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

J. A. Ferreiro, N. G. Powell1, N. Karabetsou2, J. Mellor2 and R. Waters3,*

Department of Functional Biology, University of Oviedo, Oviedo 33006, Spain, 1Department of Obstetrics and Gynaecology, Medical School, Cardiff University, Cardiff CF14 4XN, UK, 2Department of Biochemistry, Oxford University, Oxford OX1 3QU, UK and 3Department of Pathology, Medical School, Cardiff University, Cardiff CF14 4XN, UK

Received November 25, 2005; Revised and Accepted January 25, 2006

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).

*To whom correspondence should be addressed. Tel: +44 2920744848; Fax: +44 2920744276; Email: watersrl@cf.ac.uk

© The Author 2006. Published by Oxford University Press. All rights reserved.

The online version of this article has been published under an open access model. Users are entitled to use, reproduce, disseminate, or display the open access version of this article for non-commercial purposes provided that: the original authorship is properly and fully attributed; the Journal and Oxford University Press are attributed as the original place of publication with the correct citation details given; if an article is subsequently reproduced or disseminated not in its entirety but only in part or as a derivative work this must be clearly indicated. For commercial re-use, please contact journals.permissions@oxfordjournals.org
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, ade2-101, Δhis3-200, lys2, trp1, gcn5Δ) and PSY316-ada2A: (MATα, leu2-3, leu2-112, ura3-52, ade2-101, Δhis3-200, lys2, trp1, ada2A) 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/m² 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 [32P]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 T₅₀% value; i.e. the time required to repair 50% of the lesions present immediately after treatment (31). T₅₀% 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).
**MET16 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^PdATP labelling was carried out with the following primers: MspI-A: 5’-Biotin-GATAACGTGTTTTTGGTGGAACATCACCTATTGATTCTAAAT-3’ for the TS and MspI-B: 5’-Biotin-GATAACGTGTTTTTGGTTATACGTGAATGGTTTGATT 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’-CATCCCG-GCTTATACGTGAATGGTTTGATTTTAG-3’) primers. Probes for ACT1 were similarly prepared using the following primers: 5’-Biotin-GCCGGTTTTGCCGGTGACG-3’ and 5’-GGGTGTTTTTACCGTGACAG-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.](image1)

![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.](image2)
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 T90% of 2.1 h, and homogeneous (Figure 5A, top). In contrast, repair in the

![Figure 4](image-url.com) 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 1–4h. 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.
The 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, $P > 0.01$). Moreover, repair in this strand is more efficient in ada2Δ than in gcn5Δ cells ($P > 0.01$). 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, $P \geq 0.05$ (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...
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 gcn5D and ada2D cells is also highly variable. However, while on average the CPDs in the ada2Δ mutant are less efficiently repaired than in the wild type ($P > 0.01$), 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 ($P > 0.05$). 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 ($P > 0.05$). 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 ($P > 0.05$) 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,
although the two strains show interesting differences. Repair in the NTS of the \( \text{ada2} \Delta \) 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 \( \text{gcn5} \Delta \) strain are repaired faster in repressing than in derepressing conditions \((P \geq 0.05)\).

**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 \( \text{gcn5} \Delta \) and \( \text{ada2} \Delta \) 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 presented 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, \( \text{gcn5} \Delta \) and \( \text{ada2} \Delta \) 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
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 gen5Δ than in ada2Δ cells. The repair data obtained at the MET16 locus in the gen5Δ mutant grown in derepressing conditions are concordant with those obtained at the MFA2 gene in the corresponding gen5Δ-MATa strain (16). Repair at the NTS of both genes is highly inefficient in the gen5Δ mutant and repair at the MFA2 coding region of the TS was shown to be 2- to 3-fold slower in the gen5Δ 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 gen5Δ 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 KPB2 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.

ACKNOWLEDGEMENTS

This work was supported by The Wellcome Trust (056922/A/99/Z/BS/DC) and by an MRC award to R.W. renewed in 2004 (G99 00118). J.A.F. was funded by an EU Marie Curie fellowship (QLK6-CT-1999-51401). Funding to pay the Open Access publication charges for this article was provided by the MRC.

Conflict of interest statement. None declared.

REFERENCES

1. Wolffe, A. (1998) Chromatin: Structure and Function. Academic Press, San Diego, CA.
2. Workman, J.L. and Kingston, R.E. (1998) Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu. Rev. Biochem., 67, 545–579.
3. Meijer, M. and Smerdon, M.J. (1999) Accessing DNA damage in chromatin: insights from transcription. Bioessays, 21, 596–603.
4. Meyer, P. (2001) Chromatin remodelling. Curr. Opin. Plant Biol., 4, 457–462.
5. Svejstrup, J.Q. (2002) Mechanisms of transcription-coupled DNA repair. Nature Rev. Mol. Cell Biol., 3, 21–29.
6. Li,S. and Smerdon,M.J. (2002) Rp48 and Rp99 mediate subpathways of transcription-coupled DNA repair in Saccharomyces cerevisiae. EMBO J., 21, 5921–5929.
7. Powell,N.G., Ferreiro,J., Karabetsou,N., Mellor,J. and Waters,R. (2003) Transcription, nucleosome positioning and protein binding modulate nucleotide excision repair of the Saccharomyces cerevisiae MET17 promoter. DNA repair, 2, 375–386.
8. Wellinger,R.E. and Thoma,F. (1997) Nucleosome structure and positioning modulate nucleotide excision repair in the non-transcribed strand of an active gene. EMBO J., 16, 5046–5056.
9. Tijsterman,M., De Phil,R., Tasseron-de Jong,J.G. and Brouwer,J. (1999) RNA polymerase II transcription suppresses nucleosomal modulation of UV-induced (6–4) photoproduct and cyclobutane pyrimidine dimer repair in yeast. Mol. Cell. Biol., 19, 934–940.
10. Harra,R., Mo,J. and Sancar,A. (2000) DNA damage in the nucleosome core is refractory to repair by human excision nuclease. Mol. Cell. Biol., 20, 9173–9181.
11. Ura,K., Araki,M., Saeki,H., Masutani,C., Ito,T., Iwai,S., Mizukoshi,T., Kancha,Y. and Hanaoka,F. (2001) ATP-dependent chromatin remodeling facilitates nucleotide excision repair of UV-induced DNA lesions in synthetic dinucleosomes. EMBO J., 20, 2004–2014.
12. Grunstein,M. (1990) Histone function in transcription. Annu. Rev. Cell. Biol., 6, 643–678.
13. Wu, J. and Grunstein, M. (2000) 25 years after the nucleosome model: chromatin modifications. *Trends Biochem. Sci.*, 25, 619–623.

14. Hara, R. and Sancar, A. (2002) The Swi/Snf chromatin-remodeling factor stimulates repair by human excision nucleases in the mononucleosome core particle. *Mol. Cell. Biol.*, 22, 6779–6787.

15. Hara, R. and Sancar, A. (2003) Effect of damage type on stimulation of human excision nuclease by Swi/Snf chromatin remodeling factor. *Mol. Cell. Biol.*, 23, 4114–4125.

16. Teng, Y., Yu, Y. and Waters, R. (2002) The Saccharomyces cerevisiae histone acetyltransferase Gcn5 has a role in the photoactivation and nucleotide excision repair of UV-induced cyclobutane pyrimidine dimers in the MFA2 gene. *J. Mol. Biol.*, 316, 489–499.

17. Ferreiro, J.A., Powell, N.G., Karabetsou, N., Kent, N.A., Mellor, J. and Waters, R. (2004) Obf1p modulates chromatin structure, transcription and repair at the Saccharomyces cerevisiae MET16 locus. *Nucleic Acids Res.*, 32, 1617–1626.

18. Steiner, D.E. and Berger, S.L. (2000) Acetylation of histones and transcription-related factors. *Microbiol. Mol. Biol. Rev.*, 64, 435–459.

19. Georgakopoulos, T. and Thireos, G. (1992) Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. *EMBO J.*, 11, 4145–4152.

20. Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F., Berger, S.L. and Workman, J.L. (1997) Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev.*, 11, 1640–1650.

21. Pray-Grant, M.G., Schiltz, D., McMahon, S.J., Wood, J.M., Kennedy, E.L., Cook, R.G., Workman, J.L., Yates, J.R. 3rd and Grant, P.A. (2002) The novel SLIK histone acetyltransferase complex functions in the yeast retrograde response pathway. *Mol. Cell. Biol.*, 22, 8774–8786.

22. Marcus, G., Silverman, N., Berger, S., Horwich, J. and Guarente, L. (1994) Functional similarity and physical association between GCN5 and ADA2-putative transcriptional adaptors. *EMBO J.*, 13, 4807–4815.

23. Candau, R. and Berger, S.L. (1996) Structural and functional analysis of yeast putative adaptors: evidence for an adaptor complex in vivo. *J. Biol. Chem.*, 271, 5237–5245.

24. Barlev, N.A., Candau, R., Wang, L., Darpino, P., Silverman, N. and Berger, S.L. (1995) Characterization of physical interactions of the putative transcriptional adaptor, ADA2, with acidic activation domains and TATA-binding protein. *J. Biol. Chem.*, 270, 19337–19344.

25. Blau, M.R. and Green, M.R. (2002) Differential requirement of SAGA components for recruitment of TAT-A-box-binding protein to promoters in vivo. *Mol. Cell. Biol.*, 22, 7365–7371.

26. Thomas, D., Barber, R. and Sordin-Kerjan, Y. (1990) Gene–enzyme relationship in the sulfate assimilation pathway of *Saccharomyces cerevisiae*. Study of the 3-phosphoadenylylsulfate reductase structural gene. *J. Biol. Chem.*, 265, 15518–15524.

27. Wang, Y., Liu, C.L., Storey, J.D., Tibshirani, R.J., Herschlag, D. and Brown, P.O. (2002) Precision and functional specificity in mRNA decay. *Proc. Natl Acad. Sci. USA*, 99, 5860–5865.

28. Kuras, L., Chérest, H., Sordin-Kerjan, Y. and Thomas, D. (1996) A heteromeric complex containing the centromere binding factor 1 and two basic leucine zipper factors, Mef4 and Met28, mediates the transcription activation of yeast sulfur metabolism. *EMBO J.*, 15, 2519–2529.

29. O’Connell, K.F., Sordin-Kerjan, Y. and Baker, R.E. (1995) Role of the *Saccharomyces cerevisiae* general regulatory factor CP1 in methionine biosynthetic gene transcription. *Mol. Cell. Biol.*, 15, 1879–1888.

30. Reed, S.H., Boiteux, S. and Waters, R. (1996) UV-induced endonuclease III-sensitive sites at the mating type loci in *Saccharomyces cerevisiae* are repaired by nucleotide excision repair: RAD7 and RAD16 are not required for their removal from HML alpha. *Mol. Gen. Genet.*, 250, 505–514.

31. Teng, Y., Li, S., Waters, R. and Reed, S.H. (1997) Excision repair at the level of the nucleotide in the *Saccharomyces cerevisiae* MFA2 gene: mapping of where enhanced repair in the transcribed strand begins or ends and identification of only a partial rad16 requisite for repairing upstream control sequences. *J. Mol. Biol.*, 267, 324–337.

32. Kent, N.A. and Mellor, J. (1995) Chromatin structure snap-shots: rapid nuclelease digestion of chromatin in yeast. *Nucleic Acids Res.*, 23, 3786–3787.

33. Teng, Y. and Waters, R. (2000) Excision repair at the level of the nucleotide in the upstream control region, the coding sequence and in the region where transcription terminates of the *Saccharomyces cerevisiae* MFA2 gene and the role of RAD26. *Nucleic Acids Res.*, 28, 1114–1119.

34. Teng, Y. and Waters, R. (2000) Gcn4 occupancy of open reading frame regions results in the recruitment of chromatin-modifying complexes but not the mediator complex. *EMBO Rep.*, 4, 872–976.

35. Balasubramanian, R., Pray-Grant, M.G., Selleck, W., Grant, P.A. and Tan, S. (2002) Role of the Ada2 and Ada3 transcriptional coactivators in histone acetylation. *J. Biol. Chem.*, 277, 7989–7995.

36. Berger, S.L., Pina, B., Silverman, N., Marcus, G.A., Agapite, J., Regier, J.L., Triezenberg, S.J. and Guarente, L. (1992) Genetic isolation of ADA2: a potential transcriptional adaptor required for function of certain acidic activation domains. *Cell*, 70, 251–265.

37. Zheng, Y., Pao, A., Adair, G.M. and Tang, M. (2001) Cyclobutane pyrimidine dimers and bulky chemical DNA adducts are efficiently repaired in both strands of either a transcriptionally active or promoter-deleted *APRT* gene. *J. Biol. Chem.*, 276, 16786–16796.

38. Yu, Y., Teng, Y., Liu, H., Reed, S.H. and Waters, R. (2005) UV irradiation stimulates histone acetylation and chromatin remodeling at a repressed yeast locus. *Proc. Natl Acad. Sci. USA*, 102, 8650–8655.