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DNA damage activates transcription and transposition of yeast Ty retrotransposons

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Summary. A set of genes isolated from *Saccharomyces cerevisiae* showed increased transcript levels after yeast had been exposed to ultraviolet (UV) light or 4-nitroquinoline-1-oxide (4NQO). Included among these *D*NA *d*amage *r*esponsive (*DDR*) genes were members of the Ty retrotransposon family of yeast. Northern hybridization analysis indicated that maximal levels of a 5.6 kb transcript encoded by the Ty elements accumulated in cells after 4 to 6 h of exposure to 4NQO. The induced levels of transcripts varied from two- to tenfold for different Ty probes although similar kinetics and dose responses were observed for transcripts hybridizing to the different Ty family members. Pulse labeling experiments suggested that the accumulation of Ty transcripts was due, in part, to an increased rate of Ty message synthesis. Transposition of Ty elements to two target loci encoding distinct alcohol dehydrogenase enzymes, *ADH2* and *ADH4*, was examined in cells exposed to increasing doses of UV light or 4NQO. The frequency of Ty insertion into these genetic regions following DNA damaging treatments increased by as much as 17-fold compared with untreated cells. These results provide direct evidence that transposable elements can be activated by physical and chemical mutagens/carcinogens and that transpositional mutagenesis is induced by these agents in *S. cerevisiae*.

Key words: DNA damage – Ty retrotransposon – Transcription – Transposition

Introduction

Ty elements represent a multigene family in yeast consisting of approximately 35 members dispersed throughout the haploid genome. The intact elements contain direct repeats (δ regions) of approximately 330 bp flanking a central core region of 5.3 kb termed epsilon (ε region). Transcription initiates in the 5′ δ region, extends through the ε region and terminates in 3′ δ sequences (Elder et al. 1983). DNA sequence analysis has revealed that Ty elements share many of the structural and organizational features of mammalian retroviruses. For example, a reverse transcriptase is encoded by the more distal *tyb* open reading frame. The structure of this enzyme reveals significant homology to other reverse transcriptases (Clare and Farabaugh 1985). Moreover, expression of the *tyb* open reading frame depends upon a shift in translation frame by a mechanism that may be similar to that utilized in mammalian retroviral gene expression (Mellor et al. 1985).

Ty elements transpose in yeast at low frequency and Ty RNA has been shown to be a structural intermediate in the transposition process (Boeke et al. 1985). By fusing a single Ty element to the galactose-inducible *GAL1* promoter of yeast, it was possible to induce high levels of Ty transposition after growth in galactose. Moreover, this activation worked in *trans* in that other Ty elements located within the genome underwent increased movement.

Boeke et al. (1988) have recently demonstrated that not all Ty elements in yeast are functionally competent for transposition due to mutations and sequence alterations within these elements. Thus there appears to be considerable functional as well as DNA sequence heterogeneity among the members of this family although the δ elements are a highly conserved feature of all Ty elements examined (Roeder and Fink 1983).

When Ty elements transpose, they often insert into the 5′ regulatory regions of their target genes. The insertion of Ty elements upstream of the *CYC7, HIS3, HIS4, ADH2, ADH4* and *LYS2* genes results in altered expression of these loci (Williamson 1983). For example, an insertion of Ty into the 5′ noncoding region of the *CYC7* gene results in a 20-fold overproduction of the iso-2-cytochrome *c* respiratory protein (Errede et al. 1980). Similarly, Ty transposition into the regulatory regions of two distinct glucose repressible alcohol dehydrogenase loci, *ADH2* and *ADH4*, leads to constitutive expression of these genes (Paquin and Williamson 1986). In other instances, however, Ty transposition leads to decreased expression of neighboring genes (Roeder et al. 1980). In all of these cases, the regulatory effects result from increased (or decreased) levels of transcription of the affected genes which initiate at the normal start sites (Williamson 1983; Silverman and Fink 1984). In the case of *CYC7* activation by Ty, two short DNA sequences from the δ and ε regions have been shown to mimic the regulatory effects of the intact 5.9 kb Ty elements (Company and Errede 1987).

Exposure of yeast cells to a variety of DNA damaging agents stimulates transcription of a diverse set of genes, termed *DDR* genes (for *D*NA *d*amage *r*esponsive) (McClanahan and McEntee 1984). The initial characterization of some of these *DDR* genes indicated that they contained repetitive DNA sequences. Furthermore, Northern hybridization studies indicated that these repetitive *DDR* elements

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hybridized to a large 5.6 kb transcript, the size expected for Ty-related sequences. Recently, Rolfe et al. (1986) demonstrated that levels of Ty1 RNA increased in cells following irradiation with ultraviolet (UV) light. Taken together, these results argue that DNA damage or DNA damage-induced perturbations of DNA metabolism in yeast influence expression of the Ty retrotransposon family.

In this report, we have extended our original observations concerning activation of repetitive *DDR* genes by DNA damage and have examined additional features of Ty transcript accumulation following treatment with 4-nitroquinoline-1-oxide (4NQO). Because of the role of Ty RNA in its transposition, we have also investigated the effects of UV irradiation and 4NQO treatment on the frequency of Ty transposition to two distinct chromosomal regions encoding different alcohol dehydrogenase enzymes, *ADH2* and *ADH4*. The frequencies of Ty-induced mutations at these loci are stimulated up to 17-fold by UV irradiation and up to 12-fold by 4NQO exposure. These results argue that chemical and physical mutagens/carcinogens can stimulate transposition of retrotransposons in yeast.

Materials and methods

Yeast strains. The laboratory strains of *Saccharomyces cerevisiae* used were M12B (*α ura3–52 trp1–289 gal2*; Treger et al. 1988) 315-1D (*α trp1 ura1 adh1 – A1*; Paquin and Williamson 1984), and diploid strain AP1 (a/α *ade2-1*) and its asporogenous derivative AP1 (α/α *ade2-1*; Clancy et al. 1983). Strain 315-1D contains an integrated copy of plasmid YIpID36 at the *ADH1* locus which disrupts this gene.

Plasmids. Plasmid pBRS13Ty1 (Cameron et al. 1979) contains the entire S13Ty1 element cloned into pBR322. Plasmid B131Ty2 contains a Ty2 (Ty917) element cloned into pBR322. The pBR78A, pBR11A, pBR47E and pBR178C plasmids contain *Hin*dIII fragments of the *DDR78A, DDR11A, DDR47E* and *DDR178C* repetitive genes (McClanahan and McEntee 1984) cloned into the *Hin*dIII site of pBR322. Plasmid pBR322-*ADH2*-BS contains the *ADH2* gene and approximately 1.4 kb of upstream sequences cloned into the *Bam*HI site of pBR322 (Williamson et al. 1981). The plasmid pYA4-2 contains the *ADH4* gene on a 2.5 kb *Eco*RI-*Bam*HI fragment cloned into pUC8 (Paquin and Williamson 1986).

Chemicals. 4NQO was purchased from Sigma Chemical Company (St. Louis, Mo). A 1 mg/ml solution of 4NQO in absolute ethanol was prepared immediately prior to treatment of yeast cells. Antimycin A (Boehringer Mannheim) was present at a final concentration of 1 μg/ml in YPD plates.

Media. All media reagents were obtained from Difco Laboratories (Detroit, Mich). YPD plates contained 1% yeast extract, 2% Bacto-peptone, 2% glucose and 2% Bacto-agar. Liquid YP media were supplemented with either 2% glucose (YPD) or 5% glycerol (YPG).

UV irradiation and 4NQO treatment. Yeast strain 315-1D was grown overnight in YPG and diluted into YPD medium. After five doublings at 30° C or at a cell density of approximately 1×10^7 cells/ml, the cells were collected by centrifugation at room temperature, washed with 0.9% NaCl, and resuspended in 0.9% NaCl at a density of 10^7 cells/ml. Aliquots (25 ml) were irradiated in sterile plastic petri dishes (Falcon 1058) using a low pressure mercury lamp (254 nm). Cells were gently agitated during irradiation and were collected by centrifugation at room temperature (5000 *g*, 5 min). Survival of cells was measured by spreading dilutions of cells onto YPD plates. Antimycin A resistant mutants were selected on YPD plates containing 1 μg/ml antimycin A.

In preliminary experiments there was considerable variation (greater than 100-fold) in the frequency of spontaneous antimycin A resistant mutants. In four separate experiments the values ranged from 2.3×10^{-7} to 2.5×10^{-5}. A series of dilution and mixing experiments indicated that the efficiency of recovery of antimycin A resistant mutants decreased when the number of cells plated exceeded 10^7 cells/plate. In all the experiments reported here, no more than 5×10^6 cells were spread per plate in order to avoid this cell density artifact.

In order to determine whether the incubation period was important, mutants were examined after 7 and 14 days of incubation at 30° C. In several experiments, the number of antimycin A resistant mutants increased between 7 and 14 days. However, a molecular characterization of these strains indicated that the proportion of Ty induced mutations at *ADH2* and *ADH4* was the same in mutants examined at 7 days and at 14 days. The values reported here were obtained from antimycin A resistant mutants isolated after 14 days of growth at 30° C. The frequency of antimycin A resistant mutants was obtained by dividing the number of colonies growing on YPD-antimycin A plates by the number of colonies obtained on YPD plates and correcting for the dilution factor.

Yeast cultures growing exponentially in YPD medium at 30° C were exposed to 4NQO. The length of exposure and final concentration for individual experiments are given in the text. For preparation of RNA, 50 ml aliquots of 4NQO-treated or control cells were collected by centrifugation at 4° C, washed once in 0.9% NaCl and RNA extracted as described below.

Pulse labeling of yeast cells. Yeast strain M12B was grown to a density of $1–2 \times 10^7$ cells/ml in SD minimal medium supplemented with 24 μg/ml tryptophan and 10 μg/ml uridine. The 30 ml culture was divided into three equal aliquots and 4NQO was added to two of the samples to a final concentration of either 0.5 μg/ml or 1.0 μg/ml. [^{3}H]Uridine (2 mCi, ICN) was added to each of the cultures and incubation was continued at 30° C with vigorous shaking. Cells were collected by centrifugation (5000 rpm, 5 min), washed with 0.9% NaCl and either frozen in dry ice/ethanol or used immediately for RNA isolation. Labeled RNA was extracted by glass bead disruption (McClanahan and McEntee 1984) and used to hybridize to S13Ty1 DNA that had been immobilized on nitrocellulose filters as described (Zitomer et al. 1979).

Isolation of RNA. Total cell RNA was extracted from control and drug-treated cells by glass bead disruption. Purified RNA was stored at −70° C in distilled water. Poly(A)-containing RNA was purified by chromatography on oligo(dT) columns as described (Aviv and Leder 1972).

Northern hybridization analysis. Total RNA (10–100 μg) or poly(A)-containing RNA (4 μg) was fractionated by elec-

trophoresis in formaldehyde-agarose (1%) gels, transferred to nitrocellulose and hybridized with nick-translated probes as described (McClanahan and McEntee 1984).

Isolation of DNA from antimycin A resistant mutants. Individual antimycin A resistant colonies were repurified on selective media and inoculated into YPD medium (5 ml). After 2–4 days of growth of 30° C, cells from the saturated culture were collected by centrifugation (5000 *g*, 5 min) and DNA was extracted using the procedure of Holm et al. (1986).

Southern hybridization analysis. Genomic DNA samples (10–40 μg) were digested to completion with a two- to four-fold excess of *Hin*dIII or *Sac*I restriction enzyme over a period of 4 to 6 h of incubation at 37° C. The digested DNAs were size fractionated by electrophoresis in 0.7% agarose gels. The conditions for electrophoresis, transfer of DNA to nitrocellulose and hybridization with nick-translated plasmid probes were essentially as described by Maniatis et al. (1982).

Results

The repetitive DDR *genes are members of the Ty gene family*

The *DDR* genes were originally isolated from two differential hybridization screenings of yeast genomic DNA (McClanahan and McEntee 1984). In the first screening, mRNA was prepared from yeast cells that had been irradiated with UV light (254 nm) and allowed to recover for 60 min before RNA was prepared. It was anticipated that genes responding rapidly to DNA damage would be represented in this experiment. We have described two single copy *DDR* genes, *DDRA2* and *DDR48*, which were isolated in this screening (McClanahan and McEntee 1986). A second screening was performed using cDNA probes prepared from mRNA of 4NQO-treated yeast cultures. However, unlike the first experiment, the cells were exposed to the mutagen/carcinogen for 6 h prior to RNA extraction. This experiment yielded several *DDR* clones which hybridized to large (5.6 kb) polyadenylated RNAs that were two- to tenfold more abundant in 4NQO-treated cells than in control cultures. These *DDR* clones also hybridized to several (greater than ten) genomic restriction fragments indicating that they contained repetitive DNA sequences (McClanahan and McEntee 1984). Because the well-characterized Ty elements encode transcripts of similar size and are present in the yeast genome in multiple copies, a hybridization study was performed to investigate the relationship of the repetitive *DDR* genes to Ty elements. In this experiment the Ty-containing plasmid pBRS13Ty1 was digested with *Eco*RI and *Sal*I and the fragments separated by electrophoresis in a 1% agarose gel and analyzed by Southern hybridization. The double digestion of the insert produces three fragments with sizes of 1.3, 1.6 and 2.5 kb. Because of the rearrangement of Ty sequences in this clone, only the 2.5 kb *Sal*I fragment contains δ sequences whereas the 1.3 and 1.6 kb restriction fragments are derived from the ε core region (Cameron et al. 1979). The results obtained using different *DDR* genes as probes are tabulated in Table 1. Three of five *DDR* clones hybridized exclusively to the 2.5 kb fragment containing δ sequences. In addition, the *DDR11A* clone hybridized to both the 2.5 and 1.6 kb fragments indicating more extensive homology within the ε region. Surprisingly, the *DDR78A* clone did not hybridize with any of the Ty restriction fragments. Furthermore the *DDR78A* probe did not hybridize with the Ty2 clone, B131, which was recognized by four other *DDR* clones. These results suggest that the *DDR11A, DDR83D, DDR47E* and *DDR178C* clones are derived from the δ regions of Ty elements.

Table 1. Hybridization between repetitive *DDR* sequences and Ty elements

DDR probe (kb)	Ty DNA			
	S13Ty1 (1.3)	S13Ty1 (1.6)	S13Ty1 (2.5)	B131Ty2
DDR11A (4.5)	−	+	+	+
DDR83D (2.7)	−	−	+	+
DDR78A (0.5)	−	−	−	−
DDR178C (2.0)	−	−	+	+
DDR47E (1.4)	−	−	+	+

The *DDR* probes were radiolabeled by nick translation and hybridized to Ty1 or Ty2 plasmid DNAs. S13Ty DNA was digested with *Eco*RI and *Sal*I and fractionated on 1% agarose gels. The S13Ty1(1.3), S13Ty1(1.6) and S13Ty1(2.5) DNAs are the 1.3, 1.6 and 2.5 kb *Sal*I-*Eco*RI fragments derived from S13Ty1. The B131Ty2 DNA contained an entire Ty2 element. +, strong hybridization; −, no hybridization. The sizes of the restriction fragments are indicated in parentheses

Although cross hybridization suggested that four of five repetitive *DDR* genes were derived from Ty elements, it was not possible to establish a relationship between the *DDR78A* fragment and the Ty gene family by Southern analysis. Therefore, the 0.5 kb insert in pBR78A was sequenced using the Sanger dideoxy sequencing method. These results indicated that the *DDR78A* clone contained a 547 bp insert that shared 75% sequence identity with the δ elements of Ty (data not shown). Moreover this element contained many of the sequences that have been identified as functionally important in δ elements (McEntee and Bradshaw 1988). These results demonstrate that the repetitive *DDR* sequences, which were isolated based upon their increased transcript levels in 4NQO-treated cells, are members of the Ty retrotransposon family.

Characteristics of Ty transcript accumulation after 4NQO treatment

The accumulation of Ty-related transcripts was examined in yeast cells exposed to 4NQO for 6 h with different Ty (*DDR*) clones used as hybridization probes. As shown in Fig. 1A, there is a significant increase in the levels of transcripts hybridizing to the 78A, S13Ty1 and 178C probes. The relative increase in the level of the Ty transcript depended to some extent upon the DNA probe used and ranged from two- to tenfold when normalized to levels of transcripts homologous to a *HIS4* probe (McEntee and Bradshaw 1988; and data not shown). In the case of the different *DDR* clones, differences in transcript levels were more readily observed using poly(A)-containing RNA for Northern analysis (compare blots 1 and 2 in Fig. 1A).

The results shown in Fig. 1A demonstrate that increased levels of Ty transcripts accumulate after exposure of haploid yeast cells to 4NQO. As shown in Fig. 1B, in-

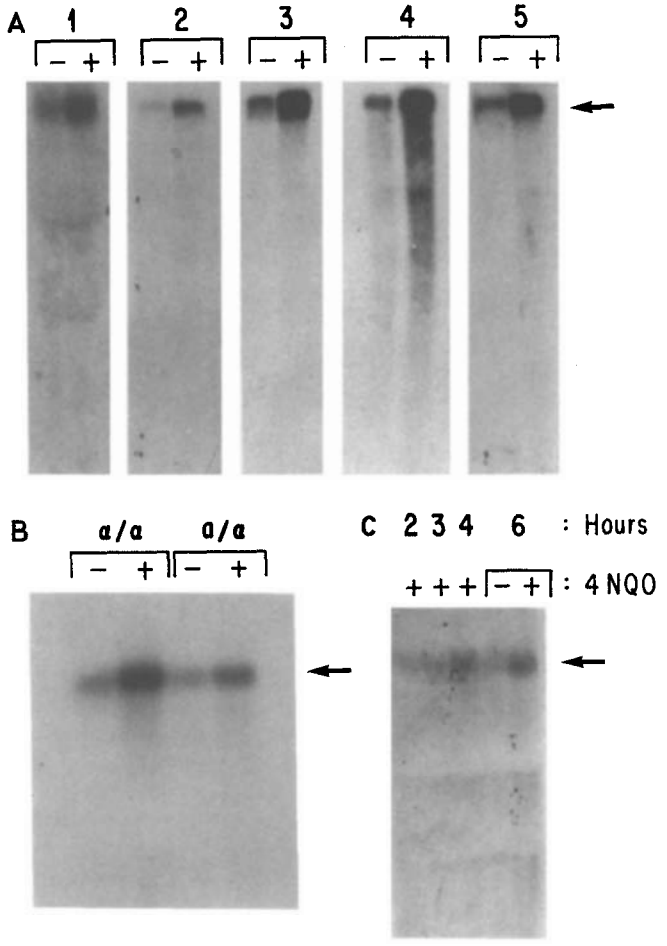

Fig. 1A–C. Induction of Ty (*DDR*) transcripts after exposure to 4-nitroquinoline-1-oxide (4NQO). **A** Yeast strain M12B was grown to a density of 10^7 cells/ml in YPD medium, divided into two portions and incubated for 6 h with (+) or without (−) the mutagen/carcinogen 4NQO (1.5 μg/ml). RNA was isolated from cells by glass bead disruption, size fractionated by electrophoresis in formaldehyde-agarose (1%) gels and transferred to nitrocellulose. In panels 1 and 3, total RNA (100 μg) was hybridized with nick-tanslated probes pBR78A and pBRS13Ty1, respectively. In panels 2, 4 and 5, poly(A)-containing RNA (4 μg) was hybridized with nick-translated pBR78A (panel 2), pBRS13Ty1 (panel 4) and pBR178C (panel 5). **B** Diploid strains API α/α and API a/α were grown to a density of 10^7 cells/ml. Half of each culture was treated with 4NQO (1.5 μg/ml, 7 h). RNA was isolated and samples (25 μg) were fractionated and analyzed as described above using nick-translated pBRS13Ty1 as the hybridization probe. **C** Strain M12B was grown in YPD medium to a density of 10^7 cells/ml and treated with 4NQO (1.5 μg/ml). Aliquots were removed at the indicated times and RNA was prepared and analyzed as described above using nick-translated pBR47E as the hybridization probe. The position of the 5.6 kb Ty transcript is indicated by an *arrow*; +, 4NQO-treated; −, control. Control experiments using an *HIS4* transcript specific probe were performed to ensure that comparable amounts of RNA were loaded in each lane

creased levels of Ty transcripts accumulated in diploid cells after 4NQO exposure although the relative increase was two- to threefold lower in a/α than that observed in isogenic α/α asporogenous cells.

The kinetics of transcript accumulation was examined in strain M12B after addition of 1.5 μg/ml 4NQO. The levels of RNA hybridizing to the Ty-related sequence pBR47E were unchanged for 2–3 h of exposure. However, after 4 h of treatment the level of transcripts increased and remained high after 6 h of 4NQO exposure (Fig. 1C). Qualitatively similar kinetics of transcript accumulation have been observed using two other Ty (*DDR*) genes as probes of Northern blots (data not shown).

Table 2. Pulse labelling of Ty-specific RNA in control and 4NQO-treated cells

Treatment	RNA (μg)	Specific activity (cpm/μg) $\times 10^{-5}$	Cpm hybridized	Fraction bound $\times 10^2$
Control	10	7.24	9136	1.26
4NQO (0.5 μg/ml, 2 h)	10	8.37	13216	1.29
4NQO (1 μg/ml, 2 h)	10	7.98	15138	1.82
4NQO (1 μg/ml, 4 h)	10	2.36	7153	3.02

Cultures of yeast strain M12B were grown in the absence or presence of 4NQO. At the indicated times, cells were labeled for 30 min with [^{3}H]uridine (200 μCi/ml) and labeled RNA was extracted as described in Materials and methods. The indicated amount of ^{3}H-labeled RNA was hybridized to nitrocellulose filter discs containing denatured pBRS13Ty1 DNA. Non-specific hybridization was determined using pBR322 DNA bound to filters. The 'cpm hybridized' is the value corrected for nonspecific hybridization to the pBR322 DNA filters

Increased rate of Ty transcription in 4NQO-treated cells

The increase in Ty transcript levels after 4NQO exposure could be due to an increased rate of Ty RNA synthesis, a decreased rate of Ty RNA turnover or a combination of both effects. In order to determine whether the rate of transcription of Ty was affected by exposure to 4NQO, we pulse labeled yeast cells with [^{3}H]-uridine for 30 min intervals at different times following 4NQO addition and examined the levels of newly synthesized Ty-specific RNA using solution hybridization. The results of this experiment are shown in Table 2. A significant fraction of labeled RNA (1.26%) in control cells hybridized to the S13Ty1 DNA on filters, consistent with the fact that approximately 10% of yeast mRNA is Ty transcript based upon Northern analysis of steady-state mRNA levels (Elder et al. 1980). In cells treated with 1 μg/ml 4NQO the amount of pulse-labeled RNA hybridizing to the S13Ty DNA increased to 1.82% at 2 h and to 3.02% at 4 h. Cells treated with a suboptimal concentration of 4NQO (0.5 μg/ml) showed only a slight increase in Ty transcript levels after 2 h of exposure. These results are consistent with Northern hybridization results demonstrating that the maximum levels of Ty RNA accumulate after 4 h of 4NQO exposure. These results support the idea that the increase in Ty transcript levels following 4NQO exposure was due at least in part to increased rates of Ty transcription.

Increased Ty transposition in UV-irradiated yeast

It has been demonstrated by Williamson and coworkers that Ty transposes to two alcohol dehydrogenase genes, *ADH2* (formerly *ADR2*) and a newly described locus, *ADH4*, which encodes a structurally distinct enzyme (Wil-

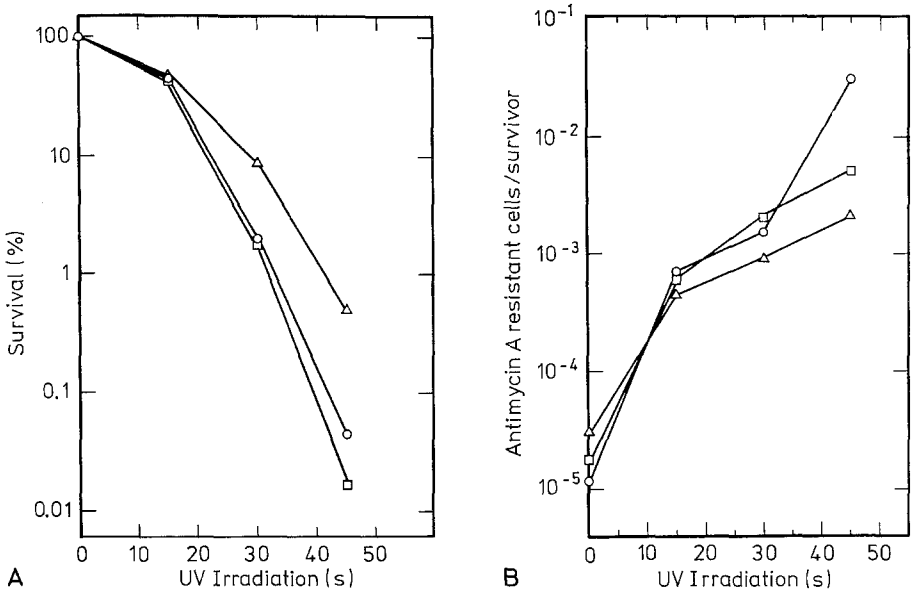

Fig. 2A and B. Survival and mutagenesis of yeast strain 315-1D following UV irradiation. Three independent cultures of strain 315-1D were grown in YPG medium to a density of approximately 10^7 cells/ml, collected by centrifugation and resuspended in 0.9% NaCl for UV irradiation. Aliquots were removed after the indicated time of UV exposure and dilutions were spread onto YPD plates to determine cell survival (**A**) and onto YPD plates containing 1 μg/ml antimycin A to measure mutagenesis (**B**). Plates were incubated in the dark at 30° C for 14 days. Independent cultures are indicated by different symbols (o, □ or △)

liamson and Paquin 1987). Under normal growth conditions, the *ADH2* gene is repressed by the presence of glucose and is expressed only during growth on nonfermentable carbon sources (Ciriacy 1975). However, in strains carrying Ty elements transposed to the *ADH2* regulatory region, this gene is expressed at high levels even in the presence of glucose. Similarly, Ty transposition to the *ADH4* locus increases expression of the *ADH4* gene. Such regulatory mutants can be isolated from strains lacking the normally constitutive alcohol dehydrogenase *ADH1* (*adh1*) by selecting for cells that grow fermentatively in glucose medium (YPD) containing the respiration inhibitory antimycin A. Paquin and Williamson (1986) have shown that a significant fraction of spontaneous antimycin A-resistant mutants contain Ty elements transposed to the *ADH2* or the *ADH4* locus.

In order to determine whether DNA damaging agents induced antimycin A resistance in yeast, a strain containing an integrative disruption of the *ADH1* gene (*adh1-1*) was exposed to increasing doses of 254 nm UV light from a low pressure Hg lamp. Survival of strain 315-1D was measured by spreading aliquots of diluted cells onto YPD plates, and mutants were selected by spreading irradiated cells onto YPD plates containing antimycin A (see Materials and methods). The results of these experiments (Fig. 2) indicated that strain 315-1D was sensitive to killing by UV irradiation and showed a dose-dependent increase in the frequency of antimycin A resistant mutants. Although exposure to UV for 30 s resulted in only 1% survival, approximately 0.1% of the survivors grew on antimycin A plates. Based upon these survival data, strain 315-1D appeared to be slightly more sensitive to killing by UV irradiation than strain M12B (data not shown).

In order to determine whether the stimulation of antimycin A resistance by UV was due to increased Ty transposition to *ADH2* or *ADH4*, genomic DNA samples were prepared from 405 antimycin A resistant mutants derived from 5 independent UV irradiation experiments. This sample included 70 independent spontaneous antimycin A resistant mutants. Each of the DNA samples was digested with excess *Hin*dIII restriction enzyme and fractionated by agarose gel electrophoresis. After transfer to nitrocellulose, the DNA on the filters was hybridized with radiolabeled *ADH2* or *ADH4* probes. The genomic hybridization patterns from 14 UV-induced antimycin A resistant mutants are shown in Fig. 3. Analysis of the *ADH2* region in these mutants (Fig. 3A) indicated that several of the strains showed alterations in the 3.5 kb *Hin*dIII fragment containing the upstream regulatory region. Those mutants that had lost the 3.5 kb *Hin*dIII fragment contained either one large fragment (approximately 9.5 kb, see lanes 7, 8, 10 and 11) or two new restriction fragments (lane 4). The sizes of the new restriction fragments were approximately 3.7 and 5.8 kb, and are consistent with the insertion of a Ty element containing a single *Hin*dIII site into the upstream region.

Analysis of the *ADH4* region of these same UV-induced antimycin A resistant mutants is shown in Fig. 3B. In 9 of the 14 mutants shown, the 3.0 kb upstream *Hin*dIII fragment was absent and one or more new restriction fragments were present. In some strains, however, *Hin*dIII digestion did not liberate any novel-sized fragments although the 3.0 kb fragment was absent (lane 12). We examined the *Sac*I restriction pattern of 5 independent mutants which lacked the 3.0 kb *Hin*dIII fragment (Fig. 4). In lanes 1 through 4 are digests of antimycin A resistant strains containing a normal 3.0 kb upstream fragment at *ADH4*. Digestion with *Sac*I produces two fragments of approximately 9.5 and 10.5 kb. The 9.5 kb fragment is derived from the plasmid-disrupted *ADH1* chromosomal locus and the 10.5 kb fragment is derived from the *ADH4* region. In lanes 5–9 are shown *Sac*I digests of genomic DNA from 5 independent antimycin A resistant strains which lacked the 3.0 kb upstream fragments of the *ADH4* region. Instead of the normal 10.5 kb fragment, these strains contained a new fragment of approximately 16 kb. The difference in the size of these large fragments, approximately 5.5 kb, is consistent with the presence of a Ty element upstream of *ADH4* in these mutant strains. As expected, the 9.5 kb fragment, derived from the *ADH1* region was unchanged in size in the antimycin A resistant mutants.

The alteration in the sizes of the upstream *Hin*dIII restriction fragments in the UV-induced antimycin A resistant strains was consistent with the insertion of Ty elements within the 5′ regions of either the *ADH2* or *ADH4* locus

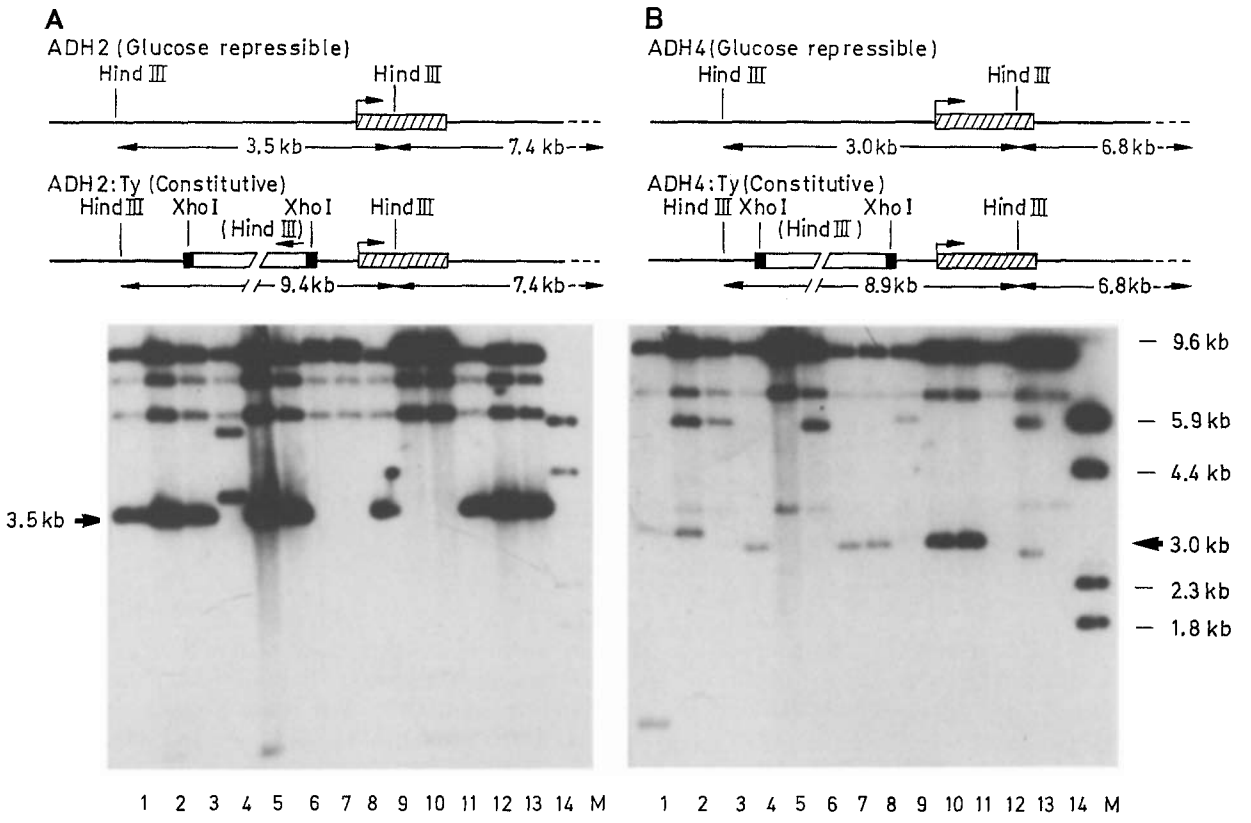

Fig. 3A and B. Southern hybridization analysis of UV-induced antimycin A resistant mutants containing rearrangements of the *ADH2* or *ADH4* chromosomal regions. Genomic DNA was prepared from 14 independent UV-induced antimycin A resistant mutants and samples (20 μg) were digested with *Hin*dIII as described in Materials and methods. Following fractionation by electrophoresis in agarose gels (0.7%), the DNAs were transferred to nitrocellulose and hybridized with a nick-translated *ADH2* probe, pBR-*ADH2*-BS (**A**) or an *ADH4* probe, pYA4-2 (**B**). The 3.5 kb *Hin*dIII fragment containing the *ADH2* promoter region is indicated by an *arrow* in **A** and the 3.0 kb promoter-containing *Hin*dIII restriction fragment is indicated by an *arrow* in **B**. Hybridization to the 9.5 kb *Hin*dIII fragment in **A** and **B** is due to homology between vector sequences and the pBR322 DNA sequences contained within the chromosomal *ADH1* gene disruption. Lane M contains molecular weight markers. The structures of the *ADH2* and *ADH4* regions are diagrammed at the top. The coding regions are designated by the *crosshatched boxes* and the Ty elements are represented by *open rectangles*. Relevant restriction sites are shown

in these mutants. Additional evidence for Ty transposition to these loci was obtained following *Xho*I restriction enzyme digestion. There are no *Xho*I sites in the upstream *Hin*dIII fragments of either the *ADH2* or the *ADH4* locus. However, *Xho*I sites are located in the highly conserved δ regions of Ty elements and transposition of Ty into the 5′ regions would introduce *Xho*I sites upstream of the *ADH2* and *ADH4* genes. As shown in Fig. 5A, B, double digestion of DNAs prepared from ten antimycin A resistant mutants demonstrated that the upstream regions of *ADH2* and *ADH4* in these mutant strains contained *Xho*I sites that were absent in the parental DNA. An examination of the restriction patterns further demonstrated that Ty transposes to a variety of sites upstream of *ADH4* whereas the sites of insertion into the *ADH2* locus appeared to be considerably more limited based upon the size range of the *Hin*dIII-*Xho*I restriction fragments. This latter result is consistent with the observation that the majority of *ADH2* constitutive mutations are caused by Ty transposition to the region between −210 to −125 relative to the start of translation (Williamson et al. 1983). Thus based on the sizes of the rearranged *ADH2* and *ADH4* regulatory regions in the antimycin A resistant mutants as well as the introduction of one or more *Xho*I restriction sites into these fragments, our results support the conclusion that Ty insertions account for a readily detectable fraction of UV-induced antimycin A resistant mutants.

Results obtained from Southern analysis of the structure of the *ADH2* and *ADH4* regions of mutants derived from five independent UV irradiation experiments are presented in Table 3. The data indicate that following UV treatment, the frequency of Ty transposition to the *ADH2* and *ADH4* loci *increased* relative to the unirradiated samples. For example, Ty insertions at *ADH2* accounted for 22% of spontaneous antimycin A resistant mutants (Table 3, experiment 1); after 15 and 30 s of UV irradiation Ty insertions accounted for 4% and 5%, respectively, of the drug resistant mutants. Nevertheless, the frequency of transposition to *ADH2* increased by 5.6-fold after 15 s of irradiation and to 11.2-fold after 30 s of UV treatment relative to the unirradiated cells. Although some variability in the induction of Ty transposition to *ADH2* and *ADH4* is observed particularly at high doses, we have detected as much as a 17-fold increase in the frequency of transposition to *ADH4* and more than an 11-fold stimulation of transposition to *ADH2* following UV irradiation.

One possible explanation for these results is that preexisting mutant cells containing Ty inserted at *ADH2* or *ADH4* were more resistant to killing by UV light and were enriched during the irradiation. This possibility was exam-

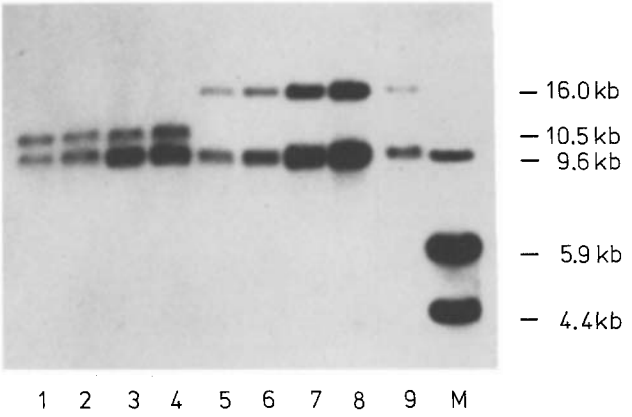

Fig. 4. Southern hybridization analysis of the yeast *ADH4* region in UV-induced antimycin A resistant mutants. Genomic DNAs, purified from five independent antimycin A resistant mutants which lacked a 3.0 kb *Hin*dIII restriction fragment from the *ADH4* region (see Fig. 3) were digested with *Sac*I, fractionated by elelctrophoresis in an agarose gel (0.7%), transferred to nitrocellulose and hybridized with nick-translated probe pYA4-2. Lanes 1–4, DNA from strains containing a 3.0 kb upstream *Hin*dIII restriction fragment; lanes 5–9, DNA from antimycin A resistant strains lacking a 3.0 kb upstream *Hin*dIII restriction fragment. The 9.5 kb *Sac*I fragment present in all lanes is due to hybridization between the vector sequences and the pBR322 DNA contained within the *ADH1* gene disruption in strain 315-1D. Lane M contains molecular weight markers

ined directly by measuring survival of parental strain 315-1D and two antimycin A resistant mutants, one containing Ty transposed to *ADH2* and the other containing a Ty insertion at *ADH4*. These results indicated that the strains containing Ty transposed to *ADH2* or *ADH4* were as sensitive as the parental strain to killing by UV irradiation (data not shown). Thus the UV exposure likely induced the Ty transpositions rather than enriched for preexisting mutants in the culture.

Recently, we have reported that treatment of yeast cells with 4NQO results in stimulation of Ty transposition (McEntee and Bradshaw 1988). As shown in Fig. 6, the frequency of Ty transposition to either *ADH2* or *ADH4* increased in a dose-dependent manner following treatment of strain 315-1D with 4NQO. As with UV treatment, 4NQO exposure induced a small fraction of Ty transpositions and

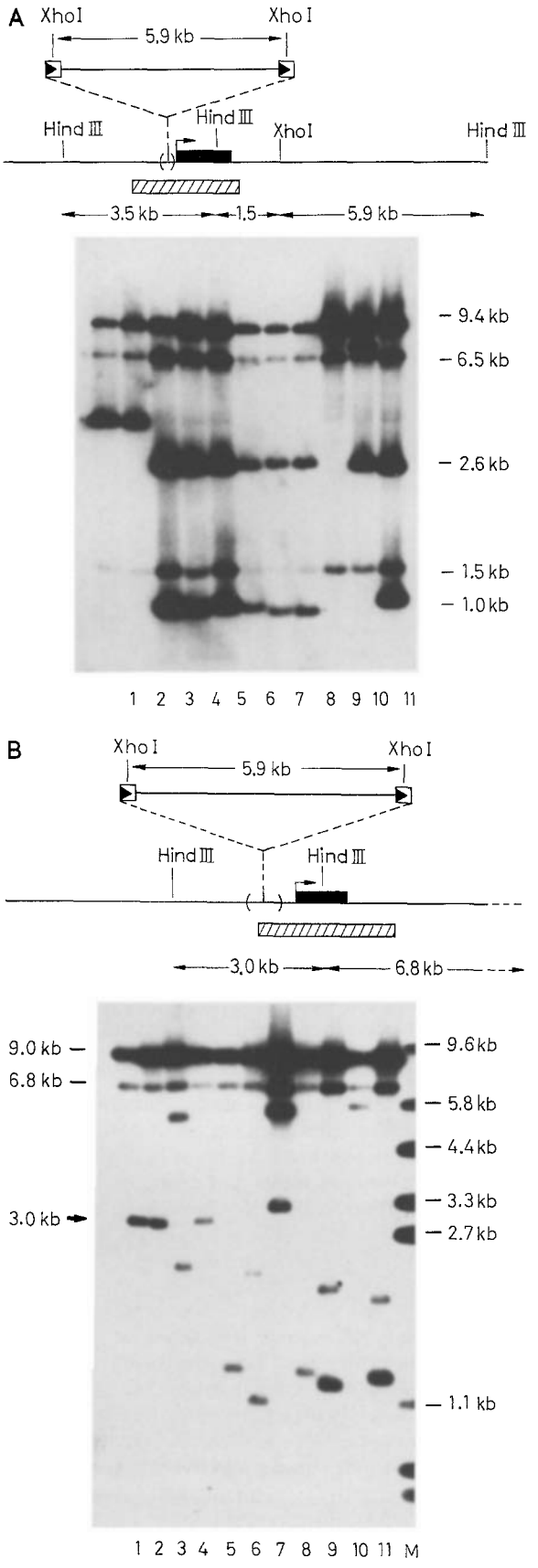

Fig. 5A and B. The rearranged *ADH2* and *ADH4* regions of UV-induced antimycin A resistant mutants contain *Xho*I restriction sites. Genomic DNAs from 18 independent UV-induced antimycin A resistant mutants containing alterations in their *Hin*dIII digestion patterns in the *ADH2* region (**A**) or the *ADH4* region (**B**) were digested with both *Hin*dIII and *Xho*I restriction enzymes and analyzed by Southern hybridization as described in Materials and methods. In **A** and **B**, lanes 1 and 2 are DNAs from parental strain 315-1D and an antimycin A resistant derivative with unrearranged *ADH2* and *ADH4* chromosomal region, respectively. In **A**, lanes 3–11 contain DNAs from independent mutants containing rearranged *ADH2* genomic regions whereas in **B** lanes 3–11 contain DNAs from strains with alterations at the *ADH4* locus. The filter in **A** was hybridized with the *ADH2* probe pBR322-*ADH2*-BS and that in **B** was hybridized with the *ADH4* probe, pYA4-2. The parental 3.5 kb upstream band from the *ADH2* locus (**A**) and the corresponding 3.0 kb fragment from the *ADH4* region (**B**) are indicated by *arrows*. The high molecular weight restriction fragments hybridizing to the *ADH2* and *ADH4* probes are due to pBR322 homology contained within the chromosomal *ADH1* gene disruption in strain 315-1D. A Ty element is shown above the *ADH2* (or *ADH4*) region in the diagrams at the top. The coding regions are indicated by the *solid black boxes* and the hybridization probes are indicated by the *crosshatched rectangles* below the line

Table 3. Frequency of Ty-induced mutations at the *ADH2* and *ADH4* genes following UV irradiation

Exposure time (s)	Survival (%)[a]	Ant A^r/ viable cell[b]	Frequency of transposition[c]	
			ADH2	*ADH4*
Expt. 1				
0	100	1.8×10^{-5}	4.0×10^{-6}	9.0×10^{-6}
15	46	6.1×10^{-4}	2.3×10^{-5} (5.6)	2.3×10^{-5} (3.0)
30	2	2.0×10^{-3}	4.6×10^{-5} (11.2)	$<4.6\times10^{-5}$
Expt. 2				
0	100	3.8×10^{-6}	8.7×10^{-7}	1.9×10^{-6}
15	36	1.9×10^{-4}	5.3×10^{-6} (6.1)	1.1×10^{-5} (5.8)
30	2	1.0×10^{-3}	$<7.1\times10^{-5}$	$<7.1\times10^{-5}$
Expt. 3				
0	100	1.2×10^{-5}	2.8×10^{-6}	6.0×10^{-6}
15	36	7.1×10^{-4}	1.5×10^{-5} (5.4)	$<1.5\times10^{-6}$
Expt. 4				
0	100	2.9×10^{-5}	6.7×10^{-6}	1.5×10^{-5}
15	48	4.6×10^{-4}	N.D.	N.D.
30	9	9.2×10^{-4}	1.5×10^{-5} (2.2)	$<1.5\times10^{-5}$
45	0.5	2.3×10^{-3}	$<2.6\times10^{-4}$	2.6×10^{-4} (17.3)
Expt. 5				
0	100	6.0×10^{-5}	1.5×10^{-5}	2.9×10^{-5}
15	40	1.6×10^{-3}	9.4×10^{-5} (6.3)	3.4×10^{-4} (11.7)

Yeast strain 315-1D was exposed to the indicated doses of UV light and antimycin A resistant mutants were selected on YPD-antimycin A (1 μg/ml) plates. Small scale DNA isolations were performed on 10–40 independent antimycin A resistant mutants from each UV dose and the presence of Ty at *ADH2* or *ADH4* was determined by Southern hybridization analysis

[a] Survival (%) is the number of colony forming units (measured on YPD plates) after the indicated UV dose relative to the untreated control

[b] Antimycin A resistant mutants per viable cell, is the number of cells growing on YPD-antimycin A plates divided by the number of survivors

[c] The frequency of transposition is expressed as the antimycin A resistant mutant frequency multiplied by the fraction of mutants that showed the appropriate structural alterations at the *ADH2* or *ADH4* locus. The numbers in parentheses indicate the induced transposition frequency after UV treatment relative to the unirradiated control value. N.D. not determined

a larger fraction of antimycin A resistant mutants showing no significant structural alterations of the *ADH2* or *ADH4* upstream regions. These mutations might have arisen from base substitution events in either the upstream regions of these genes or in genes encoding *trans*-acting regulatory factors.

Discussion

Several of the yeast DNA damage responsive or *DDR* genes isolated in this laboratory have been shown to contain repetitive DNA sequences (McClanahan and McEntee 1984). In this report, evidence is presented indicating that the repetitive *DDR* genes are members of the Ty retrotransposon family. This conclusion is based upon hybridization studies using a well-characterized Ty element (S13) as well as by DNA sequence analysis. Furthermore, following 4NQO exposure, elevated levels of a 5.6 kb transcript that hybridized to a bona fide Ty element (S13) were detected in yeast cells. This latter result is consistent with the report of Rolfe et al. (1986) that Ty-homologous transcripts are elevated in UV-irradiated cells.

The kinetics of Ty transcript accumulation following 4NQO exposure differed significantly from that of the single copy *DDR* genes, *DDRA2* and *DDR48* (McClanahan and McEntee 1986). By Northern hybridization studies, the levels of Ty-related transcripts were seen to increase after 4 to 6 h of 4NQO treatment. These maximal increases in RNA levels were detected following exposure to concentrations of 4NQO between 1.0 and 2.0 μg/ml (data not shown). This increase in the levels of cellular Ty transcript appeared to be due, at least in part, to an increased transcription rate of Ty elements based upon the pulse-labeling results shown in Table 2. The amount of Ty-specific pulse-labeled RNA isolated from haploid cells increased more than two-fold after exposure to 1 μg/ml 4NQO for 4 h. Moreover, increased levels of Ty transcripts were detected by Northern analysis in a/α diploid cells following 4NQO treatment, although the increase was less than in the asporogenous α/α diploid strain that does not repress Ty expression. Thus the effects of DNA damage can partly overcome the transcriptional regulatory effects of the a_1/α_2 complex by a mechanism which is at present unknown.

In eukaryotes, the regulation of genes by xenobiotic agents is usually mediated through control elements located upstream of the responsive gene. In the cases of the *DDR2*

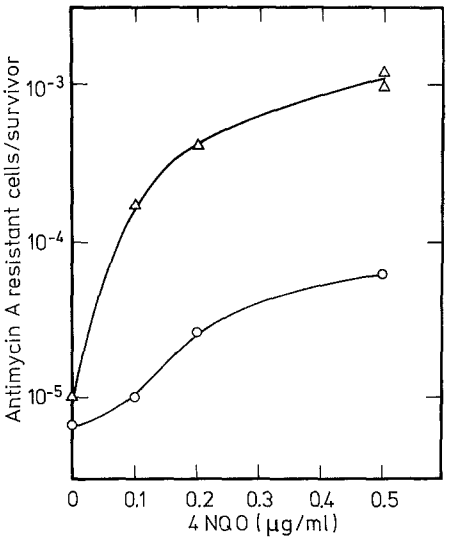

Fig. 6. Dose-dependent increase in the frequency of antimycin A resistant mutants and Ty transposition after 4NQO treatment. Yeast strain 315-1D was grown in YPG medium and exposed to the indicated concentration of 4NQO for 2 h. Antimycin A resistant mutants were selected on YPD plates containing 1 μg/ml antimycin A. For each concentration of 4NQO, approximately 35 antimycin A resistant colonies were picked, grown in YPD medium (5 ml) and genomic DNA isolated as described in Materials and methods. Genomic DNA samples were digested with *Hin*dIII and analyzed by Southern hybridization as described in the text. △, total antimycin A resistant mutants; ○, antimycin A resistant mutants containing Ty inserted at either *ADH2* or *ADH4*

and *DDR48* genes, we have localized the important *cis*-acting sequences that are necessary for DNA damage regulation approximately 200 bp upstream of structural sequences (N. Kobayashi, T. McClanahan, J. Treger, K. McEntee, unpublished results). Ty elements, unlike many well-characterized yeast genes, contain regulatory sequences within the transcription unit. DNA sequences necessary for cell type specific transcriptional regulation, for example, are located within ε sequences (Errede et al. 1985; Rathjen et al. 1987). It seems possible, therefore, that the sequences governing the transcriptional response of Ty to DNA damage are also located within the ε core rather than in the conserved δ elements. If, in fact, this notion is correct, then it suggests that individual Ty elements could respond differentially to DNA damage due to the considerable sequence diversity among ε regions of different Ty family members (Boeke et al. 1988). Thus, transcription of some Ty elements might be stimulated significantly by DNA damage whereas other elements might show little or no response. Similarly, differences in ε sequences of the individual Ty members could also contribute to possible differential post-transcriptional effects of DNA damage for different elements. The different levels of transcript accumulation observed when the different repetitive probes are used in the hybridizations (see Fig. 1 A) is consistent with there being regulatory heterogeneity among these elements. Experiments are in progress to identify the *cis*-acting regions needed for the transcriptional response of Ty elements to DNA damage. Preliminary evidence (V. Bradshaw, unpublished results) indicates that the conserved δ elements by themselves do not contain sufficient regulatory information for the DNA damage response of Ty elements. By way of contrast, the long terminal repeat (LTR) of the human retrovirus, HIV, appears to contain sequences that are necessary and sufficient for induction of pro-viral transcription by DNA damaging agents (Valerie et al. 1988).

UV irradiation stimulated the frequency of antimycin A resistant mutants more than 100-fold in yeast. Southern hybridization analysis was used to identify structural alterations in the *ADH2* and *ADH4* genes of these UV-induced mutants and provided evidence that increased transposition of Ty to these regions occurs. Two properties of the observed rearrangements indicated that they were likely due to Ty insertion: (i) the size of novel restriction fragments was approximately 5.9 kb larger than the parental fragment, the size expected for a Ty element; and (ii) the mutants contained one or more *Xho*I restriction sites within the inserted region whereas no *Xho*I sites were detected in the unrearranged parental upstream regions. The presence of *Xho*I sites, which are relatively rare in the yeast genome, is consistent with the introduction of δ sequences within the upstream regions. By examining almost 400 UV-induced mutants that were selected for antimycin A resistance we found that the frequency of Ty transposition to the *ADH2* and *ADH4* loci increased as much as 11-fold and 17-fold, respectively, after UV irradiation (Table 3). Exposure of cells to 4NQO also stimulated transposition to these loci by as much as 12-fold (Fig. 6). It is also apparent from our analysis that UV (as well as 4NQO) induced a significant fraction of antimycin A resistant mutations that were not due to Ty insertions or other large chromosomal rearrangements at the *ADH2* and *ADH4* regions. These mutants could result from base alterations in *trans*-acting genes or from small sequence alterations in the upstream regions of these dehydrogenase loci. Russell et al. (1983) have characterized two spontaneous 'promoter-up' mutants that express the *ADH2* gene at elevated levels in the presence of glucose but which contain no Ty sequences as judged by Southern hybridization. These mutants were found to contain limited sequence alterations in a region containing 20 consecutive adenine residues. Additional experiments will be required to characterize these UV-induced regulatory mutants.

The expression and transposition of Ty elements are carefully regulated in yeast. Although Ty RNA represents approximately 10% of the total message of yeast cells, the proteins encoded by these elements, including reverse transcriptase, are expressed at very low levels (Garfinkel et al. 1985). Although increasing the level of Ty RNA in cells by galactose induction of a *GAL*-Ty fusion increased the level of transposition more than 20-fold, results of recent experiments have indicated that the increase in Ty RNA per se is unlikely to account for this increased transposition (Curcio et al. 1988). Thus, DNA damage may stimulate transposition by altering one or more post-transcriptional steps as well as by influencing the level of Ty RNA. For example, it might alter the level of host factors that are required for transcription and transposition (Winston et al. 1984) or enhance the rate of frameshifting that is needed for translation of the Ty-encoded reverse transcriptase (Wilson et al. 1986). At present it is not known what effects, if any, DNA damage stress has upon these processes. Nevertheless, although the mechanism(s) by which DNA damage stimulates Ty transcription and transposition is not understood, these results demonstrate that physical and chemical mutagens can activate the expression and movement of retrotransposons in a eukaryotic cell.

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