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Differential repair of UV damage in *Saccharomyces cerevisiae*

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ABSTRACT

Preferential repair of UV-induced damage is a phenomenon by which mammalian cells might enhance their survival. This paper presents the first evidence that preferential repair occurs in the lower eukaryote *Saccharomyces cerevisiae*. Moreover an unique approach is reported to compare identical sequences present on the same chromosome and only differing in expression. We determined the removal of pyrimidine dimers from two identical α-mating type loci and we were able to show that the active *MAT*α locus is repaired preferentially to the inactive *HML*α locus. In a *sir-3* mutant, in which both loci are active this preference is not observed.

INTRODUCTION

In recent years evidence has been presented for the occurence of preferential repair of active genes after UV irradiation in mammalian cells. (For reviews see Hanawalt, 1987 and Bohr and Wassermann, 1988; 1,2) In most studies the repair of transcriptionally active genes, such as *DHFR*, in CHO and human cells was compared with the repair of either the genome overall or repetitive α-sequences upstream and downstream of the the active gene (3,4). In this way a repair domain was characterized within the *DHFR* gene of human cells (5). By comparing the transcriptionally active and inactive proto-oncogenes *c-abl* and *c-mos* differential repair in favour of the active *c-abl* gene was observed (6). Moreover active genes that are positioned close to the matrix are repaired preferentially (7).

With retrospect to the observation that UV-induced pyrimidine dimers block transcription—but not necessarily replication—in mammalian cells, preferential repair might be the molecular basis for the observation that inhibition of RNA synthesis is relieved well before pyrimidine dimers are completely removed from the genome overall (8). Regarding these data it was suggested that the survival of mammalian cells after UV damage might be dependent on the timely preferential repair of active, possibly matrix-bound, genes (7,9,10).

In this context it is interesting to know whether preferential repair occurs in the lower eukaryote *S. cerevisiae* in which pyrimidine dimers form a block for transcription as well as for replication (11,12), whereas also a much larger part of the genome is transcriptionally active than in higher eukaryotes.

To assay preferential repair in yeast we compared the removal of UV-induced damage from the mating type loci of yeast. Haploid yeast cells can be of two different mating types, **a** or α, which on mating produce **a**/α diploids, finally leading to sporulation. One mating type can be interconverted to the other in a process which is governed by the mating type cassette system. Three complete copies of the mating type loci are present on chromosome III (figure 1) of which only the *MAT* locus is expressed, whereas both *HML*α

and *HMRa* (the silent copies of *MATα* and *MAT***a** respectively) are repressed by the action of of the various *SIR* gene products that alter the chromatin structure (13,14,15,16). *HMLα* and *HMRa* serve as the donor sequences which can be transposed to the *MAT* expression site initiated by the HO recombinase, a process which is called mating type switching (17).

We used this system of two identical loci on the same chromosome (18) to study the removal of UV-induced damage from the silent *HMLα* in comparison to the active *MATα* in an α haploid strain. Using this assay it is shown that under normal conditions the active *MATα* locus is repaired preferentially to the inactive *HMLα* locus. Preferential repair was not observed in a *sir-3* mutant in which, due to derepression, both loci are active.

The model system described in this paper provides a new possibility to investigate the role of *RAD* gene products in the DNA metabolism of yeast.

MATERIALS AND METHODS

Yeast strains

Yeast strains used in thisı study are listed below:

Strain	*Genotype*	*Source*
K107	*α ho gal mal*	A. Klar (16)
K108	*αho gal mal sir-3*	A. Klar (16)

Culture medium

All strains were grown in complete medium (YEPD) as previously described by Yang and Friedberg (19).

DNA isolation

DNA was isolated according to Sherman et al (20) and purified on CsCl gradients (21).

UV irradiation

Exponentially growing yeast cells diluted in ice-cold PBS were irradiated with 254 nm UV (Philips T UV 30W) at a rate of 2 J/m^2/s. Subsequently cells were collected by centrifugation, resuspended in the original growth medium, and incubated for various times in the dark at 28°C prior to DNA isolation.

T4 endoV isolation

T4 endoV was isolated from *E.coli* cells containing a plasmid with the *DenV* gene that can be induced by IPTG (a kind gift of Dr.J.K. de Riel). The enzyme was purified according to Nakabeppu et al (22) to a purity of 95% as was shown by silverstained gels.

α specific probe

From a plasmid pAK5 (Dr.A. Klar) which contains the XhoI fragment of *HMLα*, we subcloned a ScaI fragment containing parts of X, Yα and Z1. Subsequently a DraI fragment, which is internal in Yα and strictly α-specific, was inserted into pUC19.

Southern blot analysis showing the removal of ESS

Genomic DNA was cut with restriction endonuclease HaeII which generates a 4.0 kb *MATα* fragment and aı 4.4 kb *HMLα* fragment. DNA samples were then divided in two equal parts, one of which was incubated with T4 endoV, and both were loaded on denaturing agarose gels according to Bohr et al (3). After electrophoresis the DNA was transferred to Genescreen plus (NEN) and hybridized with the α specific probe. The autoradiograms were scanned with a LKB ultroscan XL. The measurements (in absorption units: AU) were used to calculate the percentage of DNA which is not sensitive to T4 endoV with the following equation: % of DNA sensitive to T4 endoV = AU[fragment+T4 endoV] /AU[fragment-T4 endoV]. Corrections were made for the fact that the smaller *MATα*

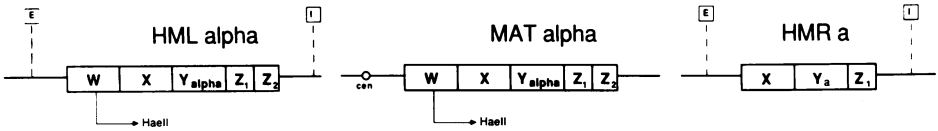

Fig 1. Schematic representation of the three mating type loci on chromosome III of *S.cerevisiae*. W, X, Z1 and Z2 are identical regions in all three loci. Two divergent transcripts, α_1 and α_2, start in Yα and run into resp. Z1 and the W/X border. The E and I boxes (silencers) are necessary for *SIR* mediated repression of the *HML* and *HMR* loci. In an α strain only the *MAT*α locus is transcriptionally active (13,14,25,28). Restriction with endonuclease HaeII generates a 4.4 kb fragment from the *HML*α locus and a 4.0 kb fragment from the *MAT*α locus. Figure is not drawn to scale. The loci are at least 150 kb apart with *MAT*α being in the middle of chromosome III close to the centromere (cen).

derived band is located in the smear of the *HML*α derived DNA upon T4 endoV fragmentation by subtracting the AU just above the *MAT*α band from the AU of the *MAT*α band. From the lanes containing DNA from irradiated cells that were not incubated to allow repair the initial amount of dimers per fragment is, according to the Poisson distribution, given by the $-\ln$ AU[fragment+T4 endoV]/ AU[fragment$-$T4 endoV].

RESULTS

Genomic DNA was isolated from UV irradiated cells after various post-irradiation incubation times. DNA was then digested with restriction endonuclease HaeII, subjected to electrophoresis on agarose gels to separate the *MAT*α and *HML*α fragments and visualized by hybridization with an internal Yα-specific probe. Restriction endonuclease HaeII generates *MAT*α and *HML*α fragments of 4.0 and 4.4 kb respectively. Since UV-induced pyrimidine dimers in DNA constitute T4 endonuclease sensitive sites (ESS) the process of repair of this type of damage can be monitored as the removal of ESS (3). The presence of ESS can be visualized on denaturing gels after T4 endoV incubation, resulting in cleavage of these fragments and subsequent loss of hybridization.

A pilot experiment using a wide range of UV doses showed the simultaneous disappearance of both the *MAT*α and *HML*α fragment when DNA was isolated immediately after UV irradiation (data not shown). This indicates that the initial number of dimers is the same in the *HML*α and *MAT*α loci and thus independent of the chromatin structure, as was also shown by others using different systems (3).

The removal of ESS after UV irradiation from the *HML*α and *MAT*α loci in the different strains used is shown in the figures 2 and 3. The differences indicated in these figures between *HML*α and *MAT*α fragments are a very accurate measure for differential repair of expressed and non-expressed genes as the genes are identical apart from expression. Moreover the fragments on which these genes are situated have undergone exactly the same experimental procedure and are shown in the same lane. The total amounts of removed ESS in these figures are however approximations since the genomic yeast DNA has not been prelabeled and slight variations may occur in the total amount of DNA loaded on the different lanes.(see also Discussion)

The removal of ESS from the *HML*α and *MAT*α loci in strain K107 after a UV dose of 150 J/m^2, resulting in 5% survival, is shown in figure 2a. From densitometric measurement (see Materials and Methods) the initial amount of dimers was 2.3 dimers per fragment. The 4.0 kb *MAT*α fragment is repaired for ca. 30% (also detected by

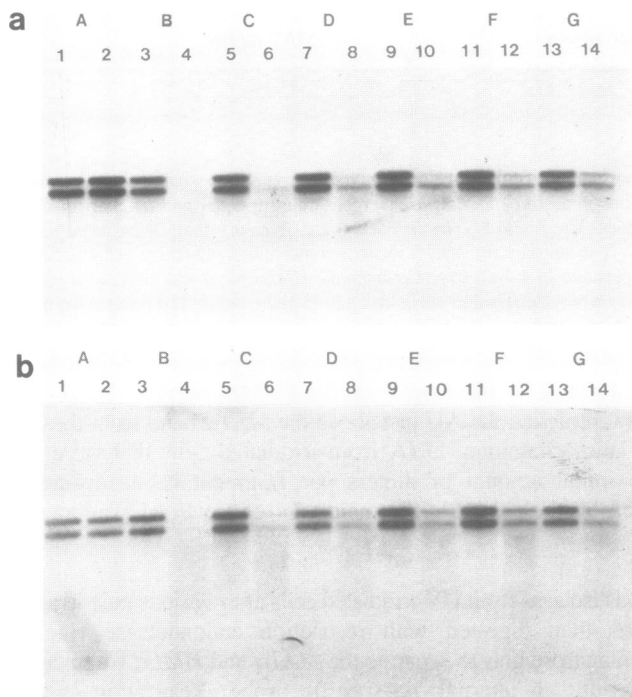

Fig 2a Southern blot analysis showing the removal of ESS (see text) from *HMLα* and *MATα* loci of *S.cerevisiae* strain K107 cells (16). The upper band is the *HMLα* fragment and the lower band the *MATα* fragment, resp. 4.4 and 4.0 kb. Sample A contains DNA from unirradiated yeast cells. Samples B to G contain DNA from 150 J/m^2 UV-irradiated yeast cells, that were incubated at 28°C to allow repair for increasing periods of time: B t=0^h; C t=2^h; D t=4^h; E t=5^h; F t=6^h and G t=9^h. Samples in all even lanes were pretreated with T4 endoV (+), as opposed to the odd lanes, which were not (−). The figure shows a typical result obtained with strain K107 under these conditions (repeated 4 times).
2b Southern blot analysis showing the removal of ESS from *HMLα* and *MATα* loci of *S.cerevisiae* K108 cells (*sir-3*; 16). For details see the legend of Fig 2a above. The experiment with K108 was repeated 4 times.

densitometry) after 2 hours post-irradiation incubation and repair reaches ca. 60% after 9 hours. In contrast the 4.4 kb *HMLα* fragment is repaired to a much lesser extent, i.e. 14% after 4 hours and 36% after 9 hours (figure 3a). Clearly, the pyrimidine dimers in the active *MATα* derived fragment are removed more rapidly than those in the inactive *HMLα* derived fragment.

We then examined the removal of pyrimidine dimers from *HMLα* and *MATα* in strain K108, a *sir-3* mutant of K107. Due to derepression in this strain both *HMLα* and *MATα* are active (as well as *HMR***a**) and presumably have the same chromatin structure. At all times after UV irradiation with a dose of 150 J/m^2, generating an initial amount of 2.7 dimers per fragment, the *MATα* and *HMLα* fragments are repaired to the same extent as shown in figures 2b and 3b. One could argue that the absence of differential repair of the *MATα* and *HMLα* fragments could be due to the simultaneous expression of **a** and α genes in the *sir-3* mutant, which causes a diploid-like phenotype as diploid-specific genes are activated. However we consider this as very unlikely as K107 and K108 have the same

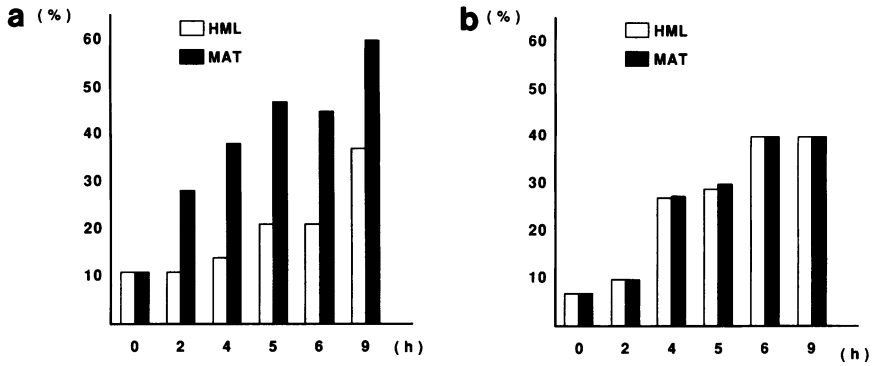

Fig 3 The figures 3a and 3b show the percentages removal of DNA resistant to T4 endoV from the *HMLα* and *MATα* fragments of strain K107 (3a:*SIR*) and K108 (3b:*sir-3*) at various post-irradiation incubation times. The percentages were obtained by scanning (using a LKB ultroscan XL) the autoradiograms shown in figures 2a and 2b respectively.

growth rate and show equal survival upon UV irradiation (results not shown). Therefore we conclude that differential repair in the *sir-3* strain K108 is absent because the *MATα* and *HMLα* loci are now both transcriptionally active.

DISCUSSION

The *MATα* locus codes for two transcripts, α_1 and α_2, which constitute the α phenotype. Due to repression by *SIR* gene products there is no transcription from the *HMLα* locus (16). Here we show that in the haploid α strain K107 upon UV irradiation the active *MATα* locus is repaired preferentially to the inactive *HMLα* locus. From figure 3a it can be estimated that for the *MATα* locus approximately 20% of the pyrimidine dimers are repaired in 2 to 3 hours, whereas for the *HMLα* locus approximately 20% of the dimers are repaired in 6 to 7 hours, after a possible delay of 2 hours. Thus there is a 2.5 fold difference in the rate of repair between the two loci in strain K107. This difference for transcribed vs. non-transcribed genes is less than reported for rodent genes but near that reported for human genes (1,2).

In the *sir-3* mutant K108 the *HMLα* locus is no longer repressed and as a consequence both *HMLα* and *MATα* are now transcriptionally active and presumably have the same conformation. Consequently both fragments were repaired to the same extent at all times after UV irradiation. The rate of removal of ESS from both loci in K108 is slightly slower than that of the *MATα* locus in K107,1 i.e.20% in 4 hours (without delay). This might be due to the higher initial amount of ESS in K108. Both the *MATα* and *HMLα* fragments in K108 do reappear much faster than the *HMLα* fragment in K107. It seems justified to conclude that the rate of removal of ESS from both loci in K108 is of the same order of magnitude as from the active *MATα* in K107. From these data it can be inferred that the preferential removal of the pyrimidine dimers from the *MATα* locus in the *SIR*$^+$ strain is correlated with this locus being transcriptionally active and is not due to the slight difference in size of the *HMLα* and *MATα* fragments.

In the *sir-3* strain the *HMRa* locus is also derepressed resulting in a diploid-like phenotype. However this change in cellular physiology does not affect the UV survival nor the growth rate. Moreover it has been shown that in a real diploid there is no apparent change in

chromatin structure of the *MAT* loci nor an effect on UV survival (16,24). Thus the enhanced repair of the *HMLα* in the *sir-3* mutant is the result of derepression of this locus and is not related to the diploid-like phenotype of the strain.

Here we observe differences in repair of two loci with an identical sequence which presumably only differ in their conformation—as induced by the SIR proteins via silencers—resulting in the presence or absence of transcription. Therefore the general conclusion seems warranted that transcribed genes situated in active and possibly more accessible chromatin are more rapidly repaired than DNA in non-transcribed regions of the yeast genome.

For the mammalian cell recovery of RNA synthesis shortly after UV irradiation is vital and preferential repair of specific loci might be a prerequisite for enhancing survival (9,10). In mammalian cells DNA replication seems to proceed despite the presence of pyrimidine dimers. In yeast however a very small number of non-repaired pyrimidine dimers is lethal to cells (23). Therefore it is crucial to yeast cells to remove all damage from the genome, active or not. Preferential repair of active genes in yeast could contribute to survival if the products of such genes are involved in the overall DNA repair process.

It is still an open question whether the observed preferential repair has evolved as an active system that recognizes damage in active genes because they are being transcribed or is just a consequence of the open more accessible chromatin structure of active genes. *Saccharomyces cerevisiae* offers the possibility to discriminate between the two alternatives since mutants have been described in which transcription has been blocked without affecting the chromatin structure (25). Such experiments are now in progress.

The system described here allows the screening of the *rad* mutants for differential repair which might enlarge insight into the role of *RAD* gene products in the DNA metabolism in yeast. In this respect the role of RAD6, which is known to be involved in chromatin formation (26) and RAD7, which is probably bound to the nuclear matrix (27), could be of interest.

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