Base Excision Repair of N-Methylpurines in a Yeast Minichromosome

EFFECTS OF TRANSCRIPTION, DNA SEQUENCE, AND NUCLEOSOME POSITIONING*

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Base excision repair of dimethyl sulfate induced N-methylpurines (NMPs) was measured in a yeast minichromosome that has a galactose-inducible GAL1:URA3 fusion gene, a constitutively expressed HIS3 gene, and varied regions of chromatin structure. Removal rates of NMPs varied dramatically (>20-fold) at different sites along three selected fragments encompassing a total length of 1775 base pairs. Repair of NMPs was not coupled to transcription, because the transcribed strands of HIS3 and induced GAL1:URA3 were not repaired faster than the nontranscribed strands. However, the repair rate of NMPs was significantly affected by the nearest neighbor nucleotides. Slow repair occurred at NMPs between purines, especially guanines, whereas fast repair occurred at NMPs between pyrimidines. NMPs between a purine and pyrimidine were repaired at moderate rates. Moreover, a rough correlation between nucleosome positions and repair rates exists in some but not all regions that were analyzed.

Subtle alterations in DNA molecules, such as oxidized or alkylated bases, are repaired by the base excision repair (BER)1 pathway (reviewed in Refs. 1 and 2). BER is initiated by specific DNA glycosylases that release the damaged base. There are two families of alkylated base DNA glycosylases (3). Although their primary sequences and three-dimensional structures are unrelated, they may have common themes in recognition of alkylated bases and catalysis of glycosidic bond cleavage (3). Crystal structures of the human AAG protein (3) and the Escherichia coli AlkA protein (4, 5) suggest that the glycosylases flip the target base out of the DNA duplex into the active site cleft of the enzymes. The “flipping out” may occur in a later step during recognition and/or incision of damaged bases (6).

Alkylation damage to DNA occurs frequently in nature and can be introduced either by alkylating agents present in the environment or by erroneous alkylation induced by the natural methyl donor S-adenosyl-methionine (7). Simple methylating agents such as dimethyl sulfate (DMS) produce a variety of damaged bases in DNA of which N-methylpurines (N$^2$-methylguanine and N$^2$-methyladenine) or NMPs constitute approximately 90% of the alterations (8). InSaccharomyces cerevisiae, the repair of NMPs occurs mainly through the BER pathway, although nucleotide excision repair (NER) may play a role if the BER pathway is abolished (9). Initiation of this pathway in S. cerevisiae is by the action of the small (34 kDa) protein MAG DNA glycosylase (10).

Although the repair of thymine glycols is coupled to transcription in both yeast and mammalian cells (11, 12), a link between transcription and BER of other lesions has not been well established. It was found that the alkali-labile sites (representing NMPs) induced by methylnitrosourea are preferentially repaired within the active insulin gene of rat insulinoma cells (13). However, no preferential repair of these lesions induced by DMS was found in the actively transcribed DHFR gene of Chinese hamster ovary cells (14–16) or in the transcribed strand of the PGK1 gene in human cells (17).

These studies raise several questions about BER in intact cells. As a damaged base is flipped out by a DNA glycosylase during BER, do neighboring nucleotides affect the rate of BER? Is there a coupling between transcription and BER of NMPs in yeast? Furthermore, as modulation of NER by chromatin structure has been well established (18), does nucleosome positioning also modulate BER? To address these questions, we studied BER of NMPs in a minichromosome in intact yeast cells.

EXPERIMENTAL PROCEDURES

Plasmid and Yeast Strains—Strain RGY1 was made by transforming strain Y452 (MATa ura3-52 his3-1 leu2-3 leu2-112 cir*) with plasmid YRpSO1 (see Fig. 1). This plasmid was constructed and characterized by Drs. Stephano Omari and Fritz Thoma at the Swiss Federal Research Institute (ETH-Honggerberg) in Zürich, Switzerland (19).

DMS Treatment and DNA Repair Incubation—RGY1 cells were grown at 30 °C in minimal medium containing 2% glucose or 2% galactose to late log phase (O$_{600}$ ~ 1.0). The cultures were mixed with DMS at a final concentration of 0.35 mM (v/v). After 5 min of incubation at room temperature, the cells were washed twice with either ice-cold 2% glucose or 2% galactose and resuspended in the same solutions containing 100 mM hydroxyurea to prevent DNA replication during repair incubation (20). One-tenth volume of a solution containing 10% yeast extract and 20% peptone was added to the DMS-treated cultures. After 0, 1, 2, and 4 h of repair incubation at 30 °C, an aliquot was removed, and plasmid DNA was isolated as described previously (21).

Analysis of NMP Repair at the Nucleotide Level in YRpSO1—A biotinylated oligonucleotide and streptavidin magnetic bead-facilitated end-labeling technique was used (22, 23). Briefly, isolated plasmid DNA was cut with restriction enzyme(s) to release the three fragments that were analyzed. DdeI and StyI were used to release the 678-bp GAL1:URA3 fragment, StuI was used to release the 717-bp URA3-3' fragment, and BstXI and RestrI were used to release the 659-bp HIS3 fragment (see Fig. 1). The restricted DNA was cleaved at NMP sites by incubation in 1 M piperidine at 90 °C for 30 min (24). One strand of the cleaved fragments was annealed to biotinylated oligonucleotides (sequences available upon request). The annealed fragments were attached to streptavidin magnetic beads and subjected to sequencing using phosphorImager screens (Molecular Dynamics). The band intensities in the gels were quantified using ImagQuaNT (Molecular Dynamics) and PeakFit 4.0 (SPSS, Inc.) deconvolution software as described (23). Sequence markers were generated from polymerase chain reaction fragments, as described previously (22, 23).

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‡ The abbreviations used are: BER, base excision repair; bp, base pair(s); DMS, dimethyl sulfate; NMP, N-methylpurine; NER, nucleotide excision repair; TS, transcribed strand; NTS, nontranscribed strand.
RESULTS

General Features of YRpSO1—YRpSO1 is a high copy number (~50 copies/cell), autonomously replicating yeast plasmid 4263 bp in length (Fig. 1). It contains (a) the ARS1 origin of replication, flanked by the 3' end of the TRP1 gene (Fig. 1, open arrowhead) and the upstream region of the GAL1 gene (Fig. 1, near nucleosome 9), (b) a galactose-inducible GAL1:URA3 fusion gene, (c) a constitutively expressed HIS3 gene, and (d) the 5' ends of the PET56 and DED1 genes of yeast (19). Moreover, a sequence of unknown function (UNF) lies downstream of the DED1 fragment. Twenty-two nucleosomes (Fig. 1, small numbered circles) are distributed in four regions, interrupted by four nuclease-sensitive gaps (19). Following galactose induction, four nucleosomes (Fig. 1, nucleosomes 19–22) in the UNF-GAL10 region are destabilized or rearranged. Upon galactose induction, the level of GAL1:URA3 RNA increases dramatically (>150-fold) from essentially zero in glucose medium (25). However, except for the regions mentioned above, no change in nucleosome structure is observed in the minichromosome (including the GAL1:URA3 fusion gene) in galactose cultures (19), indicating that only a few of the fusion genes are transcriptionally active at any one time. This fact presumably reflects insufficient GAL4p for binding to the UASg of the GAL1–10 promoter contained in the multicopy plasmid.

Induction and Repair of NMPs at Individual Sites in YRpSO1—Three fragments of YRpSO1 (GAL1-URA3 (Del1-Syl1), URA3-3' (StuI)), and HIS3 (BstNI-BstXI)) were chosen for analysis based on their proximity to the GAL1:URA3 and HIS3 genes (Fig. 1). Representative gels showing the incidence and repair of NMPs are shown in Figs. 2 and 3. As can be seen, the induction of NMPs was primarily at G residues (i.e. compare h and G lanes). This is in agreement with past reports (reviewed in Ref. 8), as N7-methylguanines induced by DMS account for >80% of the total adducts in DNA. We note that galactose caused no obvious change in the induction of NMPs in the UASg and the promoter regions of the GAL1:URA3 fusion gene (Fig. 3). In contrast, changes in induction levels are observed in these regions of the single copy genomic GAL1 gene. As mentioned above, this may reflect insufficient amounts of GAL4p bound to the UASg during galactose induction.

The band intensities at 460 total sites in the three fragments, following 1, 2, and 4 h of repair, were quantified using peak deconvolution. A decrease in band intensities indicates that NMPs at respective sites are removed. This removal reflects the complete process of BER rather than just the excision of NMPs, as the apurinic sites generated from the excision of NMPs are also alkali-labile and unligated BER intermediates would (by themselves) be DNA single strand breaks. We detected no obvious DNA single strand breaks at any time during repair incubation if the samples were not treated with alkaline piperidine, presumably because the post-excision repair process is very fast. An example of the percentage of NMPs remaining in each strand after 2 h of repair incubation is shown in Fig. 4. As implied by this data, the time course of repair was similar at most sites in the two cultures (Fig. 4).

Effect of Transcription on NMP Repair—Removal of NMPs does not appear to be coupled to transcription, because the transcribed strands of the constitutively expressed HIS3 gene and the galactose-induced GAL1:URA3 fusion gene are not repaired significantly faster (Figs. 2–4). The fraction of NMPs remaining at each site in glucose and galactose cultures were directly compared to examine more subtle changes when transcription is induced (Fig. 5). These results indicate that a few sites in the GAL1 promoter (nucleotides 462, 502, and 507; also

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2 F. Thoma, unpublished results.
3 S. Li, R. Gupta, M. Meijer, M. Livingstone-Zatchej, F. Thoma, and M. J. Smerdon, submitted for publication.
4 S. Li and M. J. Smerdon, unpublished results.
see bands marked with bars in Fig. 3) and downstream of the GAL1-URA3 fusion gene (nucleotides 1684, 1720, and 1741) are repaired faster in galactose cultures. Because these sites are outside of the transcribed regions, this result may reflect subtle disruption of nucleosomes in these two regions that was undetected by nucleosome mapping (19).

Effect of DNA Sequence on NMP Repair—In both glucose and galactose cultures, the repair rate of NMPs varied dramatically along the three fragments (Figs. 2–4). Analysis of these variations with DNA sequence yielded an unexpected correlation between NMP sequence location and removal by BER. Most NMP sites located between pyrimidines (e.g. bands marked by small arrows in Figs. 2 and 3) are repaired much faster than NMPs between purines, although exceptions to this trend do exist (e.g. bands marked by asterisks in Figs. 2 and 3).

To determine how general this sequence effect may be, we compared the repair rates of NMPs at 371 different sites that were well separated (or easily deconvoluted) bands on sequencing gels. As can be seen in Table I, in both glucose and galactose cultures, NMPs between two purines (especially two Gs) are repaired most slowly, NMPs between two pyrimidines (C or T) are repaired most rapidly, and NMPs between a purine and pyrimidine are repaired at a moderate rate. In contrast, second adjacent nucleotides (or a combination of the first and second adjacent nucleotides) have little (or no) effect on repair of NMPs (data not shown).

Effect of Nucleosome Positioning on NMP Repair—As can be deduced from Fig. 4, a rough correlation between nucleosome positions and repair rates exists for both strands of the GAL1-URA3 fusion gene in the nucleosome regions approximately centered at nucleotides 1300 and 1460. In these regions, most
sites within nucleosomes were repaired slower, whereas most sites in the internucleosome regions were repaired faster. A rough correlation can also be deduced for the TS of the GAL1::URA3 gene in the nucleosome region approximately centered at nucleotides 430 and 610 (Fig. 4). Because nearest neighbor nucleotides can dramatically affect the repair rate of NMPs, the percentages of NMPs remaining at the 371 NMP sites after each repair time were also normalized by the mean values of the corresponding sequence contexts (Table I). After this normalization, the correlation between repair rates of NMPs and nucleosome positioning was somewhat more evident (data not shown). However, no obvious modulation of nucleosome positioning on repair was seen in the HIS3 gene and in the 5′ region of the NTS of the GAL1::URA3 fusion gene (Fig. 4).

**DISCUSSION**

We observed no obvious preferential removal of NMPs in the transcribed strands of the galactose-induced GAL1::URA3 fusion gene and in the constitutively expressed HIS3 gene of YRpSO1 (Figs. 2–5). Conversely, we have found a coupling between BER of UV-induced cyclobutane pyrimidine dimers and transcription in both of these genes. These results agree with those found for BER of NMPs in the DHRF gene of Chinese hamster ovary cells (14–16) and for the PGK1 gene in human cells (17) but differ from those obtained for the insulin gene in rat insulinoma cells (13). At present, the reason for this discrepancy is unclear, but it may reflect a difference in the mode of gene expression between these cells and/or gene types.

In contrast to the lack of correlation with transcription, repair of NMPs is dramatically influenced by nearest neighbor nucleotides (Table I). These nucleotides could have a significant effect on the energy required for flipping out NMPs during recognition and incision by DNA glycosylases (3–6). Indeed, theoretical calculations show that the stability of base stacking follows the order: purine-purine (G-G slightly > A-A) > purine-pyrimidine > pyrimidine-purine > pyrimidine-pyrimidine, with a 2-kcal/mol spread between the least stable and most stable base pairs (26). Thus, it can be predicted that NMPs between purines (especially Gs) require the most energy to be flipped out, whereas those between pyrimidines require the least energy. These predictions fit exactly with our results (Table I). However, it should be noted that some NMPs located between pyrimidines were also repaired slowly (e.g. bands marked by asterisks in Figs. 2 and 3). The slow repair at these sites may reflect other factors in chromatin (e.g. nonhistone DNA-binding proteins) that may also affect repair of NMPs.

We observed a rather weak correlation between NMP repair and nucleosome positioning (Fig. 4). However, this does not necessarily mean that histone binding to DNA has little effect on NMP repair. Although this effect has not been well studied, many reports indicate that NER is modulated by nucleosomes (reviewed in Ref. 18). Therefore, this weak correlation between NMP repair and nucleosome positioning in YRpSO1 may suggest that the effect of nucleosome positioning is partially “masked” by effects of other factors on repair of NMPs.

Finally, recently the more sensitive ligation-mediated polymerase chain reaction method was used to study BER of NMPs in human cells (17). These authors also found extreme variability in BER rate at different positions and no transcription-coupled repair in the PGK1 gene. Thus, the observations in the present work appear to be testable in mammalian cells. Clearly, it will be of interest to determine whether repair of NMPs in mammalian cells is modulated by the same pattern of nearest neighbor nucleotides as we found in yeast.

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**REFERENCES**

1. Seeberg, E., Edie, L., and Bjorás, M. (1995) Trends Biochem. Sci. 20, 391–397
2. Krokan, H. E., Standal, R., and Slupphaug, G. (1997) Biochem. J. 325, 1–16
3. Lau, A. Y., Scherer, O. D., Samson, L., Verdine, G. L., and Ellenberger, T. (1992) Cell 75, 249–265
4. Labahn, J., Scherer, O. D., Long, A., Ezaz-Nikpay, K., Verdin, G. L., and Ellenberger, T. E. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 321–329
5. Yamagata, Y., Kato, M., Odawara, K., Tokuno, Y., Nakashima, Y., Matsuushima, N., Yasumura, K., Tomita, K., Ibata, K., Fujii, Y., Nakabeppe, Y., Sekiguchi, M., and Fujii, S. (1996) Cell 86, 311–319
6. Muller, G. P., and Wilson, S. H. (1997) Biochemistry 36, 4713–4717
7. Perry, R. (1982) in DNA Damage and Repair: DNA Repair in Higher Eukaryotes (Nickoloff, D., and Hoeckstra, M. F., eds) Vol. 2, pp. 33–49, Humana Press, Totowa, NJ
8. Xiao, W., and Chow, B. L. (1998) J. Genet. 97, 92–99
9. Chen, J., Derber, B., Maskati, A., and Samson, L. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7961–7965
10. LeDouc, S. P., Patton, N. J., Nelson, J. W., Boyer, V. A., and Wilson, G. L. (1990) J. Biol. Chem. 265, 14975–14980
11. Sechietha, D. A., and Hanawalt, P. C. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3050–3054
12. Sechietha, D. A., and Hanawalt, P. C. (1999) Mutat. Res. 423, 31–37
13. Wassermann, K., Kohn, K. W., and Boyer, V. A. (1990) J. Biol. Chem. 265, 13906–13913
14. He, M., Holmquist, G. P., and O’Connor, T. R. (1998) J. Mol. Biol. 284, 269–285
15. Smerdon, M. J., and Thoma, F. (1998) in DNA Damage and Repair: DNA Repair in Higher Eukaryotes (Nickoloff, D. K., and Hoeckstra, M. F., eds) Vol. 2, pp. 199–222, Humana Press, Totowa, NJ
16. Omari, S. (1990) Chromatin Structure during Transcription in the Yeast Saccharomyces cerevisiae. Ph. D. Thesis, Swiss Federal Institute of Technology, Zurich, Switzerland
17. Aboeh, S. E., Cibulski, A., Muliver, R. A., Rajalakshmi, S., and Sarma, D. S. R. (1980) Biochemistry 19, 1382–1387
18. Smerdon, M. J., and Thoma, F. (1999) Cell 61, 675–684
19. Li, S., and Waters, R. (1996) Carcinogenesis 17, 1549–1552
20. Li, S., Waters, R., and Smerdon, M. J. (1999) Methods Companion Methods Enzymol., in press
21. Maxam, A. M., and Gilbert, W. (1980) Methods Enzymol. 65, 499–560
22. Smerdon, M. J., Gupta, R., and Murad, A. O. (1995) in DNA Repair Mechanisms, Alfred Benzon Symposium 35 (Bohr, V. A., Wassermann, K., and Kraemer, K. H., eds) pp. 258–270, Humana Press, Totowa, NJ
23. Friedman, R. A., and Heneg, R. (1995) Biophys. J. 69, 1526–1535