to the average of at least three different experiments. The statistical comparison among strains of the rate of repair of the group of CPDs present in the same region was carried out by the Student's *t*-test for matched-paired samples (H0, mean value of the differences is zero). While the statistical comparison of the repair rate of the group of CPDs present in different DNA regions in each yeast strain was carried out by the Mann–Whitney test (H0, both samples are taken from populations with identical median values).

Transcription

MET16 transcription was determined by northern blot and hybridization with probes specific for *MET16* and *ACT1*. After northern blotting, the *MET16* mRNA level was normalized against the *ACT1* level and the values in the wild type strain after growth in YPD medium were used as a reference (17).

Nucleosome mapping

The nucleosomes at the *MET16* locus were mapped by Micrococcal Nuclease (MNase) digestion of permeabilized yeast cells as described previously (17,32). Briefly, 1.8×10^9 cells from each strain were grown as above. Cells were harvested, permeabilized with zymolyase and digested with 5, 10 and 20 U of MNase for 4 min at 37°C. 'Naked' DNA samples were prepared from 9×10^8 cells treated as above, but with two phenol/chloroform extractions and an ammonium acetate/isopropanol precipitation before the MNase treatment. Samples of purified naked and chromatin genomic DNA were digested to completion with EcoRI and analysed by Southern blot with a double strand probe for the MscI–EcoRI restriction fragment (+412 to +736 nt).

Primers used for DNA labelling and RNA probe preparation

The isolation of single strand DNA corresponding to the MspI fragment of *MET16* and its 3′ end [^{32}P]dATP labelling was carried out with the following primers: MspI-A: 5′-Biotin-*GATAGCTTTTTTTTT*-GGTGGACATCACCTATT-GATTCTAAAT-3′ for the TS and MspI-B: 5′-Biotin-*GATAGCTTTTTT*-GCTTATATACGTGAATGGTTTGATT-TTTAG-3′ for the NTS, as described previously (33). Sequences in italic correspond to overhang modifications. The single-strand DNA probe specific for the *MET16* mRNA was prepared from PCR products obtained with MspI-A and MspI-C (5′-*CATCCG*-GCTTATATACGT-GAATGGTTTGATTTTTAG-3′) primers. Probes for *ACT1* were similarly prepared using the following primers: 5′-Biotin-GCCGGTTTTGCCGGTGACG-3′ and 5′-CCGG-CAGATTCCAAACCCAAAA-3′.

RESULTS

MET16 transcription

The extent of *MET16* transcription was determined in wild type, *gcn5Δ* and *ada2Δ* cells grown in complete medium, derepressing and repressing methionine conditions. In complete medium *MET16* is transcribed at similar levels in the three strains (Figure 2, closed columns). Conversely, *MET16* transcription is highly and differently affected by growth in derepressing conditions (Figure 2, grey columns); its transcription is induced 100-fold in the wild type, while in *gcn5Δ* and *ada2Δ* cells its induction is severely diminished (10- and 6-fold induction, respectively). In repressing methionine conditions *MET16* transcription is intermediate between that in YPD and derepressing conditions (Figure 2, open columns). Here, transcription of *MET16* in both mutant strains is similar, namely 2.5- to 3-fold lower than in the wild type.

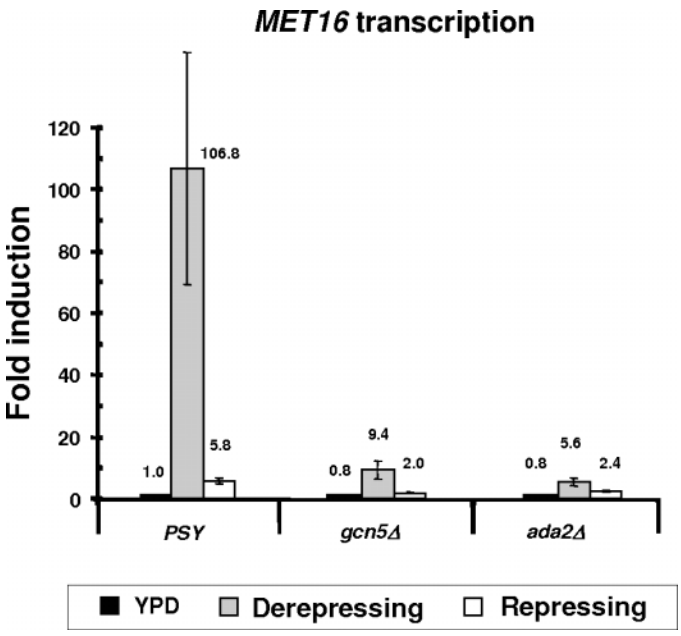

Figure 2. *MET16* transcription in *PSY*, *gcn5Δ* and *ada2Δ* cells following growth for 2 h in minimal medium with transcription derepressing (grey columns) and repressing (open columns) concentrations of methionine, as well as in YPD medium (closed columns). *MET16* transcription induction is shown relative to the level detected in *PSY* cells grown in YPD medium (1×). Values have been corrected for differences in the loading by using an *ACT1* probe. Data for each strain and growth condition correspond to the average from at least three different experiments.

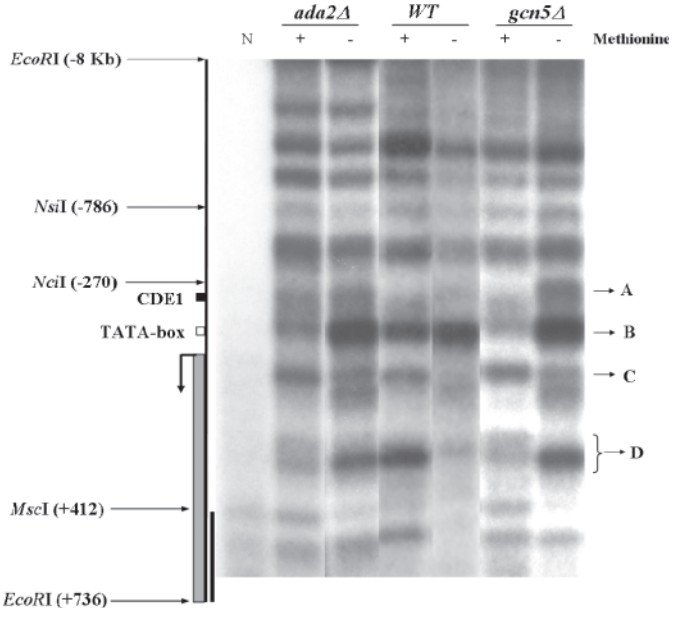

Figure 3. MNase digestion pattern of the ≈9 kb EcoRI restriction fragment including *MET16* of naked (N lane) and chromatin DNA isolated from the three strains grown for 2 h in minimal medium with transcription derepressing (10 μM, −methionine) and repressing (1 mM, +methionine) concentrations of methionine. Indicated are specific bands/regions as mentioned in the text. Relevant locus components and restriction sites are shown on the left. The black line between the MscI and EcoRI restriction sites corresponds to the probe used for the Southern blot.

Chromatin structure at the *MET16* locus

The position of the nucleosomes at the *MET16* locus was determined in the three strains and both transcription conditions by MNase digestion of the 9 kb (–8 kb to +736) EcoRI restriction fragment that includes this gene (Figure 1).

As shown in Figure 3, the pattern of bands obtained by MNase digestion is relatively similar in all strains irrespective of transcription conditions, which indicate that the nucleosomes at the *MET16* locus occupy equivalent positions. Since these MNase sensitive regions are located at exactly the same regions as in the two yeast strains analysed in our previous work (17), the three strains employed here will also have two nucleosomes positioned around the *MET16* start of transcription; i.e. the first one (nucleosome −1) between the CDE1 site and the start of the coding region (Figure 3, bands A and C) covering the TATA-box region, and the second one (nucleosome +1) inside the transcribed region between bands C and D (Figure 3).

In spite of the general similarities between the three strains after MNase mapping, two small differences dependent on the growth condition are detected. In the three strains the band B located over the TATA-box is more intense in derepressing than in repressing conditions and second, there are doublets of bands at the band C region in derepressing conditions instead of the single one present in repressing conditions.

Finally, the group of bands D present inside the coding region shows differences between strains and growth conditions as it is resolved as a single or double MNase cleavage site and with low or high intensity.

UV sensitivity in the *gcn5Δ* and *ada2Δ* mutants

Genes involved in DNA repair have been traditionally detected by the sensitivity of their mutants to UV light or chemical agents. We examined the UV sensitivity of wild type, *gcn5Δ* and *ada2Δ* cells grown in YPD medium. Deletion of either *GCN5* or *ADA2* in the strain used here does not markedly affect the sensitivity to UV-light (data not shown).

CPD repair in derepressing conditions

Figure 4 shows typical DNA repair sequencing gels for the three strains and both transcription conditions of the *MET16* TS and NTS. CPD induction in the three strains and after the two growth conditions at the MspI restriction fragment of *MET16* is similar in both DNA strands. With respect to all experiments performed, the TS of this fragment has 15–20% of the molecules damaged whereas the NTS has 14–17%.

In the wild type in derepressing conditions the rate of repair at the coding region of the TS (downstream of −38 nucleotide position) is relatively fast, with an average $T_{50\%}$ of 2.1 h, and homogeneous (Figure 5A, top). In contrast, repair in the

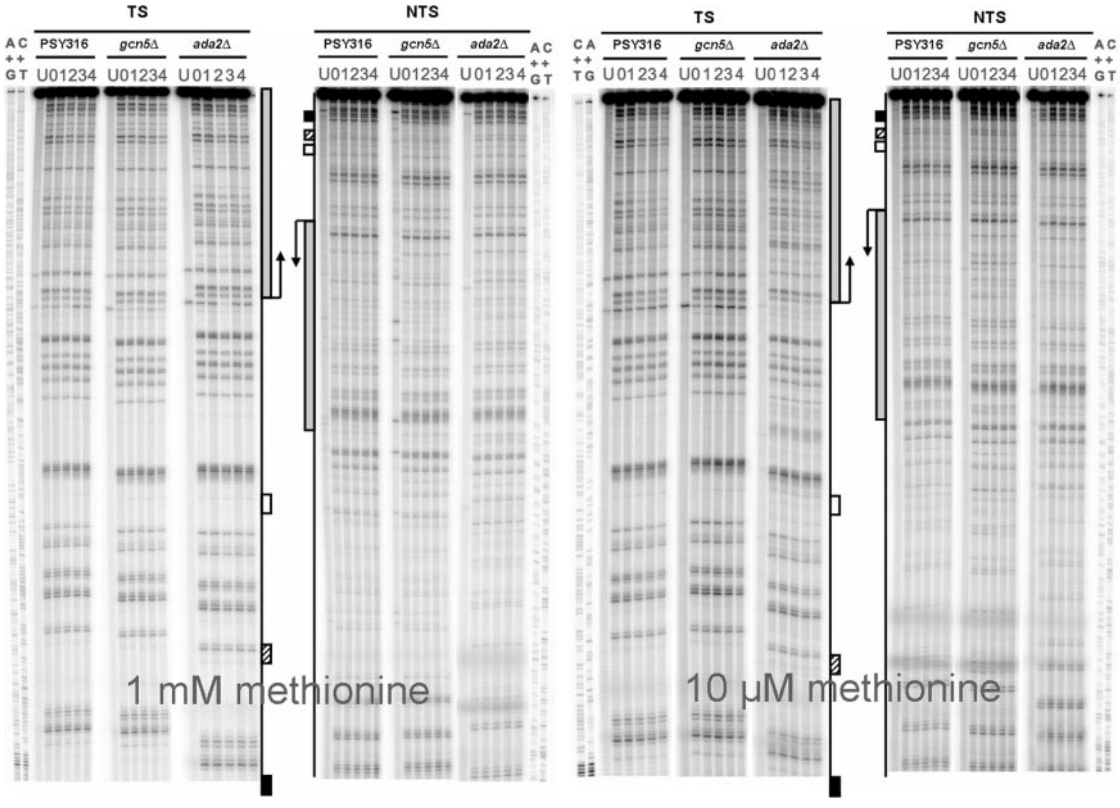

Figure 4. Representative autoradiographs at nucleotide resolution showing the repair of CPDs in the TS and NTS of the MspI fragment of *MET16* in wild type, *gcn5Δ* and *ada2Δ* cells grown in 1 mM (repressing) and 10 μM methionine conditions (derepressing). Sanger A+G and T+C sequencing ladders are included to determine the position of the CPDs induced. U, untreated cells; 0, cells treated with UV light no repair; (1–4), UV-treated cells allowed to repair the damage for 1–4 h. The intense top band corresponds to the undamaged MspI fragment of *MET16*, the bands below represent DNA fragments with a CPD lesion that was cut by the CPD-specific endonuclease. Cbf1p-binding site (closed box), Gcn4p-binding site (stripped box), TATA-box (open box) and coding region (grey box). *Note*: differences in band migration at the bottom of the autoradiographs are due to the fact that *ada2Δ* samples were run in different gels to PSY and *gcn5Δ*.

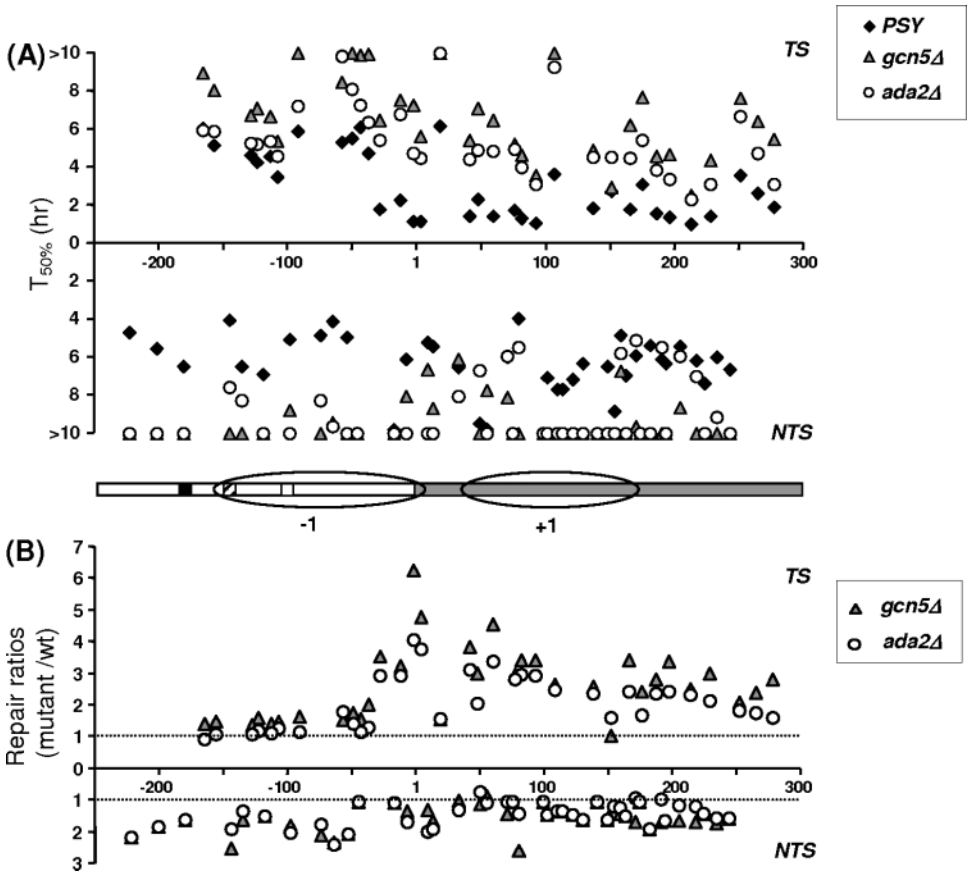

Figure 5. (**A**) Repair of individual CPDs at *MET16* versus the nucleotide position in derepressing conditions. The rate of repair in *PSY* (diamonds), *gcn5Δ* (triangles) and *ada2Δ* cells (circles) is expressed as the $T_{50\%}$ for CPDs in the TS (top) and NTS (bottom). Position 1 corresponds to the start of the coding region. Cbf1p-binding site (closed box), Gcn4p-binding site (stripped box), TATA-box (open box) and coding region (grey box). Approximate nucleosome positions are shown as transparent ovals. (**B**) Relative ratios of CPD repair between *gcn5Δ* (triangles) and *ada2Δ* (circles) versus *PSY* cells in the *MET16* TS and NTS. Values above 1 indicate faster repair in the wild type strain and those below 1 faster repair in the mutant strain.

promoter region of the TS and along the whole NTS is more variable (Figure 5A, bottom), being comparatively slow ($P > 0.001$), with an average $T_{50\%}$ of 5.2 and 6.7 h, respectively.

In comparison, the two mutant strains show less, but statistically significant ($P > 0.01$), differences in repair between the CPDs at the coding and promoter regions of the TS, i.e. 1.3- and 1.4-fold faster repair in the coding region of the *ada2Δ* and *gcn5Δ* strains than in their respective promoter regions. Similarly, repair along the NTS is 1.8- and 1.6-fold, respectively, less efficient than in the coding region of the TS, ($P > 0.001$).

Figure 5 shows that, in general, both Gcn5 and Ada2 proteins are required for the efficient removal of CPDs from both the *MET16* TS and NTS. When compared with the wild type, repair of the group of CPDs within the coding region of the TS (Figure 5A and B, top) is severely impaired in *gcn5Δ* and *ada2Δ* cells (2.8- and 2.3-fold, respectively, $P > 0.001$), whereas repair in the promoter region of this strand is moderately delayed (1.6- and 1.3-fold slower repair on average, $P \geqslant 0.01$). Moreover, repair in this strand is more efficient in *ada2Δ* than in *gcn5Δ* cells ($P \geqslant 0.01$). CPD removal in the two mutant strains is also delayed in the NTS (Figure 5A and B, bottom), $P > 0.001$. Here, the differences in repair are slightly greater in the promoter (1.8- and 1.7-fold, see Figure 5B, bottom) than in the coding region (1.4- and 1.3-fold). In contrast to the TS, repair in the NTS of *gcn5Δ* and *ada2Δ* cells is statistically homogeneous.

CPD repair in repressing conditions

When *MET16* transcription is repressed, the three strains show similar differences between repair at the coding and the respective promoter region of the TS; i.e. 1.3- to 1.5-fold faster repair in the coding region, $P \geqslant 0.05$ (Figure 6A, top). At the NTS, repair in the three strains is 1.5- to 2.1-fold less efficient than in the respective coding region of the TS ($P > 0.001$).

The repair trends along the NTS in the three strains exhibit a wave pattern supported by best fitness examination using curve analysis software (CurveExpert). There are three regions of fast repair, the Gcn4p-binding site, the first 30–70 nt of the coding region and from +170 to +225 nt positions (Figure 6A, bottom). Repair at the TS follows a similar although less obvious pattern. Since NER is less efficient at the centre of the nucleosomes cores, the data for this strand indirectly indicate nucleosomes cores can be assigned approximately to the regions between −100 to −25 nt positions, between +100 to +125 nt positions and a third nucleosome core would possibly be located over the CPD at +225 position (Figure 6A). These regions are coincident with the corresponding ones in

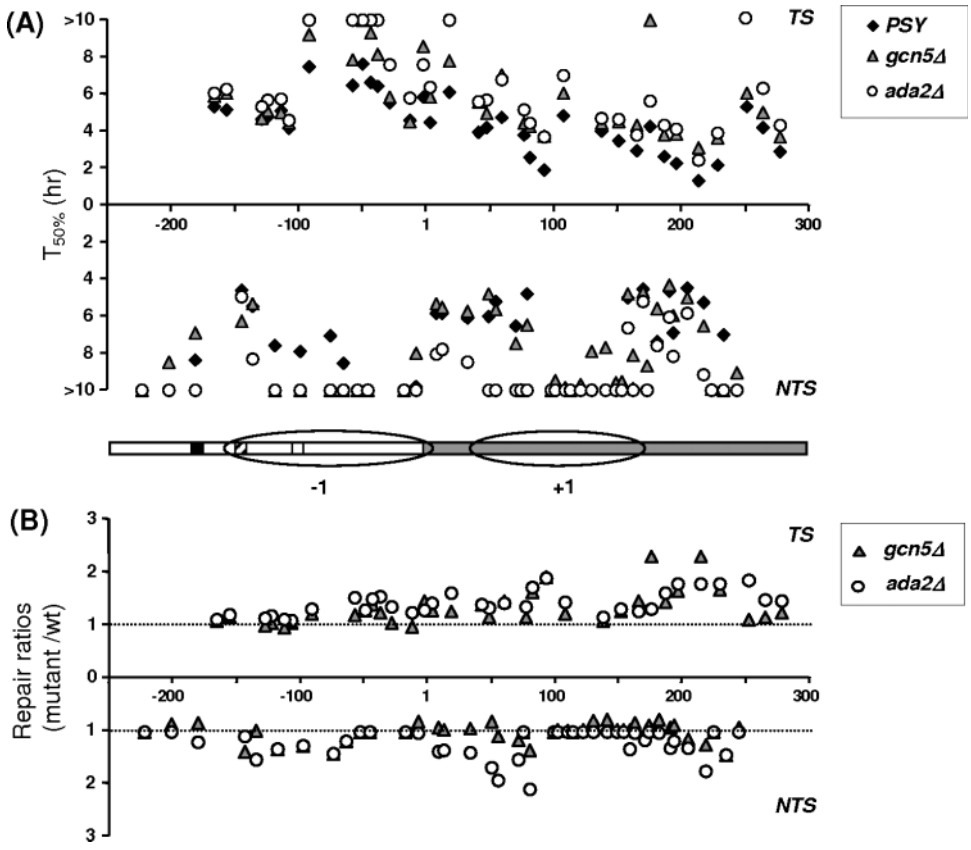

Figure 6. (**A**) Repair of individual CPDs at *MET16* versus the nucleotide position in repressing conditions. The rate of repair in *PSY* (diamonds), *gcn5Δ* (triangles) and *ada2Δ* cells (circles) is expressed as the $T_{50\%}$ for CPDs in the TS (top), and NTS (bottom). Position 1 corresponds to the start of the coding region. Cbf1p-binding site (closed box), Gcn4p-binding site (stripped box), TATA-box (open box) and coding region (grey box). Approximate nucleosome positions are shown as transparent ovals. (**B**) Relative ratios of CPD repair between *gcn5Δ* (triangles) and *ada2Δ* (circles) versus *PSY* cells in the *MET16* TS and NTS. Values above 1 indicate faster repair in the wild type strain and those below 1 faster repair in the mutant strain.

the NTS and they are similar to those nucleosome locations reported in wild type and *cbf1* mutant cells (17).

In contrast to derepressing conditions, repair along the NTS of the wild type is more variable and inefficient (Figure 6A); in this strand 43% of the CPDs require 10 h to repair half of the lesions. Similarly, repair at this strand in *gcn5Δ* and *ada2Δ* cells is also highly variable. However, while on average the CPDs in the *ada2Δ* mutant are less efficiently repaired than in the wild type ($P \geqslant 0.01$), repair in the *gcn5Δ* mutant is not statistically different from that in the wild type. Intriguingly, several CPDs present in three regions of the MspI fragment (around the −200 nt position, from -8 to +49 and from +121 to +193, are more efficiently repaired in *gcn5Δ* cells than in the wild type. These regions correspond to those that are primarily MNase-sensitive. Furthermore, although repair at the promoter region of this strand is more homogenous in *gcn5Δ* and *ada2Δ* cells, CPD repair in the coding region is more efficient in *gcn5Δ* cells ($P > 0.001$).

With respect to the rate of CPD repair along the TS, the three strains show similar trends (Figure 6A, top), although repair in the wild type at both the coding and promoter regions is slightly more efficient than in *gcn5Δ* and *ada2Δ* cells ($P \geqslant 0.05$). At the promoter region of this strand repair is faster in *gcn5Δ* than in *ada2Δ* cells ($P > 0.01$). However, repair at the coding region is statistically homogeneous in the two strains.

The level of transcription influences the rate of CPD repair

Together with the differences in CPD repair due to Gcn5 and Ada2 proteins, the rate of CPD removal is also influenced by the level of *MET16* transcription and the extent of this effect is quite different in each yeast strain (Figure 7).

Our results show that the highest differences in repair between the two growth conditions occur in the wild type strain. This is especially true for the CPDs induced in the TS (Figure 7, top); primarily these are repaired faster in derepressing than in repressing conditions ($P \geqslant 0.05$). In this strand, the highest contrast is obtained in the region around the ATG codon, where repair is 5.1-fold faster in derepressing conditions. Downstream the differences in repair start to decrease, reaching a plateau for the last 150 nt (1.5-fold difference). In contrast, the differences in repair along the NTS of the wild type ($P \geqslant 0.05$) follow a wave pattern (Figure 7, bottom). Here, repair at the proposed linker regions (see above) does not exhibit marked differences between the two transcription conditions, although surprisingly repair is somewhat more efficient in repressing conditions. Most CPDs at the nucleosome cores are repaired faster in derepressing methionine conditions.

Unlike in the wild type, repair of both strands in either mutant is little affected by changing the growing conditions,

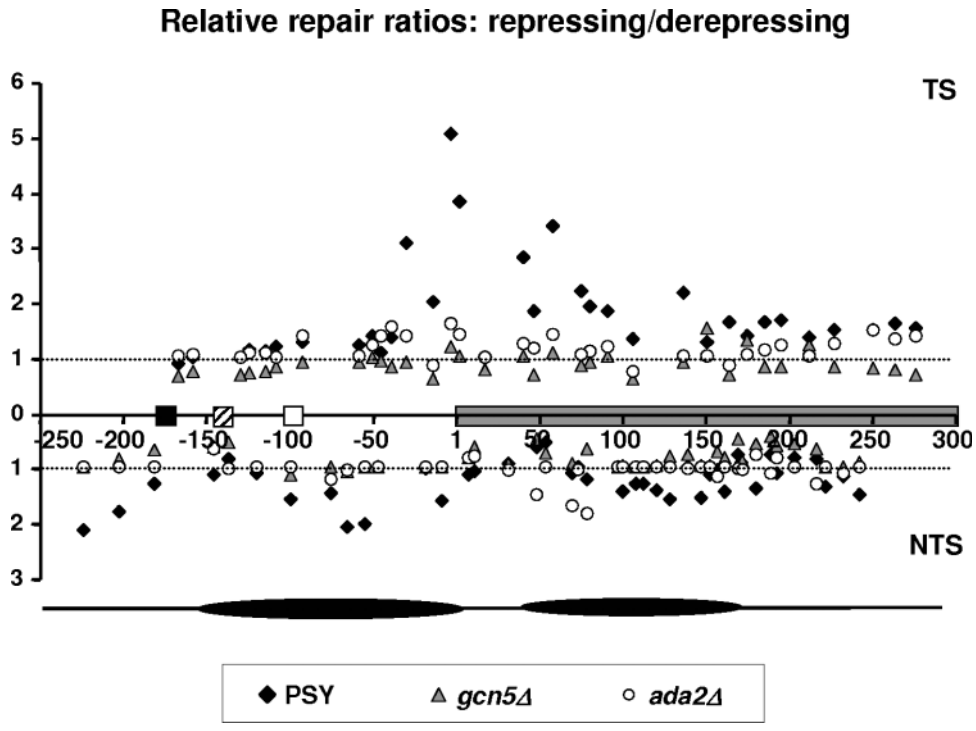

Figure 7. Relative ratios of CPD repair between *MET16* derepressing and repressing conditions in the TS (top) and NTS (bottom) of *PSY* (diamonds), *gcn5Δ* (triangles) and *ada2Δ* (circles) cells. Values above 1 indicate faster repair when transcription is derepressed, and values below 1 indicate faster repair with transcription repressed. Position 1 corresponds to the start of the coding region. Cbf1p-binding site (closed box), Gcn4p-binding site (stripped box), TATA-box (open box) and coding region (grey box). Approximate nucleosome positions are shown as closed ovals.

although the two strains show interesting differences. Repair in the NTS of the *ada2Δ* strain is not statistically different in the two growth conditions, while repair at the TS is slightly faster in derepressing than in repressing conditions ($P = 0.05$). On the contrary, CPDs at the TS and the coding region of the NTS of the *gcn5Δ* strain are repaired faster in repressing than in derepressing conditions ($P \geqslant 0.05$).

DISCUSSION

Gcn5 and Ada2 proteins modulate the full induction of *MET16* transcription

When transcription of *MET16* is repressed by growing the cells in complete medium, similar transcription levels are obtained independently of the Gcn5p or Ada2p status, suggesting that neither protein is essential for the basal transcription of *MET16*. On the contrary, in derepressing conditions *MET16* transcription is induced 11- and 19-fold less in the *gcn5Δ* and *ada2Δ* strains, respectively. This indicates that both Gcn5p and Ada2p are strongly required to obtain the full induction of this gene.

These results show that in spite of the fact that *MET16* transcription is mainly regulated by Cbf1p (28), Gcn5 and Ada2 proteins also play an important role in modulating *MET16* transcription and that their effect is highly dependent on the growth condition. This is in agreement with the known dependence of *MET16* transcription on the general control of amino acids (28–29) since this mechanism relies on the binding of Gcn4p to the AP-1 sequence, which results in the recruitment of the SAGA complex (34). Gcn5p is required for transcription of Gcn4p-dependent genes (19) and Ada2p interacts with the activation domain of Gcn4p (24). It is necessary for the HAT activity of Gcn5p (35) and to recruit the TATA-box-binding protein to Gcn5-dependent promoters (25). The results are in accord with previous data showing that mutations in *ADA2* reduce the activity of Gcn4p and that extracts from the *ADA2* mutant exhibit normal basal transcription levels but are defective in responding to Gcn4p (36).

Gcn5 and Ada2 proteins have little effect on *MET16* nucleosome positions as determined via MNase digests

We showed previously that when *CBF1* is deleted, although the number and general position of the two nucleosomes present around the start of the *MET16* coding region does not change, the MNase accessibility to the TATA-box region is strongly dependent on Cbf1p, i.e. in the presence of this protein the TATA-box is accessible, while the same region is protected in its absence (17). The three strains used here, all of them wild type for *CBF1*, show the intranucleosomal MNase-sensitive region at the TATA-box (Figure 3, band B) and bands A, C and D are located at similar positions to those present in other strains (17,29). Hence, the wild type, *gcn5Δ* and *ada2Δ* cells employed here also have two nucleosomes positioned around the *MET16* ATG starting codon.

Although the position of the nucleosomes is preserved in the three strains and either growth condition, small changes in the structure of the nucleosomes cannot be excluded. Two differences dependent on the growth medium are observed in the pattern of MNase digestion in the three strains. The

internal MNase-sensitive area present at the TATA-box (band B) is more intense in the three strains in derepressing than in repressing methionine conditions. This suggests that the TATA-box region is, as expected, more accessible when *MET16* is actively transcribed. In addition, in derepressing conditions the MNase-sensitive area C shows a doublet of bands instead of the single one present in repressing conditions. This may represent a transitory structure because they are resolved as a single band when the cells are incubated for longer in derepressing conditions (data not shown).

As happened in other yeast strains (17) the MNase-sensitive area D shows differences in the number and intensity of the bands that do not correlate with the strain or growth condition. Differences at this area could not be confirmed when the nucleosomes were mapped at high resolution (17), suggesting that they could also represent transient nucleosome structures.

These results show that in spite of the important differences detected in the level of *MET16* transcription among the three strains neither Gcn5p nor Ada2p induce large changes in the chromatin structure at the *MET16* locus. This is similar to what happens at *MET16* in the absence of Cbf1p, namely transcription is extensively affected but nucleosome positions are not markedly disrupted (17). Therefore, for *MET16* regulation both the Cbf1p-mediated mechanism and that for the general control of amino acids have similar outcomes on nucleosome positioning.

Nucleosome structure modulates CPD repair at the *MET16* locus

In repressing methionine conditions the repair trend along the NTS is strongly influenced by the position of the nucleosomes in the three strains, i.e. repair is fast at the linker regions and slow towards the nucleosome cores. This is similar to that in two different yeast strains at the same locus (17) or at the *URA3* gene (8–9).

This nucleosome modulation is not obvious in derepressing conditions in any of the three yeast strains (Figure 5A, bottom). However, in the two mutant strains the regions where repair is fast are concordant with the equivalent ones in repressing conditions, so the presence of nucleosome modulation cannot be completely discarded here. In contrast, in the wild type this nucleosome modulation seems to be primarily abolished. This is similar to in the wild type strain used to examine the role of Cbf1p in NER, where the nucleosome modulation obtained in the same derepressing medium was also less obvious than in the repressing condition (17).

Although the modulation by the nucleosomes is not evident in the TS, it is interesting to note that in repressing conditions, when the effect at the NTS is strongest, repair of the TS shows small patches of slow repair coincident with the nucleosome cores. So, in contrast to other genes like *URA3* (8–9) or *MFA2* (31), it seems that in this growth condition the fast repair at the coding region of the *MET16* TS cannot completely overcome the effect imposed by the nucleosomes.

Transcription coupled repair is stimulated in high *MET16* transcription conditions

Despite the fact that the extent of *MET16* transcription varies about 50-fold between the growth conditions and the strains employed, repair of CPDs at the coding region of the TS is always more efficient than in the respective non-transcribed regions. This fast repair constantly starts between 30 and 40 nt upstream of the start of translation as happens in other yeast strains (17) or the *RPB2* gene (6).

With the exception of the wild type strain grown in derepressing conditions, where *MET16* transcription is strongly activated, this gene is otherwise lowly transcribed and shows less than a 5-fold variation among the different conditions and strains. In spite of these differences in transcription, the faster repair at the coding region of the TS is detected at a similar relatively low level in all of them; repair at the coding region of the TS is 1.3- to 1.5-fold faster than in the promoter region. In the wild type grown in derepressing conditions, fast repair is more evident and repair at the coding region is ~2.5-fold faster than in the promoter region. However, this activation is not a general phenomenon and is mainly due to the more efficient repair of the CPDs induced around the starting ATG codon, where repair is induced up to 5-fold.

These results are concordant with our previous work where even when *MET16* is not transcribed, the CPDs at the coding region of the TS are also repaired 1.3- to 1.5-fold faster than those in the promoter region and the biggest differences in repair were obtained when the level of transcription was highest (17).

Our results indicate that when transcription of *MET16*, and possibly other lowly transcribed genes, is absent or is transcribed at very low levels, the more efficient repair of the CPDs induced at the coding region of the TS would be mainly a consequence of the chromatin structure change as suggested (37). However, when transcription is highly activated and reaches a certain threshold, the efficiency of this fast repair is further enhanced. This could be a consequence of the higher number of RNA-polymerases stalled at CPDs, together with the presence of changes in the chromatin structure that leave the DNA more accessible to enzymes involved in DNA repair. The extent of this enhanced repair depends on the action of Gcn5p and Ada2p, but this may merely reflect the extents of transcription in the presence or absence of these proteins.

The level of transcription influences the rate of CPD repair

Comparison of the repair rate in each strain between the two growth conditions shows that the biggest differences occur in the wild type, especially in the TS around the ATG starting codon. Here, repair in derepressing conditions is up to 5-fold faster than in repressing conditions. The fact that the differences in repair are bigger in the wild type than in the two mutant strains is concordant with the differences in transcription, which is highest in the wild type. Moreover, this seems mainly related to the change in the transcription extent and especially to the steps of transcription initiation and the beginning of transcription elongation (Figures 5–7). On the contrary, the differences in repair in the wild type NTS run in parallel to the position of the nucleosomes. Here, the CPDs at the nucleosome cores show more differences in repair than those at the linker regions. It is clear that at the *MET16* locus the change in the transcription extent can result in very different outcomes in the rate of repair of the two DNA strands. This suggests that in the wild type we are detecting the effect of two different factors; the change in repair at the TS would be

mainly affected by the transcription extent, whereas the changes detected in the NTS would be mainly due to the nucleosome structure.

In the two mutant strains the differences in transcription between the two growth conditions are small and concordantly, repair is quantitatively less affected. However, the effect is quite different in each strain. Repair in the *ada2Δ* mutant is only affected at the TS, where the CPDs are repaired as expected slightly, but statistically, faster in derepressing conditions. In contrast, repair in *gcn5Δ* cells at both the TS and the coding region of the NTS is faster in repressing conditions. The results in this strain are contrary to what one might expect as transcription in derepressing conditions is about 5-fold higher than in repressing conditions, but repair is less efficient. This suggests a separate role for Gcn5p in NER at the *MET16* locus when its transcription is activated by growth in low methionine conditions. This result supports the role of Gcn5p in NER identified at the repressed *MFA2* locus, where Gcn5p-mediated histone H3 acetylation is induced by UV outside of any transcriptional activation (38).

The absence of Gcn5p or Ada2p delays the rate of CPD repair

We show that deletion of either *GCN5* or *ADA2* alters the efficiency of CPD removal at the *MET16* locus and that the extent of the effect is different depending on the growth condition and the DNA strand.

In general, the influence of these two proteins is more intense when *MET16* is derepressed. In this condition, the reduction in repair is least for the CPDs in the non-transcribed regions and highest for those in the coding region of the TS. Here, although the absence of either protein has a similar qualitative effect in repair, the defect along the TS is quantitatively more pronounced by deletion of *GCN5* than by deletion of *ADA2*. This is in contrast to what is expected as transcription in this growth condition is 1.7-fold higher in *gcn5Δ* than in *ada2Δ* cells. The repair data obtained at the *MET16* locus in the *gcn5Δ* mutant grown in derepressing conditions are concordant with those obtained at the *MFA2* gene in the corresponding *gcn5Δ-MATa* strain (16). Repair at the NTS of both genes is highly inefficient in the *gcn5Δ* mutant and repair at the *MFA2* coding region of the TS was shown to be 2- to 3-fold slower in the *gcn5Δ* mutant than in the wild type, while repair at the equivalent region of *MET16* shows a 2.8-fold reduction.

In contrast to derepressing conditions, the effect of Gcn5 and Ada2 proteins on CPD repair when *MET16* transcription is repressed is much less at both DNA strands. This is concordant with the small differences detected in transcription in this growth condition. Although the effect is quite similar in the two mutant strains, repair at the non-transcribed regions is slightly more efficient in *gcn5Δ* than in *ada2Δ* cells.

Together, the results in the two mutant strains indicate that both Ada2 and Gcn5 proteins are required for efficient repair of CPDs at both strands of the *MET16* locus and that they could have overlapping effects on the rate of repair when transcription of *MET16* is activated by changing the growth conditions. One of these effects would be dependent on Ada2p, and so probably depends on the SAGA/ADA complexes. Upon *MET16* transcription induction, the activity of Ada2p is required for more efficient repair at the *MET16* TS and NTS. This may reflect the influence of this protein in creating a more relaxed chromatin state (a change small enough to be undetected by MNase mapping). The second effect would be dependent on Gcn5p and is more dramatic as this protein is required for more efficient repair at both strands of *MET16* and the repair efficiency is decreased upon transcription induction.

In conclusion, our results support the fact that Gcn5p influences the rate of NER, and at another locus, namely *MET16*, where the regulation of transcription is completely different from that at the *MFA2* gene (16). However, this is another local effect on NER via Gcn5p as NER in the genome overall is not detectably reduced nor is it diminished at the *RPB2* locus, where Gcn5p has no role in transcription (16).

Importantly, we have uncovered here a role for Ada2p in NER at the *MET16* locus; *ada2Δ* cells also have locally reduced NER, so it is possible that both proteins could act in conjunction, pointing to a role in facilitating efficient NER for some of the complexes that contain both proteins.

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