Specific transcriptional responses induced by 8-methoxypsoralen and UVA in yeast

Michèle Dardalhon1, Waka Lin2, Alain Nicolas2 & Dietrich Averbeck1

1Institut Curie Section de Recherche, UMR2027 CNRS/I.C., INSERM, LCR n°28 CEA, Centre Universitaire d’Orsay, Orsay Cedex, France; and
2Institut Curie Section de Recherche, UMR 7147 CNRS, Université Pierre et Marie Curie, Paris Cedex, France

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Abstract

Treatment of eukaryotic cells with 8-methoxypsoralen plus UVA irradiation (8-MOP/UVA) induces pyrimidine monoadducts and interstrand crosslinks and initiates a cascade of events leading to cytotoxic, mutagenic and carcinogenic responses. Transcriptional activation plays an important part in these responses. Our previous study in Saccharomyces cerevisiae showed that the repair of these lesions involves the transient formation of DNA double-strand breaks and the enhanced expression of landmark DNA damage response genes such as RAD51, RNR2 and DUN1, as well as the Mec1/Rad53 kinase signaling cascade. We have now used DNA microarrays to examine genome-wide transcriptional changes produced after induction of 8-MOP/UVA photolesions. We found that 128 genes were strongly induced and 29 genes strongly repressed. Modifications in gene expression concern numerous biological processes. Compared to other genotoxic treatments, c. 42% of the response genes were specific to 8-MOP/UVA treatment. In addition to common DNA damage response genes and genes induced by environmental stresses, a large fraction of 8-MOP/UVA response genes correspond to membrane-related functions.

Introduction

The bifunctional furcoumarin 8-methoxypsoralen (8-MOP) is a well established drug in the photochemotherapy of psoriasis and other skin diseases, including the treatment of cutaneous T-cell lymphoma (Averbeck, 1989; Zarebska et al., 2000). However, psoralsens such as 8-MOP also have a genotoxic potential that has been attributed to their ability to bind specifically and covalently to pyrimidine bases in DNA upon UV A irradiation (Averbeck, 1989). These compounds intercalate into DNA and undergo photocycladdition with pyrimidines (mainly thymidine) in a sequence-specific manner to form monoadducts and interstrand crosslinks (ICLs) (Dall’Acqua et al., 1970), for which 5′TpA sites are the preferred targets. ICLs induced by 8-MOP plus UVA (8-MOP/UVA) are critical genotoxic lesions and have strong antiproliferative effects (Zheng et al., 2006).

The DNA lesions induced upon 8-MOP/UVA exposure differ strikingly from the pyrimidine dimers induced by UV radiation, the single- and double-strand breaks and base lesions induced by ionizing radiation and the methylated bases induced by methyl methane sulphonate (MMS). Moreover, the pyrimidine photoadditions induced by 8-MOP/UVA are clearly different from the purine mono- and diadducts induced by alkylating crosslinking agents such as nitrogen mustard and cisplatin, and they are chemically more stable (Zheng et al., 2006). Due to energy transfer reactions, exposure to 8-MOP/UVA can also produce singlet oxygen, which may attack all cellular constituents (Zarebska et al., 2000). Stable cyclobutane photoadducts can also be formed with unsaturated fatty acids, thus affecting membranes (Zarebska et al., 2000).

With respect to the specificity and the complexity of the damage induced by 8-MOP/UVA, it was of particular interest to analyse the global transcriptional response of eukaryotic cells. We previously established genotoxicity levels and dose-response curves for this agent in the model yeast Saccharomyces cerevisiae (Cohen et al., 2002). We
showed by pulsed-field gel electrophoresis that 8-MOP/UVA treatment causes DNA double-strand breaks (DSBs) (Dardalhon & Averbeck, 1995; Dardalhon et al., 1998), accompanied by the induction of DNA damage response genes such as the ribonucleotide reductase subunit gene RNR2, which is involved in DNA metabolism, and the DNA repair genes RAD54 and RAD51, which are involved in the repair of DSBs by homologous recombination (Averbeck & Averbeck, 1994, 1998). However, the formation of these DSBs is indirect, as they result from the repair of primary ICLs (Jachymczyk et al., 1994, 1998). However, the formation of these DSBs is shown by pulsed-field gel electrophoresis that 8-MOP/UVA treatment causes DNA double-strand breaks (DSBs) (Darald et al., 1995; Dardalhon et al., 1998).

Materials and methods

Strains and culture conditions

The DNA repair-competent haploid strain BY4741 (MAT a, his3Δ, leu2Δ0, met15Δ0, ura3Δ) was used. Cells were precultured twice at 30 °C for 12 h in liquid minimal medium [0.67% ammonium base without amino acids (Difco), 2% glucose (Merck)] supplemented with appropriate amino acids. Cell cultures in exponential phase (final concentration 10⁷ cells mL⁻¹) were obtained in liquid YPD medium [0.5% yeast extract (Difco), 2% bactopeptone (Difco), 2% glucose].

8-MOP plus UVA (8-MOP/UVA) treatment

Cells were harvested during exponential growth phase, centrifuged, resuspended in sterile water, incubated in the presence of 5-μM 8-MOP (Sigma) in the dark for 30 min and exposed to 365-nm UVA radiation at a fluence rate of 11 J m⁻² s⁻¹ using a HPW125 Philips lamp with a Pyrex water filter. All 8-MOP/UVA experiments were performed at 20 °C. Cells were irradiated with 8-MOP/UVA and resuspended in fresh YPD at the original culture volume. Culture samples were collected for fluorescence-activated cell sorting (FACS analysis), measurements of cell survival rates, and Northern blot and transcriptome analyses at different time points after photosensitizing treatment. Experiments with 8-MOP in the dark or UVA irradiation alone were conducted for different incubation times in YPD medium.

Survival studies

Clonogenic survival of cells treated in exponential growth phase was determined as previously described by taking aliquots from treated and untreated samples (Averbeck & Averbeck, 1994). After dilution and plating on solid complete growth medium containing 2.6% agar (Difco), plates were incubated for 5 days at 30 °C. Outgrowing colonies were counted and the surviving fraction was determined.

Fluorescence activated cell sorting (FACS analysis)

Cells from exponential phase cultures were treated as previously described (Cohen et al., 2002). After sonication, cells were checked by light microscopy before analysis with a FACSCalibur apparatus (Becton-Dickinson). For each experimental point, 2 × 10⁶ cells were analyzed using CELLQUEST software.

RNA extraction and Northern blotting

Total RNA was extracted from 0.5–1.0 × 10⁸ cells using the FastRNA-Red kit (Bio101) according to the manufacturer’s instructions and subjected to Northern analysis as described (Cohen et al., 2002). Blots were successively hybridized with a radio-labeled RAD51 DNA probe (335 bp), prepared by digesting pTZ51 (a gift of M. Vedel, Institut Curie) with ClaI and BstXI, and an ACT1 probe (587 bp), prepared by digesting pSK-ACT (a gift of M. Vedel, Institut Curie) with HindIll+XbaI. Blots were scanned using a PhosphorImager system (Molecular Dynamics) and analyzed using IMAGE QUANT v.5.1 software (Molecular Dynamics). RAD51 gene up-regulation was determined as previously described (Cohen et al., 2002).
mRNA preparation for microarray analysis

Total RNA was extracted using a protocol derived from the hot acid phenol extraction method as described at http://www.molecularstation.com. Total RNA (500 to 1000 μg for each sample) was used for Poly(A)+RNA isolation with the Micro-Fast Track 2.0 kit (Invitrogen) according to the manufacturer’s protocol for yeast mRNA. Target samples for microarray analysis were prepared using an amino-allyl dye coupling protocol (http://www.molecularstation.com). Microarray analysis was performed with cells treated with 8-MOP/UVA, cells treated with 8-MOP in the dark without UVA (8-MOP/dark) or cells treated with UVA alone and incubated for 3 h or various times (1–5 h) in complete growth medium. Aliquots from each time point were used to generate cDNA probes labeled with Cy5-dUTP or Cy3-dUTP, and differentially labeled probes were combined and hybridized to yeast genomic microarrays.

Experimental design

The experimental scheme consisted of incubating cells with 5 μM 8-MOP in the dark for 30 min. Part of this material was immediately frozen and served afterwards for RNA extraction and cDNA labeling (control 8-MOP exposure), and the remainder was halved. For 8-MOP/dark treatments, one fraction was incubated in complete growth medium for 0–5 h at 30 °C. For 8-MOP/UVA treatments, the other fraction was exposed to UVA (5 kJ m⁻²) and incubated in complete growth medium for 0–5 h at 30 °C. Samples were withdrawn hourly, frozen and used for generating labeled cDNA probes. For each time point, labeled cDNA prepared from 8-MOP/dark- or 8-MOP/UVA-treated cells was hybridized with control labeled cDNA to yeast genomic microarrays. To strengthen the kinetic results, we performed additional experiments with labeled cDNA from 8-MOP/dark-treated cells and labeled cDNA from 8-MOP/UVA- or 8-MOP/dark-treated cells that were incubated for 3 h in complete growth medium. Although previous studies demonstrated that UVA radiation alone at this dose level, as well as 8-MOP/dark treatment alone, has no significant effect on survival, mutation or gene induction (Averbeck & Averbeck, 1998; Cohen et al., 2002), we also hybridized labeled cDNA prepared from untreated samples to microarrays together with labeled cDNA prepared from samples treated with UVA alone (5 kJ m⁻²) and incubated for 3 h in complete medium. In spite of the fact that changes by a less than a factor of 2 can be significant, and in order to identify only those transcripts that were specifically induced by 8-MOP/UVA treatment, genes were defined as highly differentially regulated if they exhibited an at least twofold change in the level of expression, and genes affected by exposure to UVA alone or to 8-MOP alone after incubation in growth medium were discarded. The intra-experimental variations were correct and the levels of RAD51 mRNA in each experiment were in accord with Northern measures. The mean value from three experiments with cells incubated for 3 h after 8-MOP/UVA- and 8-MOP/dark treatment is presented, as well as with cells incubated for 3 h after UVA-alone treatment.

Microarray hybridization, data acquisition and analysis

Yeast coding regions were amplified by PCR using the complete set of Yeast GenePairs Primers purchased from Research Genetics. After isopropanol precipitation, PCR products were resuspended in 3X SSC. DNA was printed on polylysine-coated glass slides or Corning Ultra Gaps II slides using a Microgrid TAS arraying robot (Biorobotics). The slides were processed, hybridized at 63 °C, and washed as described (http://www.molecularstation.com). For microarray analyses, slides were scanned using a Genepix 4000A scanner (Axon Instruments) and images were processed using GENEPIX PRO 4.0 software. Data from poor quality spots and spots with a total intensity below 500 were eliminated. The local background median intensity was subtracted from the median spot intensity before calculating the intensity ratio (Cy5 median intensity/Cy3 median intensity) for each spot. Data storage, filtering, global normalization and signal intensity ratio calculation were performed using the BASE (BioArray Software Environment) 1.0.7 program. Genes were annotated using Gene Ontology terms (Ashburner et al., 2000) provided at the Saccharomyces Genome Database website (SGD) (Cherry et al., 1998) (GO Consortium: http://www.geneontology.org; SGD: http://www.yeastgenome.org). Gene regulatory information was obtained using the Yeast Proteome Database (YPD) (Costanzo et al., 2000). Genes for which the log₂ Cy5/Cy3 ratio was >1 or < −1 (2 and 0.5-fold differences in expression, respectively) were defined as differentially regulated.

Online supplementary material

As previously described (Sollier et al., 2004), microarray details are provided at http://www.ebi.ac.uk/aerep/(query for arrays, accession number a-mexp-40). Probe details, microarray hybridization protocols and data are available at http://microarrays.curie.fr/. A list of genes with ≥2-fold altered levels of expression after 8-MOP/UVA treatment is presented in supplementary Table S1 and as a function of posttreatment incubation time in supplementary Table S2. Lists of 8-MOP/UVA response genes encoding proteins with membrane related functions and comparison with other genotoxic responses in yeast are presented in supplementary Tables S3 and S4, respectively. A Treeview analysis (Eisen et al., 1998) of 8-MOP/UVA response genes whose...
expression overlaps with that induced by histone depletion (Wyrick et al., 1999) is shown in supplementary Fig. S1.

Results and discussion

Cell cycle analysis and RAD51 mRNA expression after 8-MOP/UVA treatment of haploid yeast cells

To study transcriptome modifications after treatment with 8-MOP/UVA, we chose experimental conditions that allowed ~20% survival of haploid wild-type cells, incubation in 8-MOP (5 μM) followed by exposure to 5 kJ m\(^{-2}\) UVA. 8-MOP/UVA-treated cells did not resume growth until ~3 h posttreatment, whereas 8-MOP/dark-treated cells continued to grow (Fig. 1). FACS analysis showed that the proportions of 8-MOP/dark-treated cells in G1, S, and G2/M remained constant during the incubation period (Fig. 1). However, 8-MOP/UVA-treated cells showed a modified cell cycle distribution during posttreatment incubation (Fig. 1). Finally, RAD51 mRNA transcript levels increased immediately after 8-MOP/UVA exposure, reaching a maximum between 3 and 4 h posttreatment with an approximately fourfold difference in the expression level, whereas the level of the RAD51 transcript remained stable in 8-MOP/dark experiments (Fig. 1). Microarray analysis was thus performed under these well-characterized conditions. (Averbeck & Averbeck, 1998; Cohen et al., 2002)

Global changes in gene expression

Genes that responded similarly to 8-MOP/UVA treatment over the time course and after 3 h incubation were designated as response genes. Among the 6217 ORFs spotted on our microarrays, 157 genes exhibited a reproducible modification in expression upon 8-MOP/UVA treatment (listed in supplementary Table S1). Of these, 128 ORFs showed a significant increase in expression. The greatest extent of induction was 16-fold for RNR3, which encodes a large subunit of ribonucleotide-diphosphate reductase, and for NCA3, which encodes a member of the SUN family involved in processes such as DNA replication, ageing and mitochondrial biogenesis. Twenty-nine genes were down-regulated. The strongest repression was 3.8-fold for the ORF YGR273c of unknown function.

The analysis of the kinetics of gene induction following genotoxic damage was informative about the timing of events during the posttreatment period. Indeed, 41 genes

![Fig. 1. Responses to 8-MOP damage in wild-type cells following treatment with 5 μM 8-MOP plus 5 kJ m\(^{-2}\) UVA as compared to 8-MOP/dark-treated cells (exposure to 5 μM 8-MOP in the dark). Top: growth of 8-MOP/UVA- and 8-MOP/dark-treated cells as monitored by FACS analysis. C1 denotes a single genome content (equivalent to that of G1 haploids) and C2 denotes a double genome content (equivalent to that of G2 haploids). Middle: cell cycle profiles of 8-MOP/UVA- and 8-MOP/dark-treated cells during posttreatment incubation in complete medium as monitored by FACS analysis. Bottom: Northern blot analysis of RAD51 mRNA levels in 8-MOP/UVA- and 8-MOP/dark-treated cells. Induction was normalized to actin (ACT1) transcript levels and is given in arbitrary units.](Image)
were induced rapidly within the first hour, whereas others were induced 2 or 3 h later (supplementary Table S2). Genes already known to be involved in DNA metabolism (RNR2, RNR3, RNR4) or in DNA repair (RAD51, DUN1, DIN7) were induced after 1 or 2 h of posttreatment incubation time, respectively. Genes such as SLT2, ECM3, GUP2 and PDE2, involved in signal transduction, belong to the early genotoxic response. The high levels of expression of those genes during the first hour were not maintained throughout the entire posttreatment period but rapidly declined after reaching peak levels (data not shown). Analysis of the loci of responsive ORFs according to SGD annotations (Cherry et al., 1998) shows that they are distributed throughout the genome (data not shown).

**Cellular localization of products of 8-MOP/UVA response genes**

We analysed the cellular localization of proteins encoded by genes showing altered expression upon 8-MOP/UVA treatment, in accordance with previous determinations made by O’Shea with GFP-tagged proteins (Huh et al., 2003) (data not shown). This analysis showed that all cellular compartments, including the nucleus, are represented. Gene products located in the cytoplasm or in membranes are prominent, indicating that cellular constituents other than those involved in DNA metabolism are affected by exposure to 8-MOP/UVA. We found a higher-than-expected involvement of genes encoding membrane-bound proteins: 31.3%, vs. 23% for the genome as a whole.

**Categories of gene function affected by 8-MOP/UVA treatment**

To gain an overview of the gene functions modified after 8-MOP/UVA, we assigned them to functional categories as defined by the Gene Ontology (GO) project (Fig. 2). These assignments show that 8-MOP/UVA response genes are implicated in a wide variety of biological processes that concern three main activities: (1) DNA metabolism and cell cycle-related functions; (2) cellular functions and maintenance of cellular structures; and (3) general metabolism. 8-MOP/UVA response genes involved in DNA metabolism and cell cycle control, in cellular functions or structures and in metabolism are presented and quantified in Figs 3, 4 and 5, respectively.

**Response genes involved in DNA damage repair and the cell cycle**

Genes known to be up-regulated in response to DNA damage induced by MMS and ionizing radiation (Jelinsky et al., 2000), such as RAD51, RAD54, RNR2, RNR4, DIN7
and RNR3, were also up-regulated after 8-MOP/UVA exposure (Fig. 3). The important role of recombinational pathways in interstrand DNA cross-link repair (Saffran et al., 2004) is consistent with the induction of the recombinational repair genes RAD54 and RAD51 we observed. However, we found that genes involved in nucleotide excision repair (RAD2, RAD16 and RAD23) and postreplication repair (RAD18) taking part in repair of 8-MOP/UVA DNA lesions (Saffran et al., 2004) were unaffected.

Other notable genes relevant to DNA repair were induced, including NSE1, which encodes an essential nuclear protein that has a nonstructural role in the maintenance of chromosomes and which is a component of the SMC5-SMC6 complex involved in DNA repair, and UTH1, a member of the SUN family involved in mitochondrial autophagy, the oxidative stress response, the starvation response, mitochondrial biogenesis, and cell death. PAK1, which encodes an upstream kinase for the SNF1 complex, was also present. Moreover, we identified YRF1-2, a helicase that is highly expressed in mutants lacking the telomerase component TLC1. Other prominent genes were CDC45 and ESC8, which encode a DNA replication initiation factor and a protein involved in telomeric and mating-type locus silencing, respectively.

Among the repressed genes, RPD3 encodes a histone deacetylase, and IOC4 is implicated in chromatin remodeling. Furthermore, we observed repression of the histone genes HTA1, HTA2 and HTB2 (Fig. 3). Also, the induction of YRF1-2 as well as other subtelomeric genes such as YFL061W, YFL065C and YFL066C (supplementary Table S1) may result in structural modifications of chromatin relevant to DNA repair. These proteins share near identity to other subtelomically-encoded proteins that are members of the Y* expression cluster (Yamada et al., 1998).

Several genes involved in the cell cycle were up-regulated after 8-MOP/UVA treatment (Fig. 3). These include DUN1, which encodes a kinase required for the DNA damage-induced transcription of certain target genes and the control of the DNA damage response, SPO12, a positive regulator of the exit from mitosis, CTF19, an outer kinetochore protein that is required for accurate mitotic chromosome segregation, and CDC27, a subunit of the anaphase-promoting complex. Among repressed genes we found NBPI, which encodes a component of the mitotic apparatus, essential for the G2/M transition, and NIS1, which encodes a protein localized in the bud neck at the G2/M transition that may be involved in a mitotic signaling network.

**Response genes involved in cellular structures and functions**

Several genes involved in cytokinesis, in organelle organization and biogenesis, in the cytoskeleton structure as well as genes involved in conjugation, meiosis, spore wall formation and sporulation were modified after 8-MOP/UVA treatment suggesting that cellular integrity must be restored before cell division (Fig. 4a). Another important cellular compartment affected by 8-MOP/UVA treatment is the mitochondrion, which functions in energy metabolism and apoptosis.

8-MOP/UVA response genes whose products localize to mitochondria include DIN7, YEL047C, COT1, FMP48, PIC2, FMP50, YRO2, PUT22, ARG5, 6, FMP52 and MRP20. Also, genes involved in mitochondrial organization and biogenesis were strongly induced, including NCA3, UTH1 and YPR157W (Fig. 4a). These 8-MOP/UVA mitochondrial effects are in accord with previous work describing the induction of respiratory deficiency in yeast (Averbeck,
1989), mitochondrial dysfunction following the opening of permeability transition pores (Canton et al., 2002), and the induction of apoptosis after psoralen plus UVA treatment (Viola et al., 2004).

Cell wall maintenance genes such as WSC2, ECM13, ECM3 and GAS2 were induced, indicating that 8-MOP/UVA treatment affects cell wall components that must be reconstructed (Fig. 4a). Genes implicated in cellular functions or structures are activated or repressed in cells treated with 8-MOP/UVA. Plotted data are the mean log2 ratios. (a) Genes implicated in cytokinesis, organelle, cytoskeleton, conjugation, meiosis, sporulation, cell wall, and signal transduction. (b) Genes implicated in transcription, homeostasis, and transport. (*Genes that fall into multiple categories.)
Various biological effects, including melanogenesis (Zarebska et al., 2000), pointing to the importance of psoralens and UVA-induced photodamage of membranes.

Expression of several transcription factors was affected by 8-MOP/UVA treatment (Fig. 4b). However, by examining the Biomolecular Interaction Network Database (BIND) provided at SGD and gene regulatory information provided at YPD, we identified only a few 8-MOP/UVA response genes among their known or inferred targets. This finding suggests that the altered expression of these transcription factors does not account for the global 8-MOP/UVA transcriptional response. 8-MOP/UVA-induced genes are involved in transport (Fig. 4b). These include up-regulated genes encoding proteins with diverse transporter activities for amino acids, polyamine, hexoses, maltose, glycerol, thiamine, phosphate, protons and cations. Among homeostasis genes, several regulatory ion flux genes (involved in iron, zinc, sodium or potassium metabolism) were induced by 8-MOP/UVA (Fig. 4b). The induction or repression of transport-related genes may be necessary to maintain cellular homeostasis when membranes are damaged.

Analyses of our results reveal that membranes are important targets for 8-MOP/UVA because we find an enrichment concerning gene products located in the membranes as compared to the genome as a whole (see above). Genes encoding proteins with membrane-related functions (transport, homeostasis, signal transduction, cell wall maintenance) are numerous. The expression of several genes encoding proteins with transmembrane segments, such as YR02, YHL044W, RTA1, YCR061W, GUP2, YER113C, WSC2, YOR071C, TPO2 and PRM10, are modified after 8-MOP/UVA treatment. In addition, among the genes with expression specifically modified by 8-MOP/UVA, several are also induced by the membrane-perturbing agent chitosan (Zakraziwska et al., 2005) and/or by transient cell wall damage (Garcia et al., 2004) (supplementary Table S3). Altogether, these results suggest that 8-MOP/UVA lesions at membrane level play an important role on variations in the transcript levels. The relatively high proportion of genes with membrane-related functions in 8-MOP/UVA response genes might be explained by the fact that cellular targets affected by psoralen plus UVA treatment include not only DNA, with the induction of mono- and diadducts (Averbeck, 1989), but also cytoplasm and cell membranes, with the oxidation of phospholipids, and membrane proteins (Zarebska et al., 2000). Interestingly, some 8-MOP/UVA-sensitive mutants show specific alterations of membrane lipids (Querol et al., 1994; Brendel et al., 2003). Indeed, oxygen-dependent photoreactions between furocoumarins and cell membrane constituents can lead to lipid peroxidation and protein cross-linking, and oxygen-independent photoreactions can lead to C4 cyclo-addition between furocoumarin and

![Fig. 5. Specific genes involved in cell metabolism are up-regulated or down-regulated in cells treated with 8-MOP/UVA. Plotted data are the mean log2 ratios. (● Genes that fall into multiple categories.)](image-url)

Adducts activate protein kinase C to enhance UVA-induced melanogenesis (Zarebska et al., 2000), pointing to the importance of psoralens and UVA-induced photodamage of membranes.

Signaling was upregulated as well (Fig. 4a). These include SLT2 and PKH1 (encoding a serine/threonine MAP kinase), PBS2 (protein tyrosine kinase) and PDE2 (cAMP-specific phosphodiesterase). Interestingly, SLT2 is slightly upregulated after UVA alone. The activation of MAP kinases by 8-MOP/UVA is in line with previous work. For example, in human cells, cyclobutane adducts of fatty acids formed by UVA can be released from modified phospholipids through phospholipase A2 hydrolysis, and these adducts can act like diacylglycerol, a second messenger responsible for various biological effects, including melanogenesis (Zarebska et al., 2000). It has been proposed that 8-MOP-fatty acid
unsaturated fatty acids, thereby modulating membrane functions and intracellular pathways (Dall’Acqua & Martelli, 1991; Zarebska et al., 2000). The combined treatment 8-MOP/UVA leads to significant production of reactive oxygen species (ROS). This might be one possible explanation for the results that differential expression of significantly more genes coding for various cellular structures and functions (like membranes, cytoplasmic vesicles, transport) is found. It would be informative to use ROS scavengers before expression analysis or to compare 8-MOP/UVA effects with typical ROS-producing agents.

Response genes implicated in carbohydrate, amino acid and lipid metabolism

The lesions induced in DNA and in other macromolecules by 8-MOP/UVA treatment led to an altered metabolic program, with the induction of genes involved in carbohydrate or vitamin metabolism (Fig. 5). Moreover, genes involved in protein degradation were modified, suggesting that proteins altered by 8-MOP/UVA treatment may be targeted for removal and that the specific elimination of certain proteins may be important for cellular recovery. Indeed, evidence for new protein synthesis is suggested by the increased expression of genes involved in amino acid metabolism. Interestingly, several proteins involved in post-translational modification were up-regulated, indicating that cellular responses to DNA damage often entail processes such as phosphorylation and proteolytic cleavage that modify protein–protein and protein–DNA interactions for efficient repair.

Genes involved in lipid metabolism were also significantly induced. Modifications in the expression of genes associated with cellular metabolism were also observed after treatment with other genotoxic agents (Gasch et al., 2001). The link between modifications in gene expression and the global cellular response to 8-MOP/UVA treatment (Fig. 5) may reflect the requirement for the repair or de novo synthesis of damaged biomolecules, including lipids and proteins as well as DNA.

Comparison of the transcriptomes of cells treated with 8-MOP/UVA and other genome-wide studies as well as the transcriptomes of cells treated with other genotoxic, cytotoxic or stress-inducing agents highlights the specificity of the 8-MOP/UVA response

Comparison of our data with other transcriptome studies demonstrates that 10–20% of the genes affected by 8-MOP/UVA treatment also show modified expression over the cell cycle (Spellman et al., 1998), during the diauxic shift (DeRisi et al., 1997), in response to alpha factor (Hughes et al., 2000) or in the presence of glycosylation defects (Cullen et al., 2004). However, there was little overlap with PHO pathway-regulated genes (Ogawa et al., 2000). This expression profile suggests that in addition to the DNA damage response, a global cellular response is mounted after 8-MOP/UVA treatment.

The genes that were up- or down-regulated after 8-MOP/UVA exposure (see supplementary Table 1) were compared to those affected by other genotoxic agents (Jelinsky & Samson, 1999; Jelinsky et al., 2000; Gasch et al., 2001; Keller-Seitz et al., 2004; Mercier et al., 2004, 2005; Caba et al., 2005). Among the 8-MOP/UVA-induced genes, 58% are also induced by ionizing radiation, MMS or other agents, suggesting that the 42% remaining induced transcripts are specific for 8-MOP/UVA treatment (Fig. 6a and supplementary Table S4). However, it cannot be excluded that those transcripts could also be activated by other types of DNA lesions. Among the genes induced by genotoxic agents, we found nine (Fig. 6a) belonging to the DNA damage response (Ogawa et al., 2000). The combined treatment 8-MOP/UVA and other genome-directed agents, we found nine (Fig. 6a) belonging to the DNA damage response (Ogawa et al., 2000). The link between modifications in gene expression and the global cellular response to 8-MOP/UVA treatment (Fig. 5) may reflect the requirement for the repair or de novo synthesis of damaged biomolecules, including lipids and proteins as well as DNA.

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model of transcriptional networks activated by MMS, and some of the genes in this network were also induced (RNR2, RNR4, D1N7, D1N1, ARG3, AAD6, YLR297W and YGR146C) or repressed (ARG80, INO4 and EG172) by 8-MOP/UVA. Some 8-MOP/UVA-modified genes are also influenced by various environmental stresses (Gasch et al., 2000). The genes listed in Fig. 6b also respond to heat shock (HSP31 and TSL1), osmotic shock (YGP1), oxidative stress (UTH1), and nutrient limitation (YGP1) (Gasch et al., 2000; Causton et al., 2001; Gasch & Werner-Washburne, 2002).

According to the GO process ‘stress’, we found that in addition to the genes presented in Fig. 6b, the 8-MOP/UVA-modified genes D1N7, D1N1, NSE1, RAD51, WSC2, HTA2, HTA1 and RPD3 are also involved in stress responses. Thus, in addition to genotoxic and specific responses, a general stress response (13% of all modified genes) is mounted in S. cerevisiae cells after 8-MOP/UVA treatment.

Interestingly, a comparison of the total number of genes affected by 8-MOP/UVA alone to that affected by 8-MOP/UVA and other genotoxic agents shows that they differ with respect to intracellular localization, as defined by their GO profiles (data not shown). Gene products specifically modified by 8-MOP/UVA are in all compartments but gene products that localize to the cytoplasm, to membranes and to the nucleus are prominent. These biased localization patterns emphasize that the cellular targets affected by psoralen plus UVA treatment include not only DNA, but also proteins and lipids, with deleterious consequences for the cytoplasm and cellular membranes. Genes specifically modified by 8-MOP/UVA are likely critical for counteracting these effects as well as for ensuring the repair of DNA lesions induced by this agent.

**Involvement of chromatin-modifying activities in gene expression responses after 8-MOP/UVA treatment**

To determine whether 8-MOP/UVA response genes are affected by chromatin dynamics, we compared our data with those obtained by analysis of deletion mutants or by employing experimental conditions that interfere with chromatin structure and function (Wyrick et al., 1999; Sudarsanam et al., 2000; Meneghini et al., 2003; Kobor et al., 2004; Mizuguchi et al., 2004; Morrison et al., 2004; van Attikum et al., 2004). Twenty-seven percent of 8-MOP/UVA-modified genes were also modified in snf2 and/or swi1 deletion mutants (Sudarsanam et al., 2000), indicating that the S. cerevisiae Snf/Swi complex, which controls transcription and the chromatin structure of particular genes in vitro and remodels nucleosomes in vitro, is implicated in gene expression following 8-MOP/UVA exposure. A few genes affected by 8-MOP/UVA (about 15%) are also modified in swr1Δ cells, in htz1Δ cells and in sir2Δ cells (Meneghini et al., 2003; Kobor et al., 2004; Mizuguchi et al., 2004). This finding also suggests that under our experimental conditions, the replacement of histone H2A by H2.Z in nucleosomes as well as a Sir contribution to telomeric silencing may influence gene expression after 8-MOP/UVA treatment. In addition, there was a small overlap with the transcriptionomes of snf6 and tpr1 mutants, which are impaired in transcription (Hughes et al., 2000). Interestingly, only a few 8-MOP/UVA response genes (5%) are also affected in ino80 cells (van Attikum et al., 2004). The evolutionarily conserved INO80 chromatin remodelling complex directly participates in the repair of DSBs in yeast (Morrison et al., 2004). Based on transcriptional analysis, INO80 does not appear to regulate homologous recombination at the transcriptional level but it may influence nonhomologous end joining, which has a modest role in the repair of the photoadducts and DSBs that indirectly result from 8-MOP/UVA exposure (Cohen et al., 2002).

The most striking correlation observed was that between the transcriptional profiles caused by 8-MOP/UVA treatment and by histone H4 depletion (Wyrick et al., 1999). Approximately 62% (97/157) of 8-MOP/UVA response genes are also affected by H4 depletion (supplementary Fig. S1). Although the reduction of nucleosome content caused by H4 depletion affects many genes (c. 25%) (Wyrick et al., 1999), this overlap of 62% is highly significant ($\chi^2 = 24.9$, $P < 0.001$) and, interestingly, largely covers the entire spectrum of biological functions ranging from DNA damage response genes to membrane-specific genes. This correlation may reflect changes in chromatin structure that are likely required to process the ICLs induced by 8-MOP/UVA, which ultimately leads to DSB formation (Dardalhon et al., 1998).

**A specific 8-MOP/UVA transcriptional profile**

The damage response to the genotoxic agent 8-MOP/UVA includes expression of genes involved in DNA maintenance and repair. However, our results also reveal that membranes are important targets for 8-MOP/UVA as we find an enrichment concerning the membranes as compared to the genome as a whole. Modifications of membrane lipids and proteins and of mitochondrial functions are closely linked to the effective processing of 8-MOP/UVA-induced DNA photolesions by these repair systems. In addition, the unexpected connection between H4 depletion and the transcriptional effects caused by 8-MOP/UVA is very interesting and warrants further studies.

Interestingly, mutants deleted for some 8-MOP/UVA-inducible genes are sensitive to genotoxic agents. For example, according to YPD gene information, mutants of NSE1, ESC8, EG172, SPO12, YCR146C, YML002W, YMR144W, YLL029W and YPS1 are sensitive to MMS,
mutant of YNR068C is sensitive to gamma radiation, and mutants of YCR061W, STP4 and YHL044W are sensitive to cisplatin. As described by Birrell et al. (2002) transcriptional regulation does not necessarily predict the importance of a gene in resistance to a genotoxic agent. To further define the relationship between induced genes and genotoxic consequences after 8-MOP/UVA treatment, the survival responses of mutants deleted for genes that are specifically induced by 8-MOP/UVA will be of particular interest.

Our elaboration of the 8-MOP/UVA transcriptional response is the first step in identifying the mechanisms involved in the effectiveness and genotoxic consequences of photochemotherapeutic 8-MOP/UVA treatments. However, the use of a haploid strain may not fully represent the ploidy-specific response of human cells. Therefore, further work using a homologous diploid yeast strain will be of interest.

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Authors contribution

Both laboratories 1 and 2 contributed equally to this work.

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**Supplementary material**

The following supplementary material is available for this article:

**Table S1.** List of genes with altered expression after 8-MOP/UVA treatment.

**Table S2.** Genes with altered expression after 8-MOP/UVA treatment as a function of post-treatment incubation time.

**Table S3.** 8-MOP/UVA response genes encoding proteins with membrane related functions.

**Table S4.** Comparison with other genotoxic responses in yeast.

**Fig. S1.** 8-MOP/UVA response genes that overlap with genes modified by H4 depletion as described by Wyrick et al., 1999.

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