NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

SCREENING FOR YEAST GENES WHICH ARE INDUCED BY DNA DAMAGING

AGENTS

Jeana Neculcea

A Thesis

in

The Department

of

Biology

Presented in Partial Fulfillment of the Requirement
for the degree of Master of Science at
Concordia University
Montréal, Québec, Canada

August 1990

ISBN 0-315-64710-8

ABSTRACT

Screening for yeast genes which are induced by DNA damaging agents

I examined how gene expression in the simple eukaryote Saccharomyces cerevisiae (yeast) was affected by different agents that cause intra-cellular damage. DNA damage affected the expression of all the genes that I examined, however, different genes were affected in different ways. 4 nitroquinoline 1-oxide (4NQO) strongly induced the LEU2 and UBI4 genes, weakly induced the TMP1 , HIS3 and TOP2 genes and repressed the H2B and UBI3 genes. The UBI4 gene was induced by all the damaging agents tested, which is consistent with the UBI4 gene being a yeast stress response gene. The regulatory mechanism responsible for cell cycle stage dependent expression of TMP1 is independent of the regulatory mechanism resulting in induction of TMP1 following treatments which cause DNA damage. My results suggest that the expression of the UBI3 and UBI4 genes are coordinated so that when one of these genes is expressed at high levels the other is expressed at low levels.

ACKNOWLEDGEMENTS

I would like to gratefully express my thanks and appreciation to Dr.R.K.Storms, for his invaluable help and assistance throughout this study. I am most grateful to him for careful reading and minute criticism of this thesis.

In particular, I am grateful and I would like to thank Dr.M.B.Herrington and Dr.P.Widden for their helpful discussions.

I would like to express my gratitude to Dr.J.Fraser for her comments, and her moral support. I am grateful to J.Basso for his helpful discussion, for his prompt, and courteous help and for his friendship.

I would like to thank the people in Dr.Storms' lab., Dr. E.McIntosh, L.Lee, T.Downing, and P.Poon for their friendship, the atmosphere they created in the lab., and for their helpful comments, and to everybody in the Biology Department which helped me with a kind smile.

I would especially like to thank my family for their constant moral support, confidence, and minute-by-minute encouragement.

TABLE OF CONTENTS

TABLE OF CONTENTS (cont'd)

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

(A) DNA-repair, a cellular response for cell integrity.

DNA, the informational material of organisms, is a dynamic structure where transcription, replication and recombination must take place with a high degree of fidelity. Living organisms are constantly exposed to chemical and physical agents that can alter the normal structure of DNA (Friedberg, 1985). Since maintaining the normal structure is essential for the functioning and reproduction of organisms, many DNA-repair systems have developed during evolution.

The demonstration in the last twenty years that many human diseases result from mutations which alter cellular DNA-repair mechanisms, and the correlation between these mutations and the incidence of cancer, provides impetus for further research into understanding DNA-repair processes (Friedberg, 1985).

Extensive genetic and biochemical evidence clearly demonstrates the importance of the DNA-repair mechanisms to all living organisms, and provide us with some understanding of these mechanisms.

Several observations indicate that the repair of DNA is indeed complex. For example, it is known that in living cells there are many DNA-repair systems, including excision repair, nucleotide excision repair,

recombination repair, and error prone repair (Linn, 1982). Some of these DNA-repair systems are an essential part of cellular metabolism and are not only necessary to maintain the genetic integrity of the organism but also for cell viability. These systems include proteins that are not only important for DNA-repair but are also involved in the ongoing metabolism of most cells, such as proteins essential for the synthesis of DNA precursors, for DNA replication, and proteins involved in the synthesis of other basic metabolites. Other systems include those proteins which are exclusively involved in DNA-repair mechanisms, such as the enzyme involved in the photoreactivation of pyrimidine dimers and the proteins which recognize apurinic sites.

Not only can DNA-repair genes be categorized but so can the source of DNA damage. Friedberg (1985) grouped the sources of DNA damage into two classes, spontaneous and environmental. Environmental damage is caused by chemical and physical agents which alter DNA structure, whereas spontaneous damage does not require the action of an outside agent. The number of environmental agents known to cause DNA damage has increased dramatically in the last few decades. Many highly industrialized areas are now very polluted and levels of environmental contaminating agents that cause DNA damage now induce damage at rates exceeding the natural capacity of cells

to repair damage (Friedberg, 1985). The apparent loss of ozone from the earth's atmosphere at the South Pole and at the North Pole could increase the dose of UV radiation reaching the earth's surface in these areas. Increased UV radiation could have an important impact on all exposed organisms. One result could be to increase the rate of skin cancer. To better prepare ourselves for the increased risks associated with increased levels of DNA damage due to environmental factors it is important that we better understand how organisms repair damaged DNA.

To date the best understood DNA-repair systems are those of the prokaryote Escherichia coli (Kimbal, 1987). However, the greater complexity, nuclear structure, genome size, chromosome organization and the much greater number of genes involved in the repair of DNA in eukaryotes means that many aspects of DNA-repair in eukaryotes can only be elucidated by studying these processes in a eukaryote.

B.DNA-repair in yeast cells.

Recently, the yeast Saccharomyces cerevisiae has been used as a model system for the study of DNA-repair in eukaryotes. The advantages of using yeast are: (i) Yeast is single celled and is therefore relatively simple. (ii) Yeast can be grown under defined experimental conditions. (iii) Yeast is a eukaryote and therefore the results of experiments with it can be

extrapolated to higher organisms. (iv) Yeast is well defined genetically. (v) A large collection of mutant strains, abnormally sensitive to killing by DNA damaging agents is available, and these mutants have been analyzed for epistatic interactions. (vi) The existence of ingenious plasmids and transformation systems for yeast mean that the powerful recombinant DNA technology can be used to study DNA-repair in yeast. (vii) Many of the genes involved in DNA-repair in yeast have been isolated and partially characterized. (viii) Yeast lends itself to a wide range of genetic manipulations like tetrad analysis, and engineering strains using gene cloning and transformation.

Early research on DNA-repair in S. cerevisiae included the isolation of a series of repair-deficient mutants. These mutants were isolated by screening for yeast strains that were more sensitive to either radiation or chemical mutagens. These mutants (RAD-mutants) were classified as belonging to one of 3 repair pathways by determining the relative sensitivity to DNA damaging agents of strains carrying one rad-mutation versus strains carrying two mutations. More then 30 mutant loci have been analyzed by this method. According to this analysis if the double mutants were no more sensitive than each of the two single mutants, the two affected genes are considered "epistatic" and

presumed to code for products that function in the same repair pathway.If the double mutant has a sensitivity that is the sum of the sensitivities for the two single mutants ("additive"), the affected genes code for products acting in two different pathways. If the sensitivity of the double mutant is more than additive ("synergism") the mutations are in genes that code for products that effect two different repair pathways that compete for the same lesion (Haynes and Kunz, 1981).

The 3 repair pathways identified in this way have, more recently, been defined as repair systems. These repair systems are: the excision repair system, the recombination repair system and the error-prone repair system. These 3 epistasis groups have been assigned names based on one of the genes that have been found to fall within each group. These groups are: excision repair (Rad3), recombination repair (Rad52), and error-prone repair (Rad6) (Friedberg, 1988).

In yeast, much is known about the molecular biology and biochemistry of the excision repair pathway but not much is known about the recombinational repair and error-prone repair systems (Friedberg, 1988). I will quote only one of his statements: " The same general thrust with the RAD52 and RAD6 groups of genes is likely to be equally successful, although the lack of suitable complementation phenotypes appears to be

impeding substantial progress with the latter". In yeast, unfortunately, until now, the biochemistry of the repair pathways are not well understood. From the more than 50 loci found to be involved in cellular responses to DNA-damage, in only 10 genes is the functional role of the gene product is known. It is known that, RAD3 codes for DNA dependent ATPaseIII, CDC8 for thymidylate kinase, CDC9 for DNA ligase, RNA2 and RNA1 code for subunits of ribonucleotide reductase, UBI4 codes for ubiquitin, RAD52 for a product needed for a functional endo-exonuclease, RDA6 for a ubiquitin-conjugating (E2) enzyme, POL1 for DNA polymerase I, and RAD9 for a factor needed to arrest cells in the G2 phase after DNA damage.

The Rad3 epistasis group is involved in the nucleotide excision repair pathway. The mutants in this group are sensitive to UV, defective in excision of pyrimidine dimers and sensitive to several chemicals which produce helix-distorting lesions. As in other organisms, in yeast, the nucleotide excision repair pathway involves several steps, including the DNA incision at pyrimidine dimers by an incision endonuclease, the excision of damaged DNA by an excision exonuclease, and the repair resynthesis and DNA ligation (Linn, 1982).

In the Rad52 epistasis group the mutants are

sensitive to ionizing radiation, and are involved in DNA strand-break repair by a recombinational repair system. This group is also involved in the control of a minor pathway for UV damage repair (Burtscher et al., 1988).

In the Rad6 epistasis group, the mutants are sensitive to both UV and X-rays. Mutations in genes in the Rad6 group are pleiotropic, resulting in decreased mutagenesis, increased spontaneous recombination and defective sporulation. This group of mutants is involved in error-prone repair, because strains with defects in the Rad6 class of genes produce few or no mutations when treated with UV. All postreplication repair of UV damage is Rad6 dependent and there is believed to be more than one Rad6 dependent error-prone repair pathway. Finally, some genes in the Rad6 group, for example RAD8 and RAD18 , may also be involved in error free repair (Montelone et al., 1981).

The analysis of yeast rad mutants and the assignment of their mutations to the different epistasis groups indicates how complex the cellular DNA-repair mechanisms are. More that 50 yeast loci have been shown to be involved in DNA-repair. This is many more loci than have been identified in E. coli. This is not surprising since the chromosomes of eukaryotes have a much more complex organization than do the chromosomes of prokaryotes.

Many of the yeast genes involved in DNA-repair have been cloned and their nucleotide sequences determined. In addition, some repair enzymes have been purified, their amino acid sequences partially determined and their biochemistry studied. In addition, several new genes were identified because they were DNA damage-inducible (DIN). Ruby et al.(1985) found 4 DIN genes when they tested 500 S.cerevisiae genes for their ability to be induced by DNA damage. Using these results they predicted that if S.cerevisiae has 10,000 genes than 80 should be DIN genes. It has subsequently been shown that several of the original RAD genes are inducible.

Robinson et al.(1986) showed that RAD2 is induced by DNA damaging agents. RAD2 belongs to the Rad6 epistasis group. This gene and at least 4 others RAD1 , RAD3 , RAD4 and RAD10 , are required for an early event in nucleotide excision repair. The authors suggested that there is some similarity between the yeast RAD2 type genes and the E.coli S.O.S. response genes. This is based on the magnitude of their induction, the kinetics of their induction with different mutagens, and the phenotype of strains harbouring mutations in these genes.

Cole (1987) showed the induction of the RAD54 gene by DNA damage, but not the RAD52 gene. These genes are involved in the recombinational repair pathway (Rad 52

group). As in E.coli , it is believed that deoxyribonucleases (DNases) are required for recombinational and excision DNA repair (Chow and Resnick, 1988). They showed the necessity of a functional RAD52 gene for an endo-exonuclease activity.

Johnson et al.(1986) showed that the DNA ligase gene of S.cerevisiae (CDC9) is induced in response to DNA damage. The induction of the CDC9 gene was shown in exponential and stationary phase cultures. The induction in stationary phase was 9 fold while in exponential phase cells it was only 3 fold.

Elledge et al. (1987) showed that the ribonucleotide reductase gene (RNR2) of S.cerevisiae is a DNA damage inducible gene. The RNR2 gene codes for the small subunit of ribonucleotide reductase. The same authors showed that the CDC8 gene is also induced by DNA damage. The CDC8 gene codes for thymidylate kinase.

Treger et al.(1988) showed that the UBI4 gene is induced by DNA damaging agents. The UBI4 gene is a gene of the ubiquitin gene family in S.cerevisiae .

In recent years much research has been done on the ubiquitin system. Ubiquitin seems to be very important, because it has been highly conserved in organisms as diverse as man, yeast and maize. Ubiquitin is a 76-residue polypeptide and is found in cells both as free ubiquitin and conjugated with other proteins. Its

universality and its conservation during evolution made many researchers believe that it must play a fundamentally important function in eukaryotes. The conjugation of ubiquitin to other cellular proteins is facilitated by a series of biochemical reactions involving three different enzymes. Ubiquitinated proteins (Ub-proteins) can have two fates. First, when abnormal or damaged proteins are conjugated with ubiquitin they are degraded (Rechsteine, 1987). Second, the conjugation of ubiquitin to normal proteins apparently does not affect their stability but may affect their biological activity. For example Ub-conjugated histone proteins and cell surface proteins that are Ub-conjugated are stable (Rechsteiner,1988). In vitro experiments have demonstrated that the ubiquitination of proteins requires ATP as an energy source. Therefore all ubiquitin-dependent proteolysis is also ATP dependent. The functional significance of ubiquitination in vivo still remains unknown, however, considerable research has been performed on the analysis of ubiquitin gene expression and on the biochemistry of the formation of ubiquitin protein conjugates.

The molecular analysis of ubiquitin gene expression was initiated in S.cerevisiae . In S.cerevisiae a ubiquitin gene family, consisting of four ubiquitin genes, UBI1 , UBI2 , UBI3 and UBI4 exists. The

UBI4 gene codes for five identical repeats of the ubiquitin-coding sequence. These five repeats are arranged in a head to tail fashion. The UBI4 gene is regulated in response to stresses. All environmental stresses tested caused a large increase in UBI4 gene expression (Finley et al., 1987). In exponential cultures UBI4 is expressed at low levels but its expression is strongly induced when cultures enter stationary phase, it is also strongly induced when log phase cultures are treated with stress inducing agents (Treger et al., 1988).

The biochemical analysis of ubiquitin conjugation has demonstrated that ubiquitin conjugation involves a multi-enzyme pathway. The first step in this pathway requires the enzyme E1. E1 is an ubiquitin-activating enzyme. The second step in the pathway requires enzymes which have been designated E2. E2 enzymes are ubiquitin-conjugating enzymes which catalyze the conjugation of ubiquitin to proteins. The E3 enzymes are for the removal of ubiquitin from ubiquitin protein conjugates. In yeast, two genes coding for different E2 enzymes have been identified. The fact that the DNA-repair gene RAD6 has been shown to code for a ubiquitin-conjugating (E2) enzyme shows that ubiquitin dependent protein conjugation is probably very important for the repair of DNA damage. It would be interesting to

determine whether ubi4 mutants, which have already been shown to be sensitive to DNA damaging agents, will demonstrate other features that are typical of the Rad6 epistasis group.

The RAD6 gene was shown to be induced by DNA damage (Kupiec et al., 1986) and to be cell cycle regulated, with the peak of expression in late S phase to early G2 phase. Its induction after DNA damage was thought to reflect only the arrest of cells in G2. The RAD6 protein catalyzed the transfer of ubiquitin to the histone proteins 2A and 2B (Sung et al., 1988). The role, if any, that the ubiquitin-histone 2A and 2B conjugates play in error-prone repair is not understood. One suggestion is that the ubiquitination of H2A and H2B affects chromatin structure and that this allows the access of repair enzymes to damaged DNA.

C. The experimental approach.

The objective of the research presented in this thesis was: first to identify genes whose expression was altered when yeast cells were treated with agents that cause DNA damage, and then to begin a detailed analysis of their expression.

As mentioned above the DNA ligase gene(CDC9), the thymidylate kinase gene (CDC8) and a gene encoding the small subunit of ribonucleotide reductase (RNR2) are all induced by DNA damaging agents. Since these genes all

code for products involved in chromosome replication, I asked whether other genes involved in chromosome replication would also be induced. Therefore I analyzed the expression of three other genes involved in chromosome replication and DNA precursor synthesis to determine whether they are induced by 4 nitroquinoline 1-oxide (4NQO), an agent that mimics UV irradiation. Since this type of damage is mainly repaired by the excision repair system, I have expected that genes induced by this agent would be involved in the excision repair of DNA damage. I screened the expression of the TMP1 , H2B and TOP2 genes. The TMP1 gene codes for thymidylate synthase and is cell cycle regulated. The TMP1 gene, like the CDC8 and CDC9 genes, which are also cell cycle regulated, is maximally expressed around the G1/S phase boundary of the cell cycle. The H2B gene codes for histone 2B, which is a component of chromatin. H2B is also cell cycle regulated and its maximal expression in the cell cycle is after the maximal expression of the TMP1 , CDC8 and CDC9 genes. The TOP2 gene codes for topoisomerase II. Its role is believed to be to relieve the torsional stress in DNA that is generated by the DNA unwinding associated with DNA replication and transcription. The UBI4 gene, a ubiquitin gene that is induced by stress, was used as a positive control.

Once it was determined that the expression of these genes was affected , I wanted to see if other cell damaging agents also affected their expression. By using different damaging agents it is possible to see if the expression of these genes is turned on by agents that produce damage repaired by one or by more than one repair system. For this analysis I used the following agents: methyl methane sulfonate (MMS), methotrexate and heat shock.

Finally, since the expression of several of the DIN genes is cell cycle stage-dependent, and since DNA damage often causes cells to arrest at a particular stage of the cell cycle, I wanted to know whether cell cycle stage-dependent gene expression and DNA damage inducibility were related or independent processes. This was done by treating cells arrested at specific stages of their cell cycle with DNA damaging agents.

METHODS AND MATERIALS.

1. Strains.

The strains of *S.cerevisiae* used in this study and their genotypes are shown in table 1.

2. Growth media

In all our experiments YNBD medium (yeast nitrogen base 6.7 g, glucose 20 g/l) was used. Adenine and uracil 2 g/l; histidine, lysine and tyrosine 5 g/l were added whenever required.

3. Growth of yeast strains.

-Asynchronous and synchronous cultures.

The wild type strain was inocculated with 5 µl of an overnight culture in 100 ml medium in a 250 ml flask and incubated at 30°C with shaking. The cell damaging agents were added when the exponentially growing cultures were at an OD600 of 0.15-0.2, and after 24 hours for stationary phase cultures. At 30 minutes intervals samples of 25 ml were taken and the cells harvested by filtration and processed for RNA analysis.

Strains cdc28 and cdc15 were inocculated with 100 µl of an overnight culture in 200 ml of medium in 500 ml flasks and incubated at room temperature with shaking. At an OD600 of about 0.2 the cultures were shifted to the non-permissive temperature (37°C). After 3 hours at the non-permissive temperature 4NQO was added at a final concentration of 0.25 µg/ml. At 30 minutes intervals

TABLE 1
Saccharomyces cerevisiae strains used

Strain	Mutant	Genotype
S288C		α , SUC2, mal, mel, gal2, CUP1
A364A	17-17	a, cdc15-1, ade1, ade2, ura1, his7, lys2, tyr1, gal1
A364A	185-3-4	a, cdc 28-4, his6, ura1

samples of 25 ml were taken and the cells harvested by filtration and processed for RNA analysis.

4. Heat shock treatment

Cultures grown at 30°C were shifted to 37°C. At 30 minutes intervals 25 ml. samples were taken and the cells were harvested by filtration. Cells were quickly frozen in liquid nitrogen and kept at -80°C until RNA extraction was to be performed.

5. Treatment with DNA damaging agents

Treatment with 4-nitroquinoline 1-oxide (4NQO) was performed as described by Elledge et al., 1987. 4NQO was added at a final concentration 0.25 µg/ml to a 150 ml mid-log phase culture. At t=0, 30, 60 and 90 minutes 25 ml samples were harvested by filtration and processed for RNA analysis.

Thymidylate starvation was induced as previously described by Ruby et al.(1985) the only difference being the use of liquid media. A mid-log phase culture of strain S288C was subjected tc thymidylate starvation by addition of methotrexate and sulfanilamide at a final concentration of 35 µg/ml and 5 mg/ml respectively. 25 ml culture samples were harvested by filtration at t=0, 30, 60 and 90 minutes and processed for RNA analysis.

Treatment with methyl methane sulfonate (MMS) was performed as described by Johnson et al. (1986). A mid-log phase culture of strain S288C was exposed to

0.18% MMS. Culture samples of 25 ml were harvested by filtration at t=0, 30, 60 and 90 minutes and processed for RNA analysis.

5. RNA isolation and hybridization analysis

A screening procedure which assayed for gene expression at the level of their transcripts was used. This screening can now be performed easily because the sequences of many yeast genes are known and sequence-specific deoxyoligonucleotide probes can be easily synthesized. The screening was performed using the wild type strain S288C, where the pattern of gene expression should not be altered by the presence of mutations.

RNA manipulations were done as described by McIntosh et.al. (1988). Hybridization was done using dry agarose gels containing 10 µg of total RNA in each lane. Synthetic deoxyribonucleotides were used as hybridization probes. Each probe used was about 21 base pairs long and was complementary to the coding region of the gene of interest. The sequences of the synthetic deoxyribonucleotides used are shown in table 2.

The probed gels were also stained with ethidium bromide to detect ribosomal RNA. The bands of mRNA and rRNA were analysed by densitometric scanning. To control for the quantity of mRNA in each of the samples the ratio mRNA/rRNA was used.

TABLE 2

The sequence of the synthetic deoxyribonucleotides used for hybridization and the name of gene product.

Name of: probe	gene product	Sequence of the synthetic deoxyribonucleotides
P-H1B	histone H1B	5'-GGAAGTCTTTTTAGCGGCTGG-3'
P-HIS3	imidazoleglycerol-P dehydratase	5'-TACTAGGGCTTTCTGCTTCTG-3'
P-LEU2	beta-IPM dehydrogenase	5'-GACTCGATAGCAGCACCACC-3'
P-TMP1	thymidylate synthetase	5'-AGACTCAACGTACCAAGTGCC-3'
P-TOP2	topoisomerase II	5'-CAGAGGCGCTTACCGCTTCAG-3'
P-UBI3	ubiquitin	5'-CTTGTGGTTAGCCAAAACACCAGCACC-3'
P-UBI4	ubiquitin	5'-ATGCAGATTTTCGTCAAGACTTTGACCGGT-3'

RESULTS.

I. Screening for genes that are induced by 4 nitroquinoline 1-oxide, a UV mimicking agent.

A. Genes associated with amino acid synthesis.

For these experiments I wanted a negative control, that is a gene whose expression is not altered when yeast is treated with the DNA damaging agent 4NQO. Since Ruby et al. (1985) showed that HIS3 expression did not change following 4NQO treatment, I began my experiments by using HIS3 transcript as a negative control. HIS3 transcript increased 4.4 fold after 4NQO treatment (Fig.1A)(Table 3).

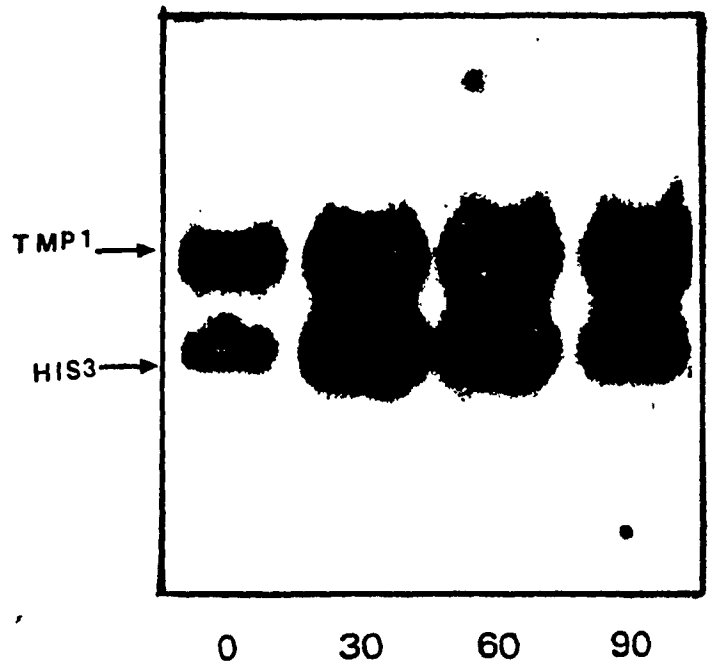
A second experiment followed LEU2 expression. No difference in LEU2 expression was observed after a 30 minutes exposure to 4NQO (Fig.1B). However, a 10.7 fold increase in the level of LEU2 message was observed after 60 minutes, and then levels remained high relative to the level in the untreated control even after 90 minutes.

None of these genes' transcriptional expression was found to be a good negative control. Therefore to control for the quantity of RNA in each of the samples were normalized either by the same amount of RNA for each sample as determined by OD 260 nm. or for some experiments the probed gels were also stained with ethidium bromide to detect ribosomal RNA (Table 3). The results of this analysis (Fig.2B) showed that each of the samples

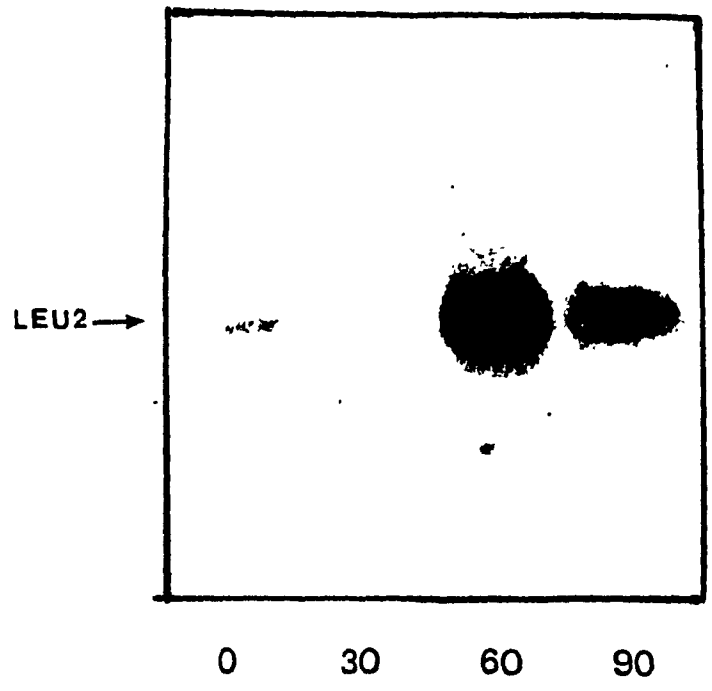
FIGURE 1

Levels of HIS3 (Panel A) and LEU2 (Panel B) transcripts following the addition of 4NQO. 4NQO was added at a final concentration 0.25 µg./ml. to a 150 ml. mid-log phase culture of strain S288C at t=0 minutes. At t=30, 60 and 90 minutes 20 ml. samples were harvested and processed for RNA analysis. The times following the addition of 4NQO is indicated below each lane. The probes used were P-HIS3 (Panel A), and P-LEU2 (Panel B).

A



B



electrophoresed had essentially the same quantity of ribosomal RNA and that the quality did not vary.

B. Genes associated with DNA synthesis and chromosome replication.

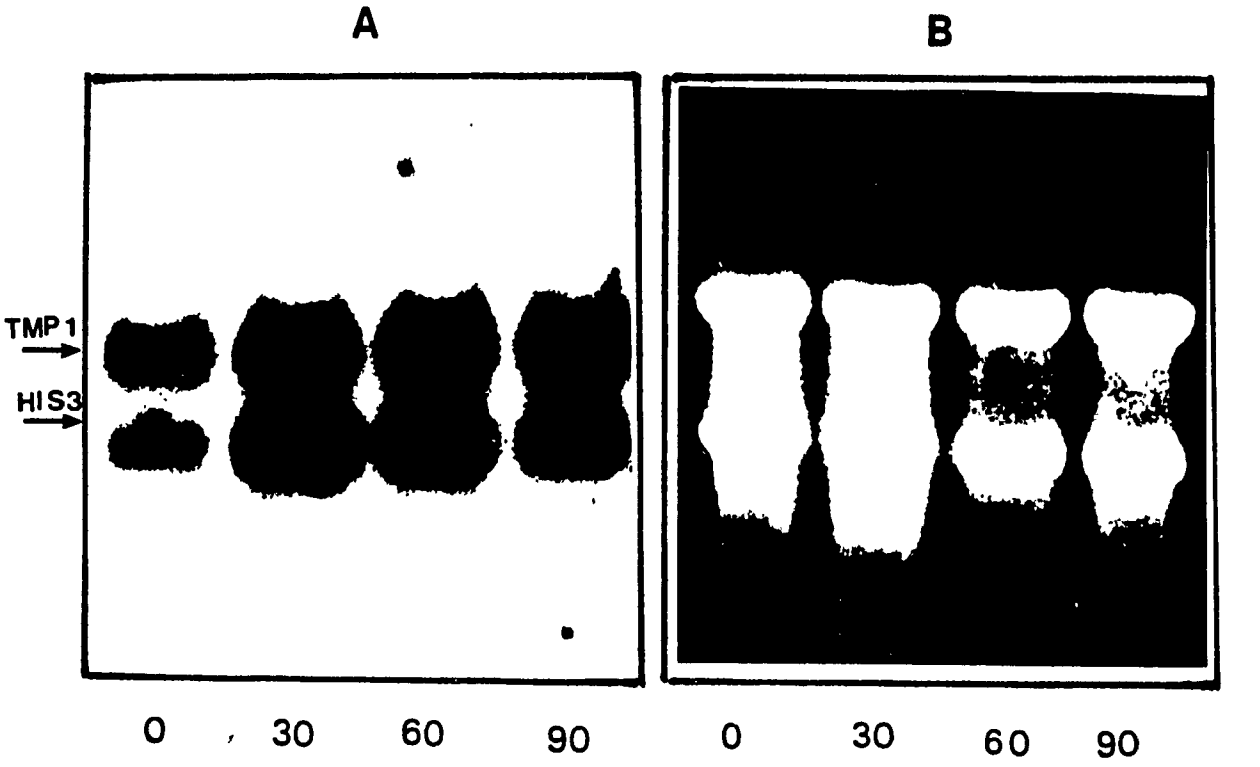
Since several genes involved in DNA metabolism are known to be induced by DNA damaging agents, it seemed that the TMP1 gene might also be induced by treatments that induce DNA damage. Hybridization analysis of the RNA (Fig. 2A, the same as Fig.1A) showed that the amount of TMP1 transcript increased after 4NQO treatment. The amount of TMP1 transcript was 3.0 times greater after 4NQO treatment than prior to 4NQO treatment. The elevated level of transcript continued for at least 90 minutes.

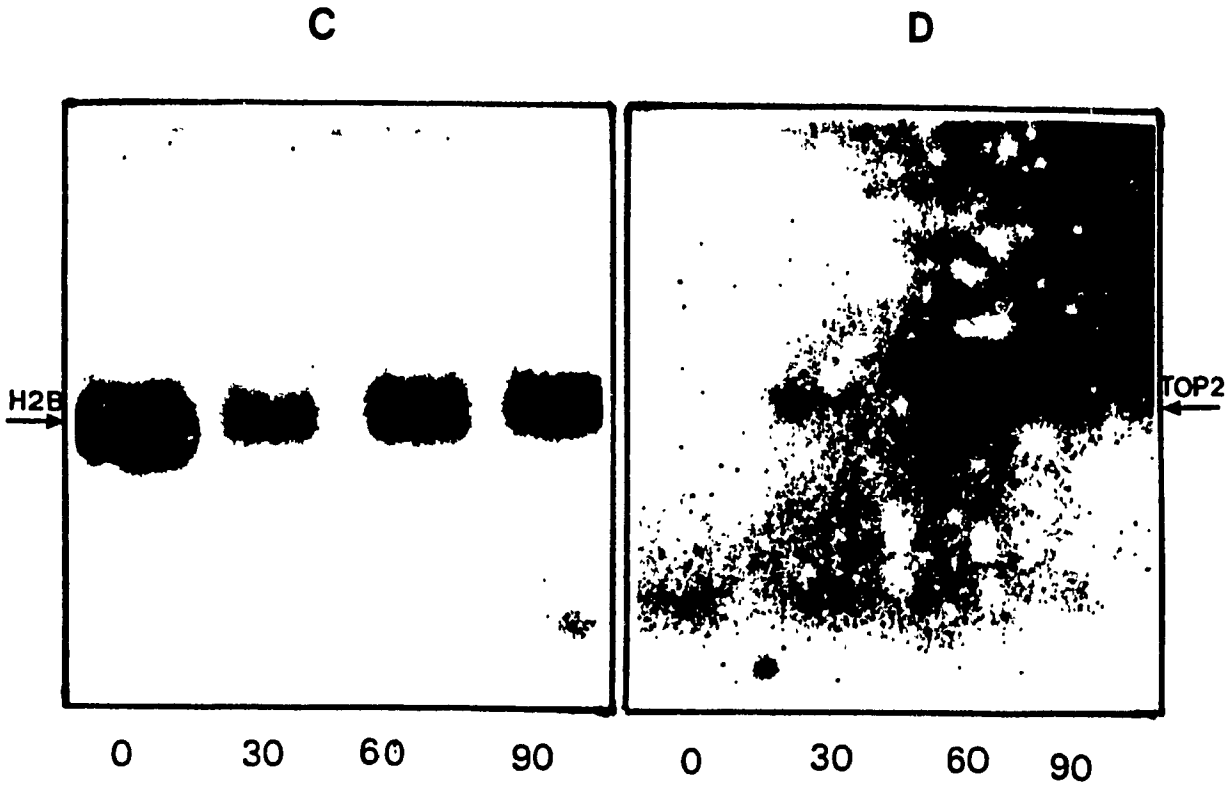
I also monitored the expression of the cell cycle regulated H2B gene at various times during 4NQO treatment (Fig.2C). At the level of its transcript the expression of the H2B gene was reduced to 0.5 times by 30 minutes after the addition of 4NQO. After 60 minutes in the presence of 4NQO H2B transcript increased to a new steady-state level which was maintained until at least 90 minutes.

TOP2 transcript was not detected in mid-log-phase sample harvested before the addition of the DNA damaging agent 4NQO (Fig.2D). 30 minutes after 4NQO was added topoisomerase II transcript had increased to detectable

FIGURE 2

Levels of TMP1 and HIS3 (Panel A), H2B (Panel C), and TOP2 (Panel D) transcripts following treatment with 4NQO. 4NQO was added at a final concentration 0.25 µg./ml. to a 150 ml. mid-log phase culture of strain S288C at t=0 min. At t=30, 60 and 90 minutes 20 ml. samples were harvested and processed for RNA analysis. The times following the addition of 4NQO is indicated below each lane. The probes used were P-TMP1, P-HIS3 (Panel A), P-H2B (Panel C) and P-TOP2 (Panel D). Panel B represents ethidium bromide stained ribosomal RNA which was used to assess the amount of RNA loaded.


A
B
TMP1
HIS3
0
30
60
90
0
30
60
90


C
D
H2B
TOP2
0
30
60
90
0
30
60
90

levels. 60 minutes after 4NQO was added TOP2 transcript was 14.7 times higher than the levels present at 30 minutes. High levels of transcript continued for at least 90 minutes.

C. Genes associated with stress response.

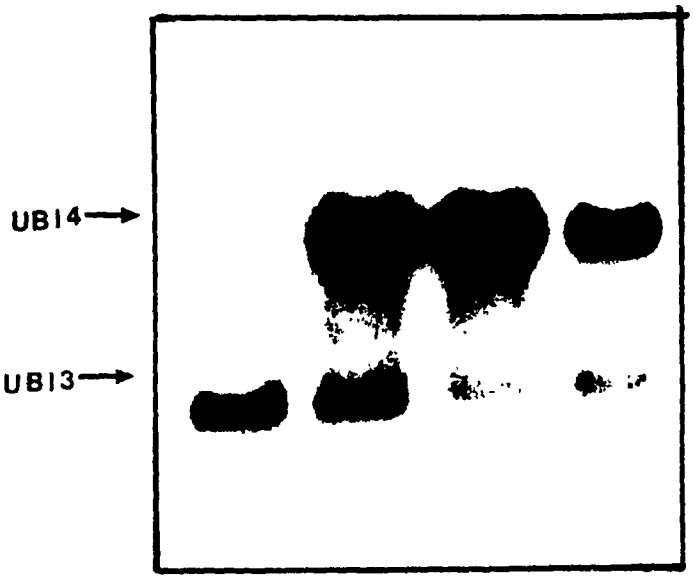
In YNBD medium in mid-log phase the UBI4 gene was expressed at a very low level (Fig.3A). The expression of UBI4 transcript increased 28.4 fold during the first 30 minutes after 4NQO was added to the culture. After one hour, in the presence of 4NQO, UBI4 transcript peaked (41.8 times), and after 1.5 hours it began to decrease (19.0 times)(Table 3).

Below the 1.5 Kb hybridization band representing the UBI4 transcript there was a second band corresponding to the 0.7 Kb UBI3 transcript. That this band represented the UBI3 transcript was shown in two ways. First, an oligonucleotide (P-UBI3) specific for UBI3 transcript was used for hybridization analysis of yeast RNA (Fig.3B). When P-UBI3 was used as the probe only the UBI3 transcript was detected and this was at the same position as the 0.7 Kb band observed when the UBI4 complementary probe (P-UBI4) was used. In addition, when we performed RNA hybridization analysis on RNA isolated from a strain which had the UBI3 gene deleted, only the 1.5 Kb band was detected with the P-UBI4 probe (data not shown).

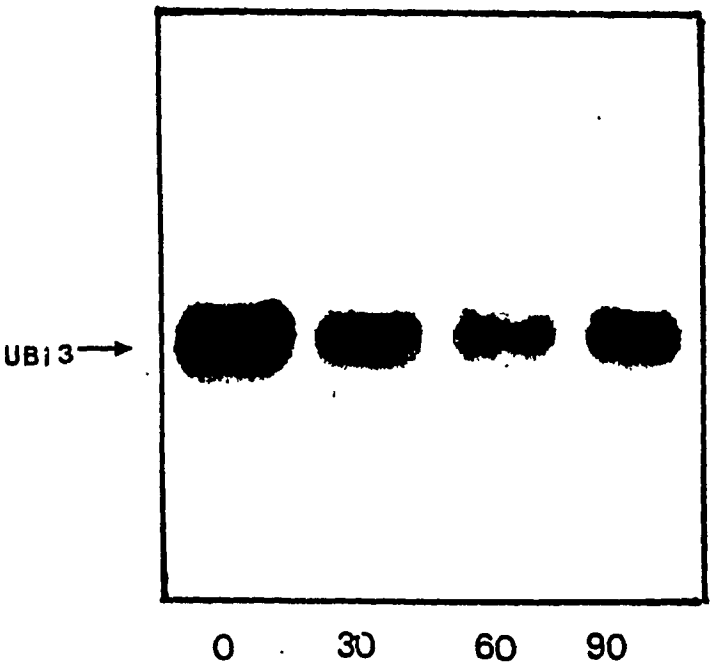
FIGURE 3

Analysis of UBI4 (Panel A) and UBI3 (Panel B) transcripts following the addition of 4NQO. This experiment was performed exactly as described in Fig.2 except that P-UBI4 was used as the probe for Panel A and P-UBI3 was used as the probe for Panel B.

A



B



Therefore, under the hybridization conditions used ubiquitin oligonucleotide P-UBI4 hybridized to both the UBI4 and UBI3 transcripts.

The UBI3 expression was high during the early log phase of batch culture growth (Fig.3B), 30 minutes after the addition of 4NQO UBI3 expression decreased to reach 0.7 times the t=0 level, and 60 minutes after the addition of 4NQO UBI3 expression began to increase.

The regulation of these two ubiquitin genes in response to the addition of the DNA damaging agent 4NQO is very different, since UBI4 was highly induced whereas UBI3 was repressed. Another interesting observation was that late in the experiment, 3 hours after the addition of 4NQO, UBI3 expression began to increase whereas UBI4 expression began to decrease, suggesting that 4NQO treatment transiently affected the expression of these genes.

From these results it can be concluded that the expression of all the genes examined was altered by the 4NQO treatment. The expression of these genes in response to 4NQO allowed us to place them into two categories, those induced by 4NQO (TMP1 , TOP2 , UBI4 , HIS3 and LEU2), and those repressed by 4NQO (H2B , UBI3).

II. The effect of other damaging agents on gene expression.

A) Heat shock.

TMP1 and HIS3 transcript levels increased 2.1 fold and 3.9 fold respectively following a heat shock (Fig.4A).

Heat shock, repressed UBI3 expression to 0.6 times the t=0 level but induces UBI4 18.7 times expression (Fig.4B). This result is reminiscent of the results obtained with 4NQO. However, the effect on UBI4 expression was less dramatic after heat shock than was the effect observed after 4NQO treatment (compare Fig.3A and Fig.4Ba).

B.Thymidylate starvation.

The effect of thymidylate starvation on the expression of TMP1 , HIS3 , UBI3 and UBI4 genes was different. During the first half hour the amount of TMP1 transcript decreased to 0.6 times the t=0 level, then after 60 minutes it began to increase. At 90 minutes levels were 2.1 times higher than they were in an early log phase culture (Fig.4Ab). HIS3 transcript levels had increased 1.5 fold by 60 minutes and 2.3 fold 90 minutes after the addition of methotrexate and sulfanilamide (Fig.4Ab). The effect of thymidylate starvation on the two UBI genes was essentially the same as the effect of 4NQO treatment (Fig.4Bb). However, induction after 4NQO treatment (41.8 times) was greater than after methotrexate

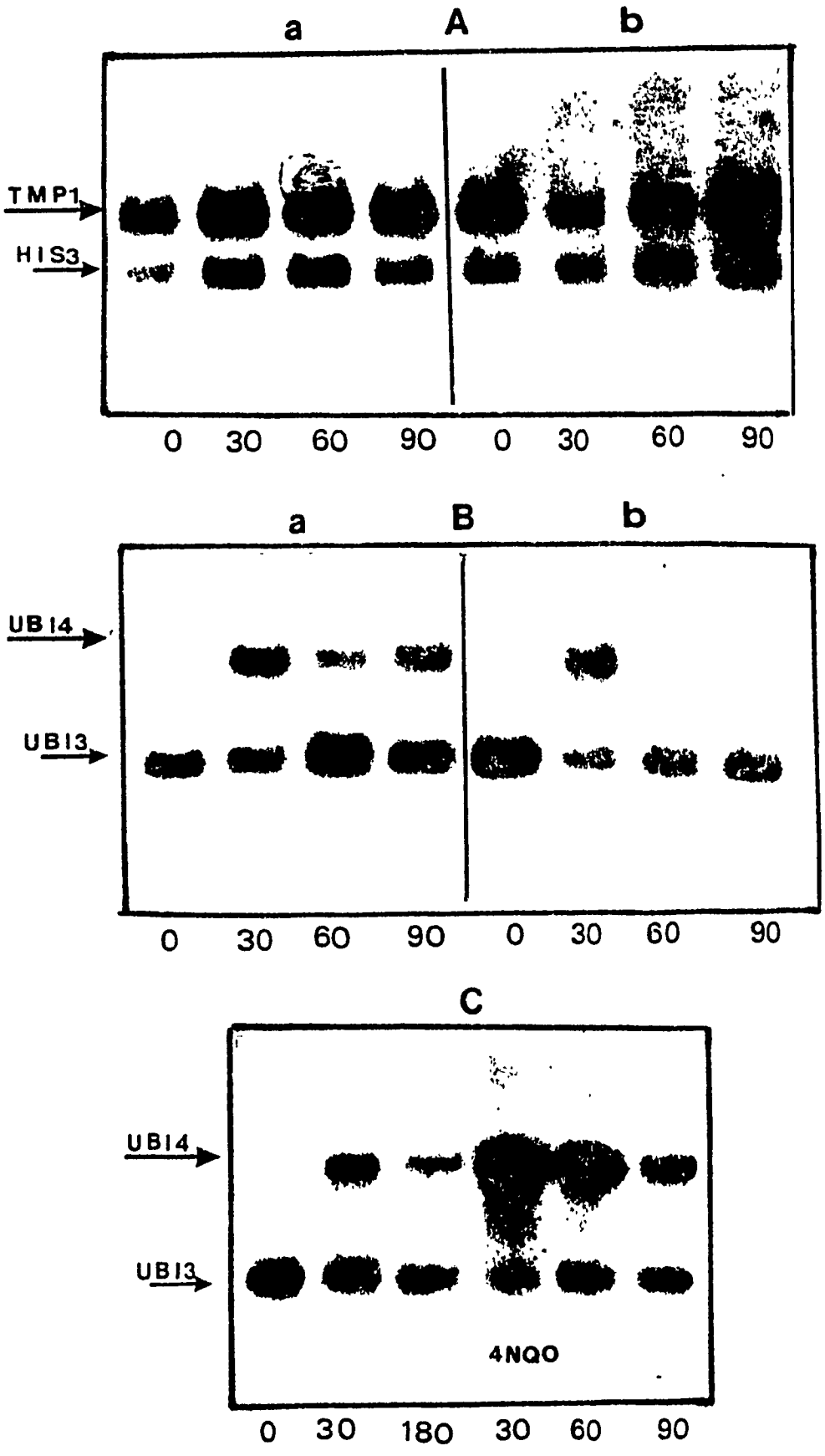
FIGURE 4A and 4B

Analysis of TMP1 , and HIS3 (Panel Aa) , UBI4 , and UBI3 (Panel Ba) transcripts levels following heat shock, and transcript analysis of TMP1 , and HIS3 (Panel Ab), UBI4 , and UBI3 (Panel Bb) following thymidylate starvation. The heat shock was induced by shifting a mid-log phase culture of strain S288C from 30°C to 37°C . Following the temperature shift 20 ml. culture samples were harvested and processed for RNA analysis at t=0, 30, 60 and 90 minutes. A mid-log phase culture of S288C was subjected to thymidylate starvation by the addition of methotrexate and sulfanilamide at a final concentration of 35 μg and 5 mg/ml. Culture samples were harvested and processed for RNA analysis at t=0, 30, 60 and 90 minutes. The time following each treatments is indicated below each lane. The probes used were P-TMP1 and P-HIS3 (Panel A) and P-UBI4 and P-UBI3 (Panel B).

FIGURE 4 (cont'd)

FIGURE 4C

Analysis of UBI3 and UBI4 transcript levels following heat shock and 4NQO treatments. A mid-log phase culture of strain S288C was shifted from 30°C to 37°C. Following the temperature shift 20 ml. culture samples were harvested at t=0, 30 and 180 minutes. At t=180 min. 0.25 µg/ml 4NQO was added. Culture samples of 20 ml. were harvested and processed for RNA analysis following the addition of 4NQO. The probes used were P-UBI4 and P-UBI3.


a
A
b
TMP1
HIS3
0 30 60 90 0 30 60 90
a
B
b
UBI4
UBI3
0 30 60 90 0 30 60 90
C
UBI4
UBI3
4NQO
0 30 180 30 60 90

treatment (22.1 times). UBI4 transcript levels after 30 minutes were 22.1 times higher but by 60 minutes had decreased to only 7.7 times the level in the t=0 sample. UBI3 expression was decreased to 0.3 times the t=0 level after 30 minutes , but by 60 minutes it began to increase.

C.Heat shock and 4NQO

Later in this thesis I will examine whether the effect of 4NQO on gene expression is cell cycle stage-dependent. Because these experiments use temperature sensitive cell division cycle cdc mutants, and arresting these mutants involves transferring them to the non-permissive temperature (37^{o}C), I needed to know how a temperature shift to 37^{o}C followed by 4NQO treatment by would affect gene expression.

The results show that UBI4 RNA was very low in the t=0 sample and increased dramatically (about 20 times) by 30 minutes (Fig.4C). Levels remained above the levels found in the t=0 sample even after 3 hours. The amount of UBI3 transcript decreased after the temperature shift and these levels were almost the same after 3 hours. 30 minutes after the addition of 4NQO UBI4 transcript had again increased (about 40 times), and the UBI3 transcript had decreased slightly. By 90 minutes UBI3 transcript was almost the same level as found before 4NQO treatment and UBI4 transcript was about one fourth the level seen 30 minutes after the addition of 4NQO. In S288C

UBI4 expression responded to heat shock differently than it did to 4NQO. The differences were: 4NQO caused the UBI4 transcript to be induced to a higher level and this level remain elevated for a longer period of time (Compare Fig.4Ba with Fig.3).

D.Methyl methane sulfonate (MMS) a X-radiation mimicking agent.

Figure 5 follows TMP1 , HIS3 , UBI4 and UBI3 expression at various time intervals after the addition of MMS to a log phase culture of S288C. The expression of both TMP1 and HIS3 genes increased 1.9 times by 30 minutes after the addition of MMS (Fig.5A). TMP1 levels continued to increase until 60 minutes, reaching 3.6 times the level found in the untreated cells. HIS3 levels did increase 3.4 times beyond the level seen at t=0 minutes. Therefore the DNA damaging agent MMS induced both TMP1 and HIS3 gene expression 3.6 and 3,4 fold respectively.

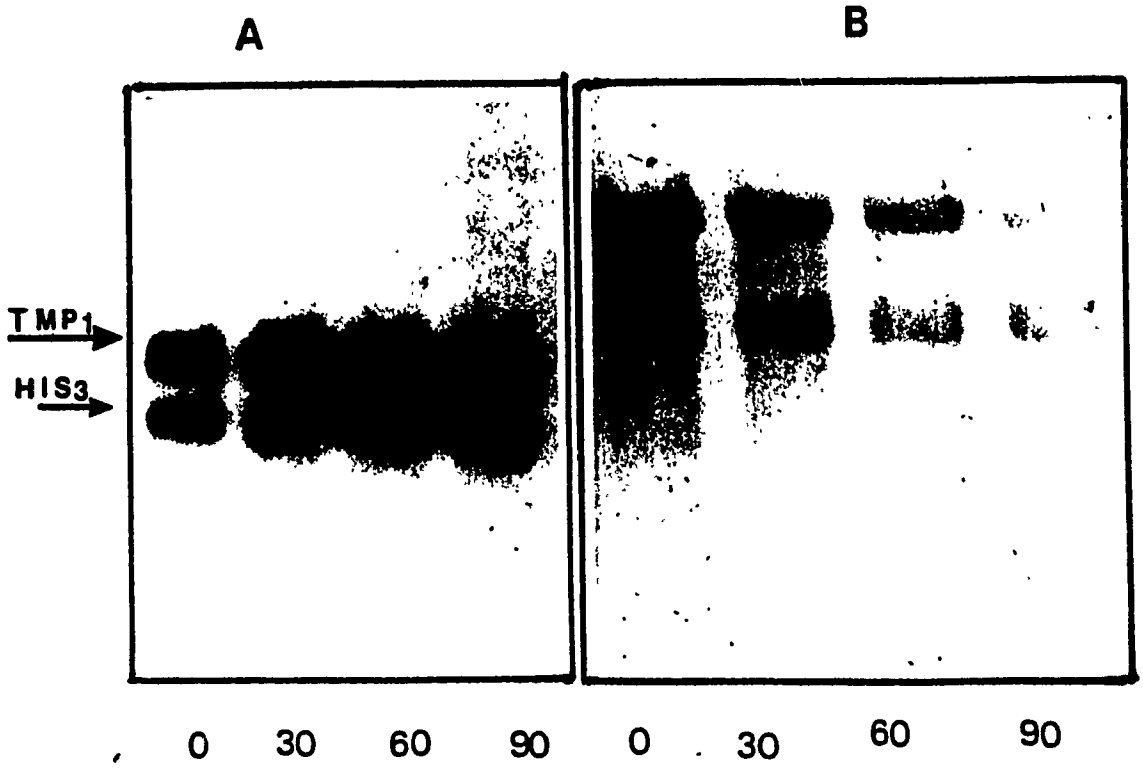
UBI4 and UBI3 expression were followed after the addition of MMS (Fig.5C). UBI4 expression was induced 7.5 fold after the addition of MMS. UBI3 expression was repressed reaching its lowest level after 90 minutes.

From these experiments it can be concluded that the expression of the TMP1 , HIS3 and UBI4 genes are induced and the UBI3 gene is repressed by the DNA-damaging agent MMS (0.5).

All the results discussed above are summarized in the

FIGURE 5

Analysis of TMP1 , and HIS3 (Panel A), UBI3 , and UBI4 (Panel C) transcript levels following treatment with MMS. At a mid-log phase culture of strain S288C was exposed to 0.18% MMS. Culture samples of 20 ml. were harvested and processed for RNA analysis at t=0, 30, 60 and 90 minutes. The time following the addition of MMS is indicated below each lane. The probes used were P-TMP1 and P-HIS3 (Panel A) and P-UBI4 and P-UBI3 (Panel C). Panel B ribosomal RNA ethidium bromide stained.


A
B
TMP1
HIS3
0
30
60
90
0
30
60
90

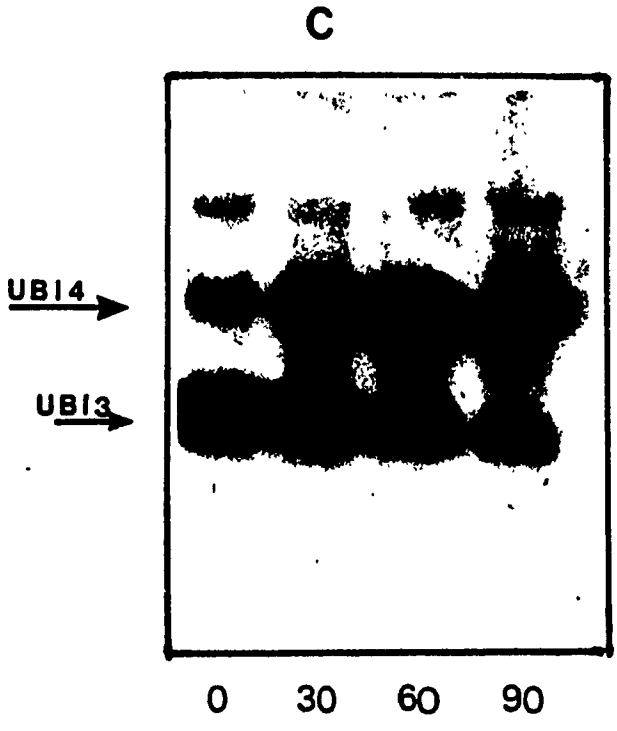

C
UBI4
UBI3
0
30
60
90

table 3 "Transcription expression of genes following cell damaged treatments." In all treatments (some repeated 2 or 3 times) with 4NQO, MMS, thymidylate starvation or heat shock the reproducibility of results as induction or repression was constant for the expression of TMP1 , HIS3 , UBI4 and UBI3 genes.

III.Preliminary investigation of the regulatory mechanisms controlling expression of DNA damage-inducible genes.

It is known that some genes, particularly those involved in the synthesis of DNA precursors and DNA replication, are cell cycle regulated (McIntosh et al.,1988). It is also known that DNA damage can arrest the progression of eukaryotic cells at specific stages of the cell division cycle. Therefore to determine whether the changes in gene expression induced by DNA-damaging agents was due to cell cycle arrest or was due directly to the DNA damaging agent, I controlled for variation caused by cell cycle stage effects by using yeast cultures arrested at specific stages in the cell cycle.

The first experiment used a stationary phase culture of strain S288C. Stationary-phase cultures accumulate cells arrested in the G1/G0 stage of the cell cycle. G1/G0 phase cells express TMP1 at very low levels (McIntosh et al.,1988), UBI3 at very low levels and UBI4 at very high levels (Treger et al.,1988).

The TMP1 and HIS3 transcript levels after 4NQO

TABLE 3

Transcription expression of genes following cell damage treatments

Treatment with	Expression of genes						
	TMP1	TOP2	H2B	UBI4	UBI3	HIS3	LEU2
4NQO	3.0	14.0	0.5	42.0	0.7	4.0	10.0
MMS	3.6	N.D.	N.D.	7.5*	0.5	3.4	N.D.
Thymidylate starvation	2.1	N.D	N.D.	22.1	0.6	2.3	N.D.
Heat shock	2.0	N.D.	N.D.	19.0	0.6	3.9	N.D.

N.D.= not determined

* = at t=0 the level of transcript was higher than in 4NQO experiment

treatment of a stationary phase culture (t=0) was very low. After 4NQO treatment their levels increased reaching 4.0 and 3.8 times the t=0 levels by 30 minutes and 7.0 and 5.5 times times respectively by t=150 minutes (Fig.6A).

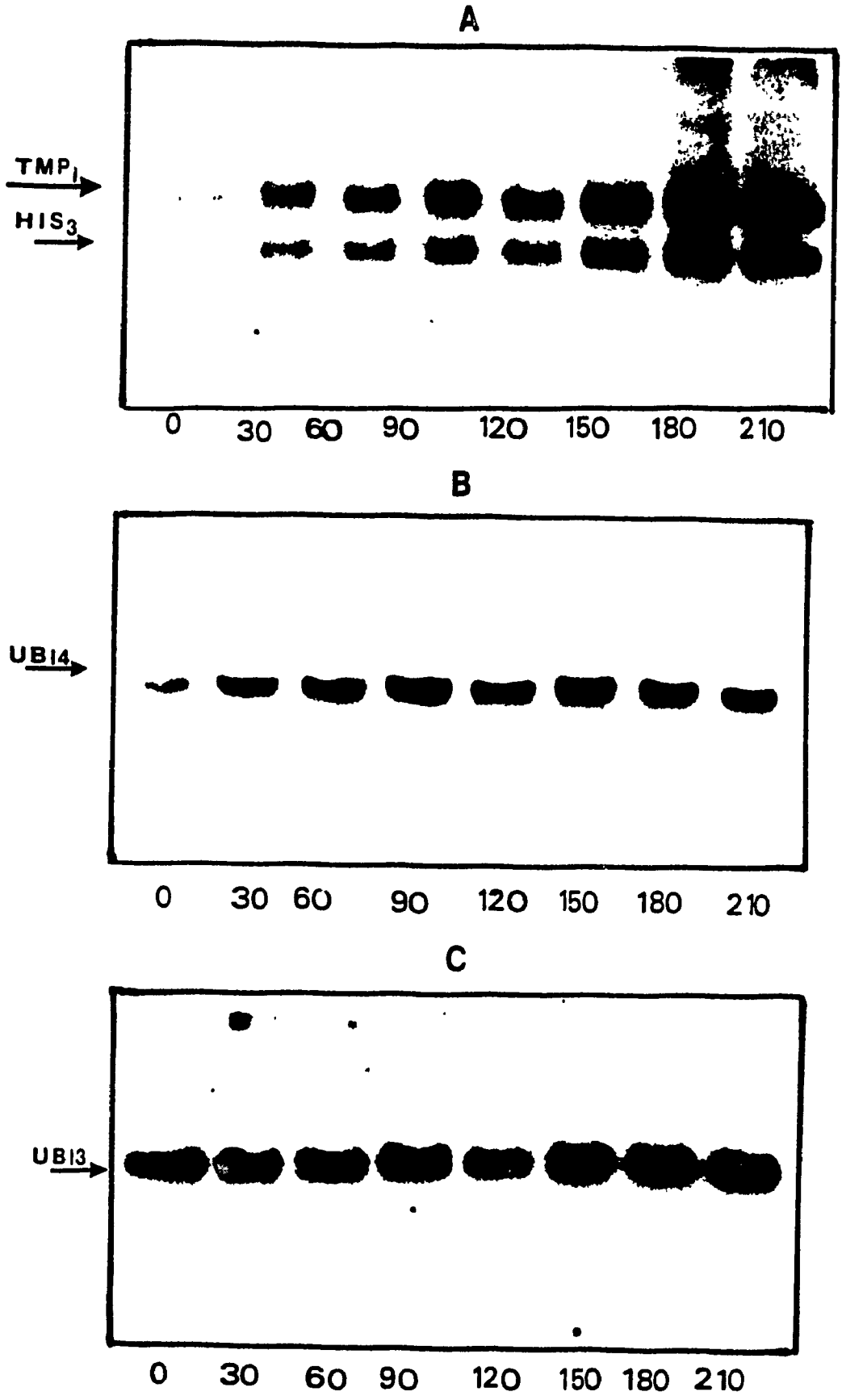
In stationary phase culture, at t=0 the UBI4 transcript levels were already very high and it increased 3.3 times during the 150 minutes following the addition of 4NQO. UBI3 transcript was present at a very low levels at t=0 and increased 1.8 times during the next 150 minutes (Fig.6A).

The last experiments were performed using two mutant strains which harbour temperature sensitive mutations. The A364 derivative 185-3-4 carries the cdc28-4 mutation, this mutation causes this strain to arrest its cell division cycle in the G1 phase when it is cultured at the restrictive temperature (37^{o}C) (Newlon, 1988). The A364 A derivative 17-17 harbors a temperature sensitive mutation (cdc15-1) which causes yeast cells to arrest their cell cycle in M of the cell division cycle (Newlon, 1988).

RNA hybridization analysis was used to follow expression of the TMP1 , HIS3 , UBI3 and UBI4 genes following 4NQO treatment of G1 and G2 arrested strains respectively. The affect of 4NQO treatment on the expression of the TMP1 and HIS3 genes G2 arrested cells is shown in Figure 7Aa. 30 minutes after the culture

FIGURE 6

Analysis of TMP1 (Panel A), HIS3 (Panel A), UBI4 (Panel B) and UBI3 (Panel C) transcript levels of strain S288C following 4NQO treatment of a stationary-phase culture. 4NQO was added at a final concentration of 0.25 µg/ml. Culture samples of 20 ml. were harvested and processed for RNA analysis at t=0, 30, 60, 180 and 210 minutes. The time following the addition of 4NQO is indicated below each lane. The probes used were P-TMP1 and P-HIS3 (Panel A), P-UBI4 (Panel B) and P-UBI3 (Panel C).


A
TMP1
HIS3
0 30 60 90 120 150 180 210
B
UBI4
0 30 60 90 120 150 180 210
C
UBI3
0 30 60 90 120 150 180 210

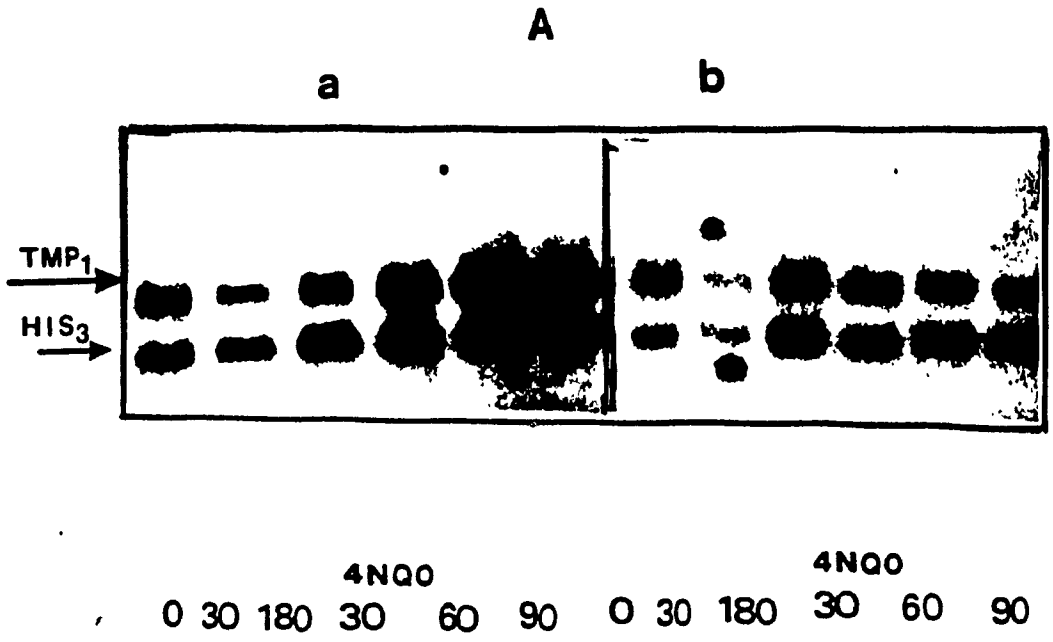
was shifted to the restrictive temperature the expression of both of these genes was repressed. After three hours at the non-permissive temperature their expression recovered to reach levels near that found prior to the temperature shift. The addition of 4NQO to these G2 arrested cells resulted in the induction of TMP1 and HIS3 . The level of these two transcripts increased by 2.8 and 1.3 times respectively during the 60 minute interval following the addition of 4NQO.

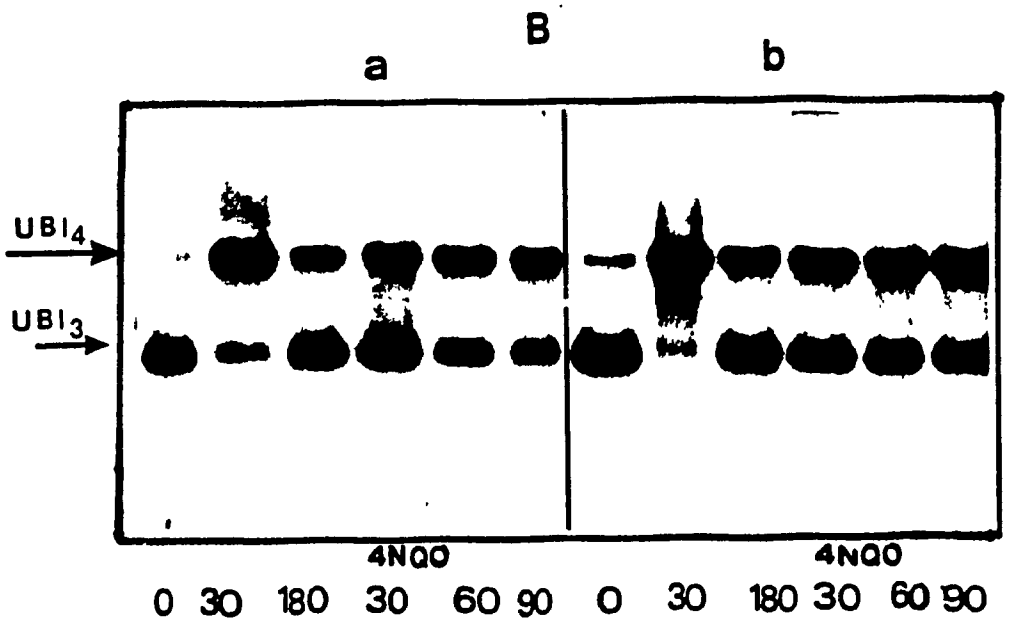
The effect of 4NQO treatment on the expression of the TMP1 and HIS3 genes in cells arrested at the cdc28 block is shown in Figure 7Ab. TMP1 levels significantly decreased during the first 30 minutes at 37°C. This was expected, since the cells were arrested in G1 at a cell cycle stage when the TMP1 gene is expressed at basal levels. In contrast, HIS3 transcript levels decreased only slightly during the same interval (better shown in Fig.8). Three hours after being transferred to the non-permissive temperature levels of both the TMP1 and HIS3 transcripts had increased above the levels seen at t=30 minutes.

30 minutes after shifting to the non-permissive temperature, UBI4 expression was induced while UBI3 expression was repressed in both of these cdc mutants (Fig.7). After three hours at the non-permissive temperature UBI4 expression dropped significantly below

FIGURE 7

Analysis of TMP1 , and HIS3 (Panel Aa), UBI4 , and UBI3 (Panel Ba) transcripts levels in cdc15 mutant strain, following the addition of 4NQO. Transcript levels of TMP1 , and HIS3 (Panel Ab), UBI4 , and UBI3 (Panel Bb) in cdc28 mutant strain, following the addition of 4NQO. The cdc mutant strains were grown at room temperature and shifted at 37°C., 180 minutes following the temperature shift 4NQO was added to at a final concentration 0.25 µg/ml. Culture samples of 20 ml. were harvested and processed for RNA analysis at t=0, 30, 180, 210, 240 and 270 minutes. The probes used were P-TMP1 and P-HIS3 (Panel A) and P-UBI4 and P-UBI3 (Panel B).


A
a
b
TMP1
HIS3
4NQO
0 30 180 30 60 90
4NQO
0 30 180 30 60 90


B
a
b
UBI4
UBI3
4NQO
0 30 180 30 60 90
4NQO
0 30 180 30 60 90

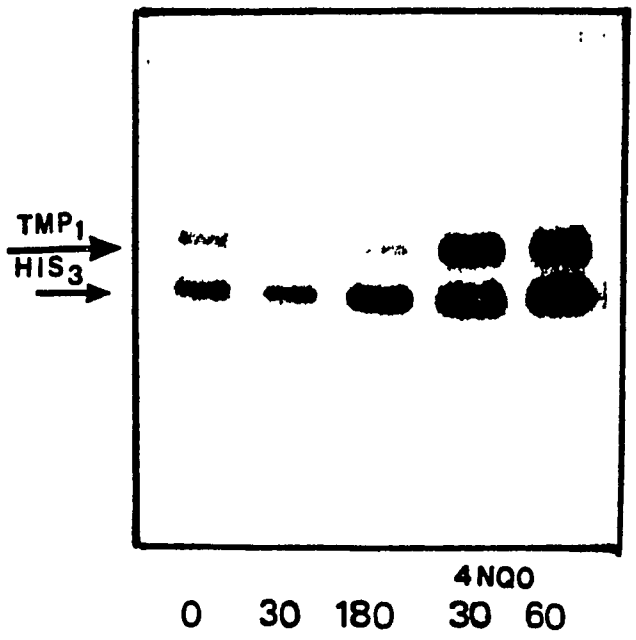
the level found at t=30 min but was still higher than the level detected in the sample taken before the temperature shift. UBI3 transcript levels increased after 180 minutes to reach levels similar to that seen before shifting to the restrictive temperature. When 4NQO was added to these cell cycle stage arrested cells the expression of UBI4 and UBI3 was not altered significantly. This is in contrast to the results obtained with the wild type strain (Compare Fig.3 with Fig.7B).

I repeated this experiment with the cdc28 mutant except the concentration of 4NQO was doubled (Fig. 8). These results showed that TMP1 transcript levels fell about 4 fold after shifting to the non-permissive temperature, but HIS3 transcript levels did not change significantly after the temperature shift. Three hours after shifting to the non-permissive temperature, TMP1 levels increased to become slightly higher than they were in the t=30 sample. In contrast HIS3 transcript increased 3-4 fold. The addition of 4NQO caused both the TMP1 and HIS3 transcript to gradually increase during the next 90 minutes (5.3 and 3.3 times respectively). Both UBI4 and UBI3 transcript increased by 60 minutes after the addition of 4NQO (2.7 and 1.7 times respectively).

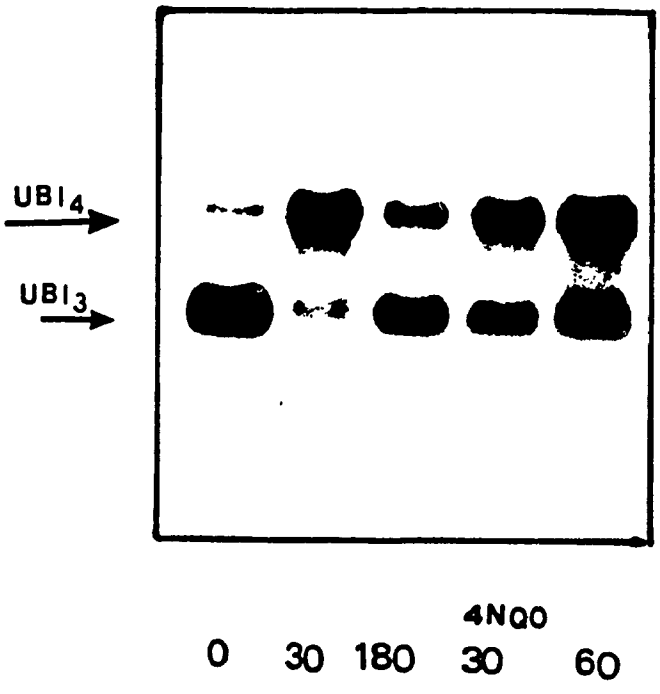
FIGURE 8

Levels of TMP1 , and HIS3 (Panel A), UBI4 , and UBI3 (Panel B) transcripts following the addition of 50 µg/ml. 4NQO to a strain harbouring a cdc28 mutation. Probes and growth conditions were as in Fig.7.

A



B



DISCUSSION

I.Screening for genes that are induced by 4NQO

The DNA-repair capacity of cells can be affected by mutations which cause defects in different aspects of cellular metabolism. In this thesis I performed preliminary analyses of the effect of DNA damage on the expression and regulation of several genes.

I screened for genes induced by 4NQO. 4NQO was used because it is believed that the DNA damage it produces is removed by the same nucleotide excision repair system that removes pyrimidine dimers. 4NQO must be activated before it is able to damage DNA. In cells this activation consists of two enzymatically catalyzed steps. First, 4-nitroquinoline 1-oxide (4NQO) is reduced to 4-hydroxyaminoquinoline 1-oxide (4HAQO). Second, another enzyme activates 4HAQO to form aminoacylated 4HAQO. Aminoacylated 4HAQO has a reactive group that allows it to covalently bind to cellular macromolecules such as nucleic acids and proteins.

Tada et al. (1975) showed that the 4HAQO-activating enzyme from yeast is seryl-tRNA synthetase. The activation of 4HAQO occurs in the presence of ATP, L-serine and Mg^{++} (Toda et al., 1975). The aminoacylated 4HAQO reacts with the guanine and adenine bases in DNA to form 4HAQO-purine adducts. It is believed that the quinoline-purine adducts are removed by the nucleotide

excision repair system, because unlike the case in wild type cells the adducts are not excised from the DNA of excision repair deficient *E. coli* and human cells (Kenago et al., 1981).

The identification of some of the genes that are induced by 4NQO treatment makes it possible to initiate studies into the regulation of the nucleotide excision repair system. The repair of DNA damage by the nucleotide excision repair pathway involves several steps. The first step involves the recognition of the damaged DNA. Next the damaged DNA strand is endonucleolitically cleaved immediately adjacent to the damaged base. Degradation of the damaged DNA initiates at the nick and an exonuclease removes the damaged DNA. Finally repair replication fills in the excised region and the repaired patch is joined at its 3' end to the parental strand by DNA ligase.

In yeast, it is known, that the DNA ligase gene *(CDC9)* is induced by DNA damaging agents (Johnson et al., 1986). It is also known that repair synthesis is needed to replace the region that was excised when the damaged DNA was removed. Since DNA precursors and DNA replication would be required for repair synthesis the genes coding for products necessary for nucleotide synthesis and DNA-synthesis might also be induced by DNA damage. Consistent with this expectation the thymidylate kinase encoding *CDC8* gene, and the *RNR2* gene encoding the

small subunit of ribonucleotide reductase are induced by DNA damage (Elleger and Davis, 1987).

This study reveals new information about the regulation of several genes. The genes tested can be divided into two categories based on their response to DNA damage; (A) genes induced by DNA damage and (B) genes repressed by DNA damage.

A. Genes induced by DNA damage.

The 4NQO treatment arrested cell cycle progression, because three hours after the 4NQO was added the cell density in the treated culture was only 75% of the density in the untreated control culture (data not shown). Wintersberger et al. (1987) showed that treatments with many DNA damaging agents caused cells to arrest cycle progression in the G2 phase. Other experiments (Mitchel et al., 1987,) found that some of the DNA damaging agents tested by Wintersberger induced error-free recombinational repair, whereas others induced error-prone repair. Together these two sets of results suggest that all types of DNA lesions cause cells to arrest cell cycle progression in the G2 phase. The fact that DNA damage causes cells to accumulate in G2 of the cell cycle suggests that DNA damage may be more efficiently repaired when cells are arrested in G2. The recent identification and characterization of the yeast RAD9 gene strongly supports the notion that G2 arrest is important for the

repair of DNA damage and survival following DNA-damage, since lesions in the RAD9 gene cause cells to be hyper-sensitive to DNA damage and incapable of arresting cell cycle progression in G2 after the induction of DNA damage.

The results using asynchronous log-phase cultures showed that TMP1 transcript is induced 3.0 fold by 4NQO. The change in TMP1 expression after 4NQO treatment could occur because 4NQO affects one or more processes that affect gene expression. For example the enzymatically activated form of 4NQO could bind to cellular macromolecules like proteins, RNA and DNA. Therefore the level of normal proteins and RNA might be lower in treated cells than in control cells. To compensate for these reduced levels the cells may induce a large number of genes including TMP1 . The periodically regulated TMP1 gene is expressed in late G1 but not in G2, therefore it would be expected that the accumulation of cells in G2 would repress TMP1 expression. Finally, the repair of DNA damage requires DNA precursors, therefore it might be expected that DNA precursor synthesis and therefore TMP1 expression might be induced by DNA lesions. This induction suggests that new thymidylate synthesis is needed for DNA-repair. This result is similar to that of Elledge et al. (1987) who showed that the thymidylate kinase gene (CDC8) is also induced 2-3 fold by exposure to 4NQO.

Thymidylate kinase is another enzyme involved in the synthesis of thymine deoxyribonucleotides. This response is not surprising, since repair replication needs new nucleotides to replace the excised sequences surrounding the damaged DNA. Other results, which will be discussed later will support this idea.

The yeast genome codes for two topoisomerase enzymes, topoisomerase I and topoisomerase II. TOP2 codes for topoisomerase II, a gene product essential during mitosis. This enzyme seems to be needed to relieve the torsional stress generated by unwinding the DNA helix during replication and transcription (Newlon, 1988). At the time of DNA-repair the unwinding of the DNA helix may be necessary to facilitate repair synthesis and the transcription of repair-inducible genes. Consistent with topoisomerase II being important for DNA-repair I found that TOP2 was induced by 4NQO. An objection to this interpretation arises because DNA-damage causes cells to arrest in the G2 phase and TOP2 is induced at this stage in normal cells.

The role of topoisomerase II in DNA-repair was studied by using inhibitors and these studies gave controversial results. A hypothesis for its role in DNA-repair was postulated after experiments using novobiocin or nalidixic acid, which are topoisomerase II inhibitors, were performed. It is believed that

topoisomerase II has a role in a pre-incision step of the excision repair process. Its role would be to change the topological state of the DNA. One objection to this hypothesis is that novobiocin is a non-specific inhibitor which also acts on a mitochondrial ATPase and through this mode of action could disrupt energy metabolism and deplete cellular ATP. Since ATP is essential for the incision step of excision repair novobiocin could act by depleting cellular ATP and not via a direct effect on topoisomerase II (Downes and Johnson, 1988). Although my results show that TOP2 is induced by 4NQO two questions remain: (i) is TOP2 important for DNA-repair, and (ii) is TOP2 induction a real response of this gene to DNA-damage or is it simply the result of arresting cell division in G2.

Another gene UBI4 was strongly induced by DNA damage. This very strong induction after damage supports the idea that its product has a key role in DNA repair. Ubiquitin's role in proteolysis suggests that UBI4 may have a role similar to that of the recA gene in E.coli .

Recently a lot of work has been done on the regulation of the ubiquitin genes of yeast. The UBI4 gene, one of four ubiquitin genes in yeast, was shown to be specifically required during stress (Ozkanak et al.1987). Consistent with its role during stress the UBI4 gene was induced by heat shock, amino acid analogues, nutrient starvation, 4NQO, MMS and MNNG (Ozkanak et

al.1987 and this thesis) These results suggested that the UBI4 gene is part of a yeast repair system similar to the SOS system in E.coli .

Two other genes, HIS3 and LEU2 were found to be induced by DNA damage. Our experiments used a minimal medium without amino acids, therefore these genes must have already been induced because amino acid biosynthesis must take place under these conditions to supply the amino acids needed for protein synthesis.

HIS3 transcript increased 4.0 fold after 4NQO treatment while LEU2 transcript levels increased 10.0 fold. Many enzymes needed in amino acid biosynthesis are under the regulation of the general amino acid control system. The expression of all genes under general amino acid control increases when cells are starved for any one of several amino acids. It may be that the HIS3 and LEU2 genes are induced by DNA damage, because DNA damage mimics an induction of the general amino acid control system. This is a hypothesis which would be useful to verify.

The induction of the HIS3 and LEU2 genes by DNA damage is surprising for two reasons. First, protein synthesis taking place in the early log phase culture would require amino acids both before and after the addition of 4NQO. In fact the induction of HIS3 and LEU2 by 4NQO seems surprising, because the amount of

protein synthesis after the induction of DNA-damage is probably less that the amount before, since protein synthesis is probably inhibited after DNA damage . Second, if DNA-damage did cause the amount of protein synthesis per cell to increase I would expect that the demand for histidine and leucine although different would increase by the same proportion.

Two possible explanations for the difference between the DNA-damage-dependent induction of HIS3 and LEU2 are; (i) the regulation of these two genes is perturbed by the DNA-damaging agents, and (ii) DNA-damage induces the synthesis of proteins that contain higher proportions of leucine relative to histidine residues than do the proteins synthesized during normal logarithmic growth.

Ruby et al. (1985) identified damage inducible genes (DIN) by screening a small library of yeast promoters for their ability to induce lacZ expression after treatment with a DNA damaging agent. They found four DIN gene promoters and seventeen other genes that were not significantly induced by DNA damage. They also showed that a HIS3'-'lacZ fusion gene was induced less than two fold after MMS and UV treatment, this is consistent with the results obtained in this thesis. Ruby et al. (1985) showed that HIS3 gene transcript expression did not change following 4NQO treatment, but they did not show this result and maybe there was weak induction which they did

not consider significant.

My results and those from the literature suggest that DIN genes can be subdivided into two classes: (i) genes that are strongly induced by DNA damage, and (ii) genes that are weakly induced by DNA damage. Why do DIN genes fall into these two classes? It may be that the the strongly induced genes code for products that are important for the repair of induced DNA damage, but are not needed at significant levels during normal growth. Therefore, to reach the levels necessary for the DNA-repair taking place after treatment with a DNA-damaging agent they must be strongly induced. The genes that are weakly induced by DNA damage may code for products involved in repairing a minor portion of the DNA damage, or having house-keeping functions required by untreated dividing cells and by cells with damaged DNA.

B. Genes repressed by DNA damage.

Although many genes are induced in response to DNA damage it is also likely that some genes will be repressed by DNA damage. I found that two genes, *UBI3* and *H2B*, were repressed by 4NQO.

The *UBI3* gene is another gene of the ubiquitin family. The *UBI3* transcript in normal cells is high during log-phase growth, whereas it is low during stationary phase (Ozkaynak et al., 1987). I found that *UBI3* expression was highly expressed before the addition

of 4NQO and was reduced to 0.7 by the addition of 4NQO.

The expression of the histone H2B gene was also repressed by 4NQO treatment. Expression decreased to 0.5 during the first 30 minutes following the addition of 4NQO and then increased.

The repression of the histone H2B gene by DNA damage can be explained as follows. First, since DNA damage usually arrests cell division there is no need for chromosome replication. Second, since the DNA helix must be accessible to repair enzymes, it may be necessary that the damaged DNA not be associated with its normal complement of histone and non-histone proteins. This lower level of H2B expression may favor a loose chromatin structure which makes the DNA helix more accessible to repair enzymes. The cell cycle is arrested by DNA damage before chromosome replication when cells try to repair DNA damage. In yeast, the RAD9 gene is required to arrest damaged cells in G2 (Weinert and Hartwell, 1988). It will be interesting to see if genes that code for the other histone and non-histone chromosomal proteins are also repressed.

I studied the effect of DNA damage on the expression of seven genes. I found that the expression of all seven genes was altered by treatment with DNA damaging agents. The genes that were induced are involved in DNA precursor synthesis, DNA replication, amino acid synthesis, and

cellular response to stress. The two genes that were repressed are required for ribosome biosynthesis and chromosome replication. Table 4 shows what is now known about gene expression following DNA damage.

II Screening 4NQO inducible genes for induction by other damaging agents.

A. Response of gene expression to different agents.

From the data presented in this thesis it can be seen that DNA-damaging agents, thymidylate starvation and heat-shock had the same general effect on the expression of the genes studied. However, there were differences in gene expression between the different treatments. This variation could occur because the modes of action of the different agents were different or it could be due to differences in the amount of damage induced by these different treatments.

Targer et al. (1988) showed that UBI4 transcript had a dose-dependent increase after exposure to different quantities of 4NQO or MNNG. These dose-dependent differences in UBI4 expression are probably due to differences in the levels of damage caused by the different doses. These differences are consistent with UBI4 expression increasing with the level of cellular damage. To date the UBI4 gene has been found to be induced by all stress factors tested (Finley et al., 1987, Treger et al., 1988 and this thesis).

TABLE 4

Genes expression following DNA damage

Ref.	Gene		Change of gene expression by	
	Nane	Function	DNA-damage	cell-cycle
1	RAD2	in incision of DNA damage	+	
2	RAD52	in endo-nuclease activity	n.i	meiosis
3	RAD6	ubiquitin-conjugating enzyme	+ ?	S to G2
4	POL1	DNA polymerase	+	upon START
5	RNR1	su. of ribonucleotide reductase	+	" "
5	RNR2	" " " "	+	" "
5	CDC8	thymidylate kinase	+	" "
6	CDC9	DNA-ligase	+	" "
7	TMP1	thymidylate synthase	+	" "
7	TOP2	topoisomerase2	+ ?	mitosis
7	H2B	H2B histone	-	after TMP1
7	HIS3	imidazoleglycerol-P dehydratase	+	
7	LEU2	beta-IPM dehydrogenase	+	
7	UBI3	ubiqutin	-	log phase
7&8	UBI4	"	+	stationary

n.i.= not induction; + = induced; - = repression.

Ref.= reference: 1. Robinson et al,(1986); 2. Cole et al.,(1987).; 3. Kupiec et al., (1986).; 4. Johnston et al.,(1987); 5. Elledge at al.,(1987); 6. Johnson et al.,(1986); 7. this thesis; 8. Treger et al.,(1988)

B. What is the role of the TMP1, HIS3 and UBI4 gene products in DNA repair ?

DNA repair genes can also be subdivided into two classes: (i) genes that code for products that are mainly required during stress; and (ii) genes that code for products that are required both during normal growth and during a stress response.

The UBI4 gene can be assigned to the first class. In log-phase cultures its expression is extremely low, whereas its expression increases drastically following the damaging treatments tested here. Consistent with this interpretation, it has been shown that the UBI4 gene is not essential during vegetative growth but is essential for survival following a number of stresses (Treger et al., 1988).

The TMP1 and HIS3 genes apparently fall into the second class. These genes are essential for the growth of yeast cells in minimal medium. One is required for dTTP biosynthesis and the other for histidine biosynthesis. My results showing that both the TMP1 and HIS3 genes are induced following DNA damage suggests that the products of these genes are important for survival following treatments that damage DNA.

Mitchel et al. (1987) showed that MNNG induced an error-prone repair system , UV induced an error free-excision repair system and gamma-radiation induced an

error free-recombinational repair system. The UBI4 gene is strongly induced by MNNG, by 4NQO, a UV-radiation mimicking agent and by MMS a gamma-radiation mimicking agent. The induction of UBI4 by all these agents suggested that the UBI4 gene product is an important component of all three repair pathways. Consistent with this idea we, (manuscript in preparation) and others (Ozkaynak et al.1987), have found that the UBI4 gene is essential for survival following the induction of DNA damage. It will be very interesting to determine whether the ubiquitin expressed from the UBI4 gene is required to form Ub-histone conjugates for gene regulation and/or making damaged DNA accessible for repair and/or Ub-dependent proteolysis of abnormal proteins.

Methyl methane sulfonate (MMS) is an X-radiation mimicking agent, therefore the majority of the damage induced by MMS is repaired by the recombinational repair system. Earlier in this study I found that 4NQO treatment alters the expression of TMP1 . 4NQO mimics damage which is repaired by the excision repair system. The induction of the TMP1 gene after 4NQO and MMS suggests that thymidylate synthase is required for the error free repair of DNA damage by both the excision repair and recombinational repair systems. It will be interesting to determine whether the TMP1 gene is also induced by damaging agents that induce the error-prone repair system.

To determine this I would follow *TMP1* expression following the addition of MNNG, an agent which causes damage that is mainly repaired by the error-prone repair system (Mitchel et al., 1987).

III. Preliminary investigation of the regulatory mechanisms controlling damage inducibility.

Very little is known about the genes involved in DNA repair in yeast. Although many genes (*RAD* genes) have been identified and shown to be important for the repair of DNA damage, the products encoded by the vast majority of these *RAD* genes have not been identified. Many laboratories are doing research directed towards the identification of the *RAD* gene products and their function. In other laboratories another approach is being used. This approach is to study the effect of DNA damage on the expression of genes which code for known products. Using this approach it was found that several genes, that are under cell cycle stage-dependent regulation, are also induced by DNA damage. The question, - are the cell cycle stage-dependent regulation and DNA damage-inducible regulation controlled by two distinct regulatory mechanisms? - can now be answered experimentally.

Wild type strains of *S.cerevisiae* lack thymidine kinase activity and are not permeable to thymidylate. Therefore yeast can not use exogenous supplies of thymidine nor thymidylate. This means that the only source

of dTTP in wild type cells is the thymidylate synthase catalyzed synthesis of thymidylate from uridylate.

The yeast thymidylate synthase encoding TMP1 gene is expressed in a cell cycle stage-dependent fashion, (Storms et al., 1984). Its periodic expression is regulated at the level of transcription with mRNA peaking at a point in the cell cycle near the end of G1 and the beginning of S phase.

The results presented in Figure 6A show that TMP1 transcript levels in stationary phase cultures are very low, however, when 4NQO is added to stationary phase cells the amount of TMP1 transcript increases 7.0 fold. The increase of TMP1 transcript after addition of 4NQO to stationary phase cultures, is strong evidence that the TMP1 gene is under both cell cycle stage and DNA damage dependent regulation, since stationary phase cultures contain yeast cells in the G1/GO stage, a stage when the TMP1 gene is turned off.

The CDC9 gene, which encodes DNA ligase and is cell cycle regulated, is also induced by DNA damaging agents (Johnson et al., 1986). This is consistent with it also being under dual regulation.

The relative levels of the UBI3 and UBI4 transcripts are very different. During log-phase UBI3 expression is high whereas UBI4 expression is very low. The opposite is true in cultures entering the

stationary-phase where expression of UBI3 is very low and UBI4 is very high. I also found that these two genes responded in opposite ways when log-phase culture were treated. That is, the UBI3 gene was repressed whereas the UBI4 gene was strongly induced. Together these results suggest that these two genes respond to the same regulatory signals but in opposite ways. However, the opposite regulation of these two genes does not always occur because when stationary-phase cells were subjected to stress both genes were slightly induced (3.3 times UBI4 and 1.8 times UBI3).

THE MAJOR CONCLUSIONS

1. DNA-damage affected the expression of all seven genes tested causing either, induction or repression.

2. Treatment of yeast with DNA damaging agents causes the UBI4 and LEU2 genes to be strongly induced and the TMP1 , HIS3 and TOP2 genes to be weakly induced.

3. DNA damaging agents repress the expression of the H2B and UBI3 genes.

4. All stress factors tested caused induction of the UBI4 gene.

5. Thymidylate synthase is induced by agents which cause DNA damage which is repaired by the two error free repair systems.

6. The TMP1 gene is under at least two independent regulatory mechanisms, cell cycle regulation and a DNA damage inducible regulation.

7. The UBI3 and UBI4 genes are coordinately regulated, when one is induced, the other is repressed and vice versa.

LITERATURE CITED

Berger, N.A., S.J.Berger,and S.L.Gerson. 1987. DNA-repair, DNA-ribosylation and pyrimidine nucleotide metabolism as targets for cancer chemotherapy. Anti-Cancer Drug Design. 2: 203-210.

Bohr, V.,and P.Hanawalt. 1984. Factors that effect the initiation of excision-repair in chromatin. 109-126. in DNA repair and its inhibition. Ed.A.Collins, C.S.Downes,and R.T.Johnson. IRL Press. Oxford-Washington DC.

Burtscher, H.I., A.J.Cooper,and L.B.Couto. 1988. Cellular responses to DNA-damage in yeast Saccharomyces cerevisiae . Mutat.Res. 194: 1-8.

Chow, T.Y.K.,and M.A.Resnick. 1988. An endo-exonuclease activity of yeast that requires a functional RAD52 gene. Mol.Gen.Genet. 211: 41-48.

Cole, G.M., D.Schild, S.T.Lovett, and R.K.Mortimer. 1987. Regulation of RAD54 and RAD52 -lacZ gene fusions in Saccharomyces cerevisiae in response to DNA damage, Mol.Cell.Biol. 7: 1078-1084

Cole, G.M., D.Schild and R.K. Mortimer. 1989. Two DNA repair and recombination genes in Saccharomyces cerevisiae RAD52 and RAD54 , are induced during meiosis. Mol.Cell Biol., 9: 3101-3104

Downes, C.S.,and R.T.Johnson. 1988. DNA topoisomerases and DNA repair. BioEssay.8: 179-184.

Downes, C.S. 1986. Molecular biology of DNA repair. Mutat.Res. 166: 221-226.

Eckstein, H. 1988 Evidence for cyclic GMP yeast Saccharomyces cerevisiae , and studies on its possible role in growth. Z.Naturforsch. 43C: 386-396.

Ellege, S.J.,and R.W.Davis. 1987. Identification and isolation of gene encoding the small subunit of ribonucleotide reductase from Saccharomyces cerevisiae : DNA damage-inducible gene required for mitotic viability. Mol.Cell.Biol. 7 :2783-2793.

Elledge, S.J. and R.W.Davis. 1989. DNA damage induction of ribonucleotide reductase. Mol.Cell Biol., 9: 4932-4940.

Finley, D, E.Ozkaynak, and A.Varshavsky. 1987. The polyubiquitin gene is essential for resistance to high temperatures, starvation and other stresses. Cell. 48: 1035-1046.

Friedberg, E.C. 1988. Deoxyribonucleic acid repair in yeast Saccharomyces cerevisiae . Microbiol.Rev. 52: 70-102.

Friedberg, E.C. 1987. The molecular biology of nucleotide excision repair of DNA: recent progress. J.Cell.Sci.Suppl.6: 1-23.

Friedberg, E.C. 1985. Preface, vii-x, in DNA repair, W.H.Freeman and Company, New York.

Game, J.C. 1983. Radiation-sensitive mutants and repair in

yeast. 109-137. in Yeast genetics - Fundamental and applied aspects. Ed. by J.F.T.Spencer, D.M.Spencer,and A.R.W.Smith Springer-Verlang New York, Berlin, Heidelberg, Tokyo.

Ganesan, A.K., P.K.Cooper, P.C.Hanawalt,and C.A.Smith. 1982. Biochemical mechanisms and genetic control of DNA repair. 313-323. in DNA repair, chromosome alteration and chromatin structure. Ed. by Natarjan A.T., G.Obe,and H.Altmann. Elsevier Biomedical press Amsterdam, Oxford, New York.

Hadwiger, J.A.,and S.I.Reed. 1988. Invariant phosphorylation of the Saccharomyces cerevisiae Cdc28 protein kinase. Mol.Cell.Biol. 8: 2976-2979.

Haynes, R.H.,and B.A.Kunz. 1981. DNA repair and mutagenesis in yeast. 371-414. in The molecular biology of the yeast Saccharomyces . Life cycle and inheritance. Ed. by J.N.Strathern, E.W.Jones,and J.R.Broach. Cold Spring Harbor Laboratory.

Hershko, A. 1988. Ubiquitin mediated protein degradation. J.Biol.Chem. 263:15237-15240.

Herskowitz, I. 1988. Life cycle of the budding yeast Saccharomyces cerevisiae . Microbial.Rev. 52: 536-553.

Hinnebusch, A.G. 1988. Mechanisms of gene regulation in the general control of amino acid biosynthesis in Saccharomyces cerevisiae . Microbiol.Rev. 52: 248-273.

James, A.P,and A.Nasim. 1987. Effects of radiation on

yeast. in The yeast. vol2. Ed.by A.H.Rose,and J.S.Harrison. Acad.Press.

Johnson, A.I., D.G.Barker,and L.H.Johnson. 1986. Induction of yeast DNA ligase genes in exponential and stationary phase cultures in response to DNA-damaging agents. Curr.Genet. 11: 107-112.

Johnston, L.H., J.H.M.White, A.L.Johnson, G.Lucchini and P.Pleviani. 1987. The yeast DNA polymerase I transcript is regulated in both the mitotic cell cycle and in meiosis and is also induced after DNA damage. Nucleic Acids Res. 15: 5945-5962.

Jentsch, S., J.P.Mc.Grath and A.Varshavsky. 1987. The yeast DNA repair gene RAD6 encodes a ubiquitin-conjugating enzyme. Nature. 329: 131-134.

Kenago, M.I., M.Tada,and Y.Korwazoe. 1981. Measurement of base damage caused by 4-nitroquinoline 1-oxide. 187-201. in DNA repair. A laboratory manual of research procedures. voll. parA. Ed E.C.Friedberg, and P.C.Hanawalt. Marcel Dekker Inc. New York and Basel.

Kimball, R.F. 1987. The development of ideas about effect of DNA repair on the induction of gene mutation and chromosomal aberrations by radiation and by chemicals. Mut.Res. 186: 1-34.

Kunz, B.A., B.J.Barclay, J.G.Game, J.G.Little,and R.H.Haynes. 1980. Induction of mitotic recombination in yeast by starvation for thymine nucleotides.

Proc.Natal.Acad.Sci.USA. 77: 6057-6061.

Kupiec, M., and G.Simchen. 1986. Regulation of the RAD6 gene of Saccharomyces cerevisiae in the mitotic cell cycle and in meiosis. Mol.Gen.Genet. 203: 538-543.

Lammers, M.,and H.Follmann. 1984. Deoxyribonucleotide biosynthesis in yeast Saccharomyces cerevisiae , A ribonucleotide reductase system of sufficient activity for DNA synthesis. Eur.J.Biochem. 140: 281-287.

Linn, S. 1982. Nucleases involved in DNA repair. 59-84. in Nucleases. Ed.by S.M. Linn,and R.J.Roberts. Cold Spring Harbor Laboratory.

Magana-Schwencke, N., J.A.P.Henrigues, R.Chanet,and E.Moustacchi. 1982. The fate of 8-methoxypsoralen photoinduced crosslinks in nuclear and mitochondrial yeast DNA: comparison of wild type and repair deficient strain. Proc.Nalt.Acad.Sci.USA. 79: 1722-1726.

Marck, M., P. Gompel-Klein, E.Hoase, J.Hietkamp, A.Ruhland,and M.Brendel. 1988. Genetic characterization of hyperresistance to formaldehyde and 4-nitroquinoline 1-oxide in yeast Saccharomyces cerevisiae . Mol.Gen.Genet. 21: 260-265.

McClanahan, T., and K.McEntee. 1984. Specific transcripts are elevated in Saccharomyces cerevisiae in response to DNA damage. Mol.Cell.Biol. 4: 2356-2363.

McClanahan, T.,and K.McEntee. 1986. DNA damage and heat

shock dually regulate genes in Saccharomyces cerevisiae . Mol.Cell.Biol. 6: 90-96.

McCready, S.J., J.M.Boyce,and B.C.Cox. 1987. Excision repair in the yeast, Saccharomyces cerevisiae . J.Cell.Sci.Suppl. 6: 25-38.

McIntosh, E.M., R,W.Ord,and K.K.Storms. 1988. Transcriptional regulation of the cell cycle dependent thmidylate synthase gene of Saccharomyces cerevisiae . Mol.Cell.Biol. 8: 4616-4624.

McIntosh, E.M., M.H.Gadsden,and R.H.Haynes. 1986. Transcription of genes encoding enzymes involved in DNA synthesis during the cell cycle of Saccharomyces cerevisiae . Mol.Gen.Genet. 204: 363-366.

Mitchel, R.E.J.,and D.P.Morrison. 1987. Inducible DNA repair systems in yeast: competition for lesions. Mut.Res. 183: 149-159.

Mitchel, R.E.J.,and D.P.Morrison. 1986. Inducible error prone repair in yeast. Suppression by heat shock. Mut.Res. 159: 31-39.

Moens, P.B., B.J.Barclay,and J.G.Little. 1981. Nuclear morphology of yeast under thymidylate starvation. Chromosome. 373: 1-8.

Montelone, B.A., S.Prakash,and L.Prakash. 1981. Recombination and mutagenesis in rad6 mutants of Saccharomyces cerevisiae evidence for multiple functions of the RAD6 gene. Mol.Gen.Genet. 184:

410-415.

Moore ,C.W. 1988. Bleomycin induced DNA repair by Saccharomyces cerevisiae ATP-dependent polydeoxyribonucleotide ligase. J.Bact. 150; 4991-4994.

Moreland, R.B., G.L.Langevin, R.H.Singer, R.L.Garcea,and L.M.Hereford. 1987. Amino acid sequences that determine the nuclear localization of yeast histone 2B. Mol.Cell.Biol. 7: 4048-4057.

Nasmyth, K., 1983, Molecular analysis of cell lineage. Nature. 302: 670-676.

Newlon, C.S. 1988. Yeast chromosome replication and segregation. Microbiol.Rev. 52: 568-601.

Osley, M.A., J.Gould, S.Kin, M.Kane,and L.Hereford. 1986. Identification of sequences in a yeast histone promoter involved in periodic transcription. Cell. 45: 537-544.

Ozkaynak, E., D.Finley, M.J.Solomon,and A.Varshavsky. 1987. The yeast ubiquitin genes: a family of natural gene fusions. EMBO J. 6: 1429-1439.

Peterson, T.A., L.Prakash, S.Prakash, M.A.Osley,and S.I.Reed. 1985. Regulation of CDC9 , in Saccharomyces cerevisiae gene that encodes DNA ligase. Mol.Cel.Biol. 5: 226-235.

Prakash, A, R.Polakowska, P.Reynolds,and S.Weber. 1983. Molecular cloning and preliminary characterization of

the RAD6 gene of the yeast, Saccharomyces cerevisiae . 559-568. in Cellular response to DNA damage. Alan R.Liss Inc. New York.

Rechsteiner, M. 1987. Ubiquitin-mediated pathways for intracellular proteolysis. Ann.Rev.Biol. 3: 1-30.

Resnick, M.A., J.Westmoreland, E.Amaya,and K.Bloom. 1987. UV-induced damage and repair in centromere DNA of yeast. Mol.Gen.Genet. 210: 16-22.

Robinson, G.W., C.M.Nicolet, D.Kalainov,and E.C.Freidberg. 1986. A yeast excision-repair gene is inducible by DNA damaging agents. Proc.Natal.Acad.Sci.USA. 83: 1842-1846.

Ruby, S.W.,and J.W.Szostak. 1985. Specific Saccharomyces cerevisiae genes are expressed in response to DNA-damaging agents. Mol.Cell.Biol. 5: 75-84.

Sassone-Corsi, P., J.Visvader, L.Ferland, P.L.Mellon,and I.M.Verma. 1988. Induction of proto-oncogene fas transcription through the adenylate cyclase pathway: characterization of a cAMP responsive element. Genes & Develop.2: 1529-1538.

Schild, D.S., I.L.Calderon, C.R.Contopoulou,and R.K.Mortimer. 1983. Cloning of yeast recombination repair genes and evidence that several are nonessential genes. 417-427. in Cellular responses to DNA damage. Alan R.Liss, Inc., New York.

Siede, W.,and F.Eckardt. 1986. Analysis of mutagenic DNA

repair in a thermoconditional mutant of Saccharomyces cerevisiae . III Dose-response pattern of mutation induction in UV-irradiated rev2 cells. Mol.Cell.Genet. 202: 68-74.

Schiestl, R.H., P.Reynolds, S.Prakash,and L.Prakash. 1989. Cloning and sequence analysis of the Saccharomyces cerevisiae RAD9 gene and further evidence that its product is required for cell cycle arrest induced by DNA damage. Mol.Cell.Biol.. 9: 1882-1896.

Sung, P., S.Prakash,and L.Prakash. 1988. The RAD6 protein of Saccharomyces cerevisiae polyubiquitinated histones, and its acidic domain mediates this activity. Genes & Develop. 2: 1476-1485.

Tada, M.,and M.Tada. 1975. Seryl-tRNA synthetase and activation of the carcinogen 4-nitroquinoline 1-oxide. Nature. 255: 510-512.

Thorner, J. 1982. An essential role for cyclic AMP in growth control: the case for yeast cells. Cell. 30: 5-6.

Treger, J.M., K.A.Heichman,and K.McEntee. 1988. Expression of the yeast UBI4 gene increases in response to DNA-damaging agents and in meiosis. Mol.Cell.Biol. 8: 1132-1136.

Walker, G.C., L.Marsh,and L.A.Dodson. 1985. Genetic analysis of DAN repair: interference and extrapolation. Ann.Rev.Genet. 19: 103-126.

Weinert, T.A.,and L.H.Hartwell. 1988. The RAD9 gene controls the cell cycle response to DNA damage in Saccharomyces cerevisiae . Science, 241: 317-322.

Wheals, A.E. 1987. Biology of the cell cycle in yeast. 284-344. in The yeast. vol2. Ed.by A.H.Rose,and J.S.Harrison. Acad.Press.

Wintersberger, U.,and A.Karwan. 1987. Retardation of cell cycle progression in yeast cells recovering from DNA damage: a study at the single cell level. Mol.Gen.Genetic. 207: 320-327.

Wittenberg, C.,and S.I.Reed. 1988. Control of the yeast cell cycle is associated with assembly/disassembly of the cdc28 protein kinase complex. Cell. 54: 1061-1072.