Published online November 30, 2005

Nucleic Acids Research, 2005, Vol. 33, No. 20 ***6635–6643***
doi:10.1093/nar/gki959

A haploid-specific transcriptional response to irradiation in *Saccharomyces cerevisiae*

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Received August 31, 2005; Revised and Accepted October 26, 2005

ABSTRACT

Eukaryotic cells respond to DNA damage by arresting the cell cycle and modulating gene expression to ensure efficient DNA repair. We used global transcriptome analysis to investigate the role of ploidy and mating-type in inducing the response to damage in various *Saccharomyces cerevisiae* strains. We observed a response to DNA damage specific to haploid strains that seemed to be controlled by chromatin regulatory proteins. Consistent with these microarray data, we found that mating-type factors controlled the chromatin-dependent silencing of a reporter gene. Both these analyses demonstrate the existence of an irradiation-specific response in strains (haploid or diploid) with only one mating-type factor. This response depends on the activities of Hdf1 and Sir2. Overall, our results suggest the existence of a new regulation pathway dependent on mating-type factors, chromatin structure remodeling, Sir2 and Hdf1 and independent of Mec1 kinase.

INTRODUCTION

The cellular response to exogenous DNA damage involves a complex combination of cell cycle arrest, the modulation of gene expression and DNA damage repair, resulting in the survival or death of the cell. Diploid *Saccharomyces cerevisiae* strains (Mat*a*/Matα) are more resistant than haploid strains (Mat*a* or Matα) to gamma rays (1). The genetic basis of this difference remains poorly understood. Diploids and haploids differ in the expression of mating-type genes and the number of chromosome copies. Various cellular processes, including mating, meiosis and budding, are directly controlled by a/α mating-type, at the transcriptional level. Recent studies have also demonstrated the importance of mating-type status in the regulation of microtubule properties (2), the maintenance of cell wall integrity (3) and DNA repair by non-homologous end-joining (NHEJ) (4). Galitski *et al*. (5) investigated the contributions of mating-type and ploidy to gene expression in three isogenic sets of yeast strains differing only in terms of ploidy, which were subjected to whole-genome expression analysis. The results obtained confirmed the existence of both ploidy-dependent and mating-type-specific gene expression patterns under normal growth conditions. We used microarray analysis and gene reporter fusions to assess the contributions of ploidy and mating-type to the transcriptional response induced by irradiation.

Gamma irradiation generates various types of DNA damage, including double-strand breaks (DSBs). A single unrepaired DSB is deleterious for cells, as it may lead to genetic instability and the loss of chromosome fragments. Such damage may occur anywhere in the genome and may have a major effect on the general organization of chromosomes in the nucleus. The DSBs caused by ionizing radiation trigger G_2/M arrest before entry into mitosis, preventing the loss of chromosome fragments during division (6,7), whereas base modifications inhibiting DNA replication activate the S phase-progression checkpoint (8). Transduction of the resulting signals is thought to require the kinase cascade, which involves the activities of Mec1p, Rad53p, Chk1p and Dun1p [reviewed in Elledge *et al*. (9)] However, the transcription factors involved in the γ-induced response at the other end of the regulation cascade have not been identified.

One of the most important defense mechanisms against the lethal effects of DSB is the repair of broken DNA by homologous recombination (HR). The abolition of radiation resistance has been observed for a number of DNA repair mutants of the *RAD52* recombinational repair epistasis group (*RAD51*, *RAD52* and *RAD54*) (10), and for *RAD50*, *XRS2* and *MRE11*, affecting the resection of DSBs (11,12). The difference in radiosensitivity between haploids and diploids seems to result mostly from the lack of a template for HR during the G_1 and early S phases of the haploid cell cycle.

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However, many studies have shown that genotype at the MAT locus also plays an important role in the response to irradiation, affecting DNA repair and the HR/NHEJ balance, (13–16). Diploid cells express the Mata1-Matα2 repressor, which turns off the transcription of a set of 'haploid-specific genes', including several components of the mating pheromone signaling pathway. NHEJ efficiency has been shown to be lower in diploid cells than in haploid cells (17,18). All the genes involved in controlling this balance have not been yet characterized. Recent studies have shown that *LIF1* and *LIF2* (*NEJ1*) are strongly regulated by mating-type, as the steady-state levels of these proteins are lower in diploid Mata/Matα strains than in haploid strains (4,19). However, mating-type heterozygosity is known to increase the frequency of HR (15,16) via Ku-dependent and -independent mechanisms (13). The available data therefore seem to indicate that both ploidy and mating-type locus affect the efficiency of DNA repair. The identification of all proteins induced by irradiation and subject to a/α regulation should further increase our understanding of the way in which the choice between repair pathways is controlled.

MATERIALS AND METHODS

Strains and culture conditions

The *S.cerevisiae* diploid FF 6053 (Mat*a*/α), and the haploids FF 18734 (Matα), FF 18733 (Mat*a*) and FF 18735 (Mat*a*/α) were used for transcriptional analysis. The FF 6053 diploid was obtained by mating two haploids (FF 18734 and FF 18733). FF 18735 was constructed by integration a plasmid (Yip5) encoding Matα into the FF 18733 haploid strain. The *hdf1* and *sir2* mutant strains are W303-1a (Matα) haploid derivatives. We analyzed URA3 gene silencing in haploid and diploid strains, using W303-1a derivatives containing a modified telomere VII-L, in which the ADH4 subtelomeric gene was replaced by the URA3 reporter gene and various portions of the X and Y′ element were inserted between the URA3 reporter gene and terminal telomeric DNA repeats (20). Yeast cells were grown exponentially in YPD medium at 30°C and oxygenated by shaking at 150 r.p.m. with a HT Infors AG shaker (Bottmingen, Switzerland).

Ionizing irradiation conditions and time-courses

Overnight exponential cultures were centrifuged, and the cell pellet was resuspended at a density of 10^9 cells/ml and irradiated (60 Gy/min and ^{137}Cs source) at room temperature in rich medium to minimize temperature and osmotic variations during treatment. Irradiated cells were plated directly on rich medium for survival analysis or immediately resuspended in rich medium at the original density for time-course experiments. Cells were irradiated at time 0, and samples were collected for microarray and cell cycle analysis at various times (0.1, 1, 2, 3, 4 and 5 h) after irradiation. Kinetic analysis was performed on strains exposed to a 200 Gy of ionizing radiation, which resulted in a cell survival rate of 25% for the two haploid strains (FF 18734 and FF 18733) and 75% for the diploid strain (FF 6053). We used DAPI staining, microscopy and FACS analysis, as described previously (21) to determine the duration of cell cycle arrest following irradiation. The transcriptional response was analyzed during this period.

Probe and microarray hybridization and data analysis

Total RNA was extracted from frozen samples by the hot phenol method. A fluorescently labeled first-strand cDNA was synthesized by RT, as described in Supplementary Data. For all microarray hybridizations, the fluorescent Cy-3-labeled cDNA control population was prepared from the same pool of total RNA extracted from five independent, exponentially growing cultures of the diploid strain (FF 6053). Hybridized microarrays were scanned with a Genepix 4000B machine (Axon Instruments). Fluorescence intensities for all spots were normalized using the location and scale normalization procedures described by Mercier *et al*. (22), details are provided in Supplementary Data.

As a unique pooled RNA sample (prepared from non-irradiated cultures of the diploid strain) was used as the reference in all experiments, we calculated a ratio by dividing the measured ratio for each irradiated haploid strain with the corresponding value for the same strain in the absence of irradiation. For this purpose, we prepared three independent normal growth cultures of each haploid strain for control experiments (0 Gy), and used the median ratio for these strains for the normalization of irradiation time-course data. Genes with expression levels differing between irradiated and non-irradiated samples by a factor of at least two for at least one time point filtering of the time-course experiment were identified as irradiation-regulated (IR) genes. Pairwise mean linkage clustering analysis was performed with Cluster (using uncentered Pearson correlation coefficients) and visualized using Treeview (23).

Measurement of telomere position effect (TPE) by analysis of reporter gene expression

TPE was assessed by analyzing variegated expression of the *URA3* gene. Cells with a repressed *URA3* gene were selected as colonies growing in the presence of 5-FOA (SC + 5-FOA), which is toxic to cells expressing a functional *URA3* gene product (24). We then distinguished ura- mutants and silenced cells by replica plating on medium lacking uracil (SC-URA). Cells growing on both SC + 5-FOA and SC-URA media were considered to have a repressed *URA3* gene. We compared the TPE in haploid and diploid strains in the absence of irradiation, using various *URA3* reporter gene constructs (detailed in Figure 4). Drop assays were performed with the URA construct, by spotting serial dilutions of three independent overnight cultures in SC liquid medium on to SC, SC-URA and SC + 5-FOA plates. The effect of irradiation dose was assessed by dilution assay for three exponential independent cultures of each strain, irradiated at different doses, serially diluted and plated on specific media to determine the percentage of cells with a repressed *URA3* gene. In parallel, we evaluated the survival rate of irradiated cells by calculating the ratio of viable cells in irradiated cultures to viable cells in non-irradiated cultures.

Online supplementary data

Details of probe, microarray hybridization protocols and data analysis are given provided in the Supplementary Materials and Methods. Haploid-specific (HS-IR) genes and their function are listed in Supplementary Table S1. A statistical

analysis of the effect of Gasch mutants on HS-IR gene expression is given in Supplementary Table S2. Supplementary Figure S1 shows Treeview expression analysis of the HS-IR genes in 300 mutants and Supplementary Figure S2 shows the chromosomal location of HS-IR genes. The raw data are available at the following URL http://microarrays.curie.fr/.

RESULTS

Differences in global responses to irradiation between haploids and diploids

We compared global gene expression responses to ionizing radiation between *S.cerevisiae* haploids and diploids by irradiating three isogenic strains: the Mat*a*/α diploid (FF 6053 strain), the Mat*a* haploid (FF 18733 strain) and the Matα haploid (FF 18734 strain). The patterns of gene expression induced by irradiation were deduced by comparing the patterns of expression of a given strain before and after irradiation. The three isogenic strains were exposed to a 200 Gray (Gy) dose of ionizing radiation. Cell survival rates were lower in both haploids (25%) than in the diploid (75%). The irradiated cells stopped dividing for about 4 h and then resumed mitosis in all three strains (data not shown). We studied the transcriptional response of irradiated cells during the full recovery period, by analyzing mRNA from samples taken immediately after irradiation and every hour for the next 5 h.

In all three strains, ionizing radiation led to significant changes in the gene expression program, with the relative abundance of about 1400 genes differing by a factor of two or more between irradiated and non-irradiated cultures. Most of these IR genes displayed rapid and strong changes in expression—a typical stress response feature that has already been reported after γ-irradiation and treatment with various other types of DNA-damaging agent (25–27). We compared the lists of IR genes for the three strains (Figure 1). Surprisingly, the two haploids had twice as many IR genes in common with each other (595) than in common with the diploid strain (291 and 265). Only 124 genes were found to be induced (or repressed) in all three strains following irradiation. Many of the genes known to be involved in inducible DNA damage repair, such as *RAD51*, *RAD54*, *RNR2*, *RNR4*, *HUG1* and *RFA2* (21,28–30), were induced in all three strains. We compared our data with published results obtained after MMS and ionizing radiation treatments in a Mat*a* strain (25). The eight genes displaying specific induction in response to DNA-damaging treatments (*RNR2*, *RNR4*, *RAD51*, *RAD54*, *PML2*, *YER004W* and *YBR070C*) were also found to be induced in our experimental conditions in the Mat*a* haploid strain. *DIN7* was the only gene of the 'DNA Damage Signature' set [see Gasch *et al*. (25)] unaffected by irradiation in our experiments.

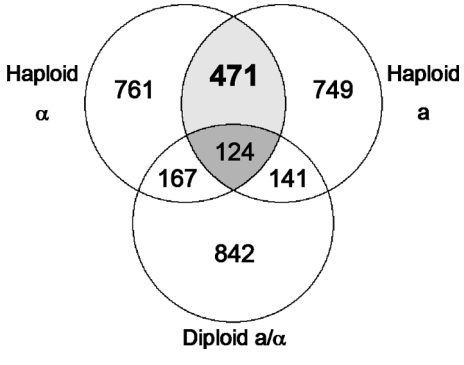

Figure 1. Venn Diagrams comparing the HS-IR genes modulated in the various strains. Circles indicate the number of genes showing changes in expression by a factor of at least two after irradiation in the haploids FF 18733 (Matα), FF 18734 (Matα) and the diploid FF 18735 (Mata/α). The intersections of the circles correspond to genes induced in at least two strains (i.e. the strains corresponding to the intersecting circles). Numbers indicate the number of genes in each group.

We focused on the 471 IR genes displaying changes in transcription after irradiation in both haploids, but not in the diploid strain. We found that 278 of these HS-IR genes were induced and 193 were repressed (Figure 1; Supplementary Tables S1).

Promoter analysis of HS-IR genes

We tried to identify transcription factors potentially involved in the regulation of HS-IR genes by analyzing the 800 bp sequence directly upstream from the coding region for consensus binding sites for known or unknown transcription factors. The Pbox, Qbox and PRE elements and sequences recognized by mating-type factor heterodimers were not significantly more frequent in the set of HS-IR genes than in the genome as a whole. This result suggests that the regulation of this response by mating-type factors is indirect. Only two sequences were found to occur at high frequency in the promoters of the 278 induced HS-IR genes: 114 genes (41%, versus 22% for the genome as a whole) contained the CTCATC sequence recognized by Rfa2. The binding of Rfa2p to upstream sequences has been shown to repress the expression of some repair genes and is decreased by UV irradiation or MMS treatment, thereby leading to the induction of these repair genes (31). We found that 81 of the 278 induced HS-IR genes possessed upstream regions containing the ATGAGC sequence, which has no known binding factor. The promoter regions of the 193 repressed HS-IR genes presented no overrepresentation of any specific sequence other than the frequent occurrence of G-rich sites.

Subtelomeric and even distributions of HS-IR genes on chromosomes

Visual inspection showed that an unexpectedly high proportion of induced HS-IR genes were located near chromosome ends ($\chi^2 = 114.6$, $P < 0.0001$; Supplementary Figure S1). Indeed, 18% of the 278 induced HS-IR genes were located within 20 kb of a telomere (subtelomeric). About one quarter (51/218) of the subtelomeric genes on our microarrays were induced by irradiation (Supplementary Table S1). The 51 subtelomeric HS-IR genes induced were evenly spread over 23 of the 32 chromosomes extremities, indicating that subtelomeric gene derepression is a general process affecting most chromosome ends (Supplementary Figure S1).

Genes controlled by the same sequence-specific transcription factor tend to be spaced at regular intervals along chromosome arms (32). We analyzed the distribution of HS-IR genes by calculating the distance between pairs of HS-IR genes from the same chromosome arm. In this analysis of coexpressed genes, eight yeast chromosome arms had too low

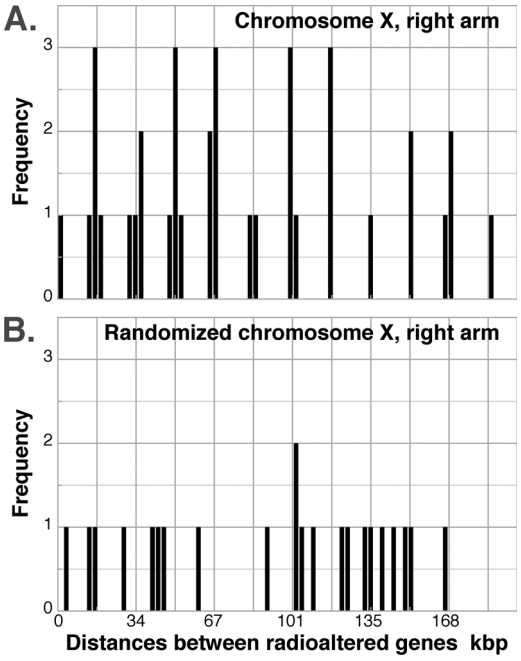

Figure 2. Distribution of distances separating radioaltered HS-IR genes along the right arm of yeast chromosome X. (**A**) Distances were measured between the starting points of the coding sequences of all gene pairs for the complete list of HS-IR genes. These distances are shown here on a bar graph, with a grid interval of 16 850 bp. Bar width (or 'bin size' for data discretization) is 2500 bp i.e. about the length of one yeast gene allowing fine distribution analysis. Varying bin size had no effect on the conclusions drawn. (**B**) As for (A), following the random attribution of gene positions. Gene content, chromosome length and target list are as in natural chromosome X. For each gene on this chromosome arm of length *L*, the randomization process replaces the start position with a random integer between 1 and *L*. Calculations used Microsoft® Excel VBA routines. The routines and data are available upon request.

a density of HS-IR genes for any firm conclusion to be drawn. Six of the remaining 24 arms displayed weak periodicity, and 18 displayed clear periodicity. For example, the HS-IR genes on the right arm of chromosome X tended to be regularly spaced, and were separated by 16 850 bp or by multiples of 16 850 bp (Figure 2A; grid step 16 850 bp). This even spacing is not consistent with the random attribution of gene positions (Figure 2B). Different periods were observed for different chromosome arms, as reported previously for coregulated genes (32). Thus, most HS-IR genes appear to be controlled by a few molecular factors involved in regular nuclear organization.

Chromatin modifying activities regulate HS-IR genes

We investigated whether all the HS-IR genes studied were regulated by the same pathway, by comparing the effect on their expression of deletions of genes encoding various regulatory proteins. We investigated microarray data for about 300 mutants (33). As expected for genes regulated by the same pathway, 134 of the 193 repressed HS-IR genes displayed similar sensitivities to a large set of mutations (Supplementary Figure S2-A), with basal expression levels decreasing for 32 mutants and increasing for 113 mutants. The number of HS-IR genes showing variation of expression was estimated and compared with the total number of genes showing variation of expression in each mutant. Only mutants giving a $P > 0.005$ in a hypergeometric test were considered (listed in Supplementary Table S2). Analysis of the molecular functions affected in mutants displaying specific HS-IR gene expression changes showed that most directly or indirectly involved chromatin remodeling and/or silencing. The largest changes in expression of repressed HS-IR genes were observed in mutants with impairments affecting chromatin (e.g. *sir4*, *rpd3*, *sin3*, *hat2*, *cyc8*, *hst3*, *ubp10* and *tup1*). The clustering of the induced HS-IR genes was consistent with a complex pattern of regulation, with very few common regulators (Supplementary Figure S2-B). However, most of the induced HS-IR genes also displayed significant changes in expression in mutants with impaired chromatin assembly and chromatin modifications (*sir2*, *sir3*, *hdf1*, *iswi1* and *isw2)* and DNA topology *(top1* and *top3*). Inactivation of the *TUP1* and *SSN6* genes encoding proteins acting as a transcription factor complex sensitive to chromatin structure (34) significantly increased the expression of repressed HS-IR genes, suggesting that irradiation may facilitate the recruitment of these repressors to the regulatory regions of HS-IR genes.

HS-IR gene expression and silencing are controlled by mating-type

HS-IR genes were identified as genes displaying changes in expression after irradiation in haploids but not in diploids. These genes seemed to be sensitive to chromatin regulation and some were subject to telomeric chromatin silencing. We investigated the contribution of mating-type status to control of the general transcriptional response to irradiation by analyzing the expression of HS-IR genes in a pseudo-diploid strain: a Mat*a* haploid strain, expressing the α factor. Control experiments involving microarray analysis confirmed that a combination of the a1 and α2 factors resulted in the repression of haploid-specific genes. As expected, the *STE2*, *STE6*, *MFA1*, *MFA2*, *AGA2*, *ASG7*, *Mfalpha1*, *Mfalpha2*, *STE3*, *FUS3* and *RME1* genes displayed similar levels of expression in the Mat*a*/α pseudo-diploid and in the diploid. Most of the IR genes shown to be induced after irradiation in haploids but not in diploids showed no induction in the pseudo-diploid strain (Figure 3). In contrast, 65% of the 124 genes induced (or repressed) in both haploids and diploids (see Figure 1), were also induced in the haploid expressing both mating-types (data not shown).

As the expression of HS-IR genes, including subtelomeric genes (Figure 3), seems to be controlled by mating-type factors, we investigated the effect of ploidy on silencing by means of reporter gene studies. Telomeric silencing at native ends has been reported to vary with gene location, depending on the combination of X and Y′ elements in yeast (35). We confirmed that this was the case in a TPE assay in strains carrying different subtelomeric sequences between the *URA3* gene and the TG_{1-3} repeat. However, for all constructs, the *URA3* gene was less strongly silenced in the diploid strain than in the haploid strain (Figure 4). Diploid-associated derepression was more pronounced in reporter constructs bearing the part of the X or Y′ element immediately adjacent to the telomere, suggesting that the natural subtelomeric sequences are involved in modulating TPE as a function of ploidy. Thus, TPE and microarray

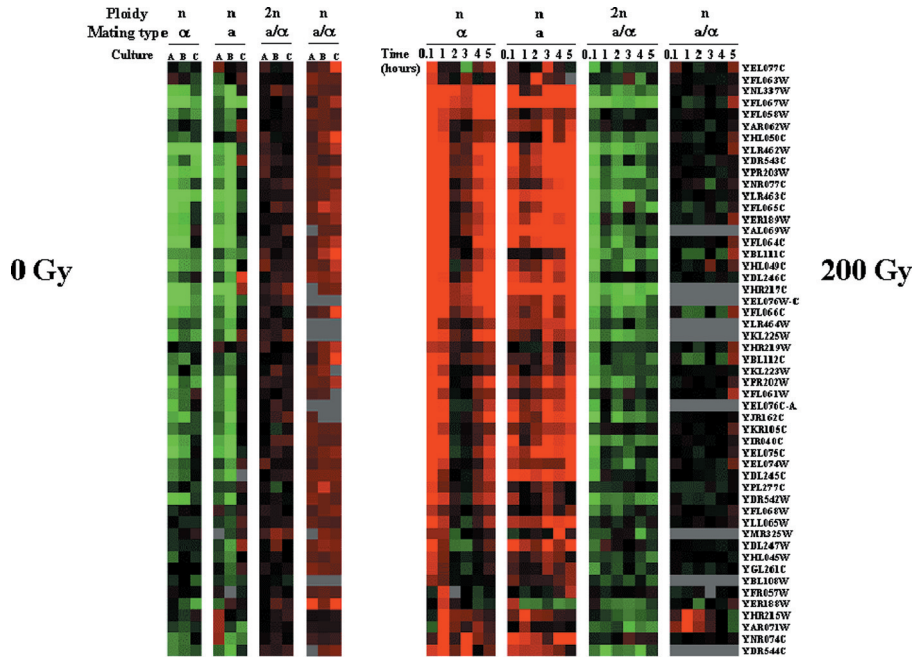

Figure 3. Analysis of the expression of subtelomeric HS-IR genes. Expression of the HS-IR genes in the four strains: the haploids (Mat α), (Mat a), [Mat a(α)] and the diploid (Mat a/α)—is shown with TreeView (23). Only data for telomeric genes are reported. The full analysis of the 471 HS-IR mutants is presented in Supplementary Figure S1. Panel A, non-irradiated cells: For each strain, RNA from three independent cultures was analyzed as described in materials and methods. Panel B, irradiated cells: RNA levels for the various genes were determined immediately after irradiation with 200 Gy (time 0.1) and after 1 h, 2 h, 3 h, 4 h and 5 h of incubation. Ratios were calculated with respect to the median of the three measures in non-irradiated cells.

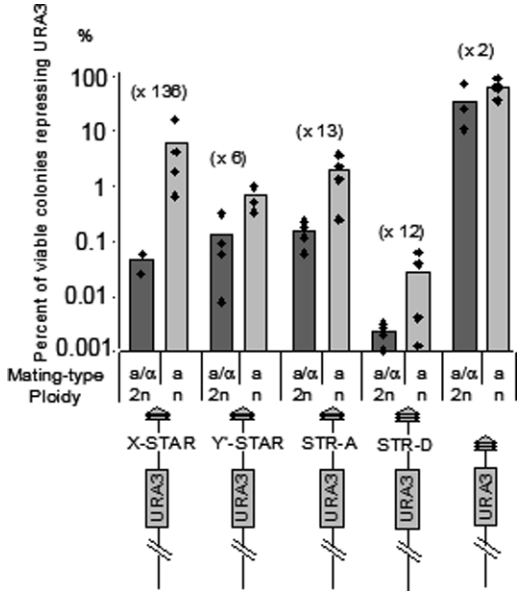

Figure 4. Measurement of TPE, using an artificial telomere-proximal gene, URA3, at tel VII-L. Various URA3 constructs were tested for TPE in haploid (gray rectangles) and heterozygous diploid (black rectangles) strains. Histogram bars represent the mean values obtained for a given strain. Each diamond indicates the proportion of colonies displaying URA3 gene repression (ratio of colonies growing in 5-FOA-SC versus SC).

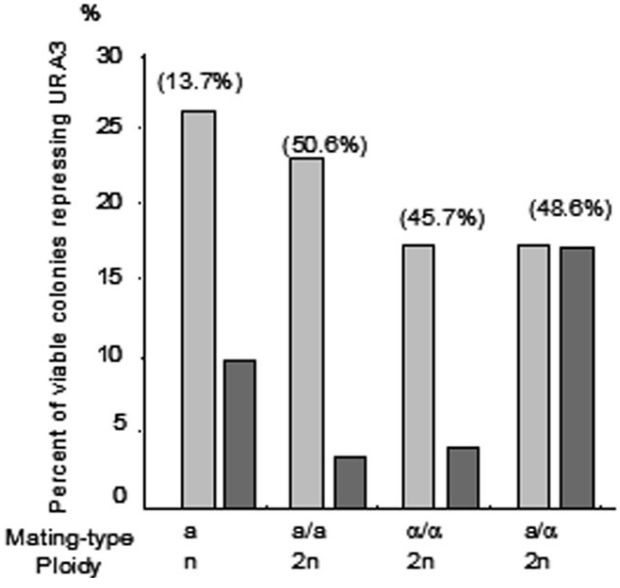

Figure 5. Effect of gamma rays on TPE in haploids and diploids with different mating-types. Four isogenic strains varying in ploidy and mating genotype, as indicated on the abscissa, were analyzed for TPE. The percentage of viable cells displaying URA3 repression in non-irradiated (dark gray) cultures and in cultures exposed to 200 Gy irradiation (light gray) was estimated by dilution assay on three independent cultures (diamonds). The cell survival rate is indicated in brackets.

analyses highlighted constitutive differences in silencing between diploid and haploid strains. As shown by microarray data (Figure 3), the silencing of subtelomeric genes measured by TPE (Figure 5) was not significantly affected by irradiation in diploids. The difference in silencing between diploids and haploids may be due to differences in the number of chromosomes or to differential regulation by mating-type factors, as shown for the HS-IR genes. We constructed diploid strains expressing only one mating-type to determine which of these two possibilities applied. The constitutive TPE in diploids

expressing only one mating-type was weaker than that in diploids, indicating that TPE may be sensitive to chromosome number. This would not be entirely surprising as the silencing protein Sir3 has already been shown to be present in limiting amounts in haploid cells (35,36). Interestingly, unlike (*a*/α) diploids, diploid strains expressing only the *a* or the α factor displayed complete derepression of the subtelomeric reporter gene after irradiation (Figure 5). This result is consistent with microarray data indicating that the response to irradiation, including the silencing switch-off, is inhibited by the expression of both mating-type factors in diploids.

The HS-IR response requires Ku70 and Sir2 but not Mec1

We further characterized the chromatin-dependent response to DNA damage by analyzing the response of *hdf1* and *sir2* mutant cells to 200 Gy of ionizing radiation over a 5 h period. These mutants displayed a much weaker overall transcriptional response than wild-type cells. Only 646 and 517 genes in the *hdf1* and *sir2* mutants, respectively, displayed radiomodulation, with only 185 genes being radiomodulated in both mutants. Only 6% of the genes insensitive to *hdf1* and *sir2* were HS-IR genes. Thus, the *hdf1* and *sir* mutations seem to block preferentially change in expression of the 471 HS-IR genes. We checked that the dependence of the gene responses to hdf1 and sir2 activity was specific to HS-IR genes, by carrying out the same analysis with the 124 genes induced in both diploids and haploids. This group was significantly less sensitive to the deletion of *hdf1* and *sir2* deletion, with only 19% (23/124) of the genes tested radiomodulated in the mutants ($\chi^2 = 14{,}2$, $P < 0.001$). These results suggest that the Sir2 and Ku70 proteins play specific roles in regulating the haploid-specific transcriptional response to irradiation.

We performed the same analysis on data published by Gasch *et al*. These authors studied the kinetics of gene expression after irradiation, for a wild-type strain and a *mec1* mutant. They found that 1369 genes were regulated by irradiation in the wild-type strain, versus only 962 in the *mec1* mutant. We found that 544 genes were radiomodulated in both strains, indicating that the expression of these genes was not entirely controlled by Mec1 kinase. We analyzed the effect of *mec1* mutation on the expression of HS-IR genes. Most (104) of the 169 HS-IR genes radiomodulated in the wild-type were also radiomodulated in the *mec1* mutant. This high proportion of Mec1-independent responses differed significantly from that reported by Gasch (544 of a total of 1369 radiomodulated genes were radiomodulated in both strains; $\chi^2 = 10.69$, $P < 0.005$). In contrast, only 22 of the 124 genes radiomodulated in our three wild-type strains were radiomodulated in the *mec1* mutant. This proportion does not differ significantly from that for the data published by Gasch ($\chi^2 = 0.9$, $P < 0.25$). Thus, *mec1* deletion has effects opposite to those of *hdf2* and *sir2* deletions, affecting HS-IR gene expression only weakly and having a strong effect on the haploid-independent response to irradiation. Global analysis of the response to irradiation in the *hdf2*, *sir2* and *mec1* mutants suggested that the response of HS-IR genes to irradiation depends on mating-type factors, Ku and Sir but not Mec1. This regulation pathway is different from the known Mec1-controlled pathway of radiomodulation in haploids and diploids and seems to be insensitive to mating-type factors, Ku and Sir proteins. Most of the genes responding to DNA damage (e.g. *RNR2*, *RNR4*, *RAD51*, *RAD54* and *RFA2*) studied by other laboratories are regulated by the Mec1 pathway.

DISCUSSION

We identified, by means of transcriptome analysis and reporter gene studies, a cellular response to irradiation dependent on chromatin structure and mating-type factors. Gene silencing has been shown to result from the inhibition of RNA pol II transcription activity by a specific compact chromatin structure. It requires the binding to histone tails of three unrelated proteins: Sir2, Sir3 and Sir4 initially recruited by Rap1. One possible mechanism accounting for the decrease in silencing after irradiation involves the repression by irradiation of genes encoding these proteins, resulting in a loosening of the compact structure of chromatin. The *RAP1* and *SIR3* telomeric structural genes displayed no change in expression following irradiation. The *SIR4* gene displayed a continuous increase in expression over the period analyzed. However *SIR4* overexpression was expected to increase silencing rather decrease it as observed, based on data for the overexpression of *SIR3* (35,36).

Many authors have highlighted the similarity between DSB and telomeric termini. Both bind proteins involved in the NHEJ repair pathway, such as the Ku heterodimer and the Mre11/Rad50/Xrs2 nuclease/helicase complex, which is thought to be involved in break religation. The Sir proteins, which are associated with transcriptional silencing at subtelomeric and mating-type loci, seem to be directly or indirectly involved in DNA damage repair. Sir4 interacts physically with Ku70, and mutations of the Sir complex result in deficiencies in the repair of linear plasmids (37–40). At both telomeres and DSBs, NHEJ and HR proteins compete in the maintenance of chromosome integrity. Ku proteins prevent HR at telomeres (41), whereas this process is the primary means of repair at DSBs (see for review van den Bosch, (42,43). The proteins binding around each site may be responsible for selecting the mechanism activated. DNA repair by HR is enhanced by mating-type heterozygosity. This increase in the rate of HR repair was not observed in an *a*/α diploid with only one mating-type, suggesting that the presence of a homologous chromosome is not sufficient to increase the rate of HR (13–15,18). Moreover, the DNA repair defect caused by the *rad51-K191R* mutant protein, which is responsible for a partial defect in ATP hydrolysis, is abolished in diploids and by mating-type heterozygosity in haploids (44). The effect of mating-type heterozygosity, which enhances repair, is not restricted to the HR pathway, because end-joining activity is also repressed in diploids (13,16,17). The abolition of radiation sensitivity in diploids has been observed for a number of DNA repair mutants (10), and in the case of the *rad18* and *rad55* diploids, is due to mating-type heterozygosity rather than ploidy (15,45). Vaillant *et al*. showed that NHEJ regulation involves the control of Lif2 protein production, which is repressed in diploids.

An analysis of published microarray data identified no known repair genes more strongly expressed in diploid strains

expressing the two mating-type alleles than in diploids expressing only one mating-type allele (46). Our data on the transcriptional response induced by irradiation showed that very few of the many genes displaying differential expression in haploids were directly involved in DNA damage repair (see Supplementary Tables S1 and S2). We performed an extensive ontology analysis on the microarray data, estimating statistical significance by means of a hypergeometric method. The processes retained were those corresponding to a number of genes significantly higher than would be expected on the basis of chance alone, considered the whole set of genes analyzed. This analysis revealed that induced HS-IR genes are involved primarily in ribosome biogenesis (23 genes, $\chi^2 = 15$), rRNA metabolism and RNA processing (28 genes, $\chi^2 = 6.5$), whereas repressed HS-IR genes are primarily involved in energy reserve metabolism (7 genes, $\chi^2 = 20.68$), mitochondrial electron transport (5 genes, $\chi^2 = 40.75$) and response to copper or desiccation (5 genes, $\chi^2 = 10{,}33$). Birell *et al*. (47) in their analysis of the transcriptional response to various DNA-damaging agents (including ionizing radiation), found no relationship between the genes required for survival following exposure to DNA-damaging agents and the genes displaying an increase in transcription after exposure. We identified 10 genes (*QCR9*, *QCR10*, *COX5B*, *QCR8*, *CYT1*, *GRX1*, *CTT1*, *SOD1*, *SOD2*, *CUP1-1* and *CUP1-2*) involved in oxidative phosphorylation, oxidative stress and Cu^{++} homeostasis that were induced after irradiation. The genes involved in these processes were recently shown to be induced by continuous exposure to low doses of ionizing radiation (22). Their contribution to the survival of irradiated cells remains unclear but it is possible that they act by regulating the free radical pool in the cell.

Our results demonstrate that chromatin structure is controlled by mating-type heterozygosity. Silencing at telomeric ends was weaker in diploids than in haploids, as shown by assessments of the expression of all genes located at subtelomeric positions and reporter gene fusions. In our reporter gene studies, we observed this ploidy dependence in different genetic backgrounds and various telomeric sequence combinations. The low level of silencing in diploids could be a consequence of saturating some silencing proteins by doubling the number of binding sites on chromosomes in diploids. However, we do not favor this hypothesis that does not explain the low level of silencing of most subtelomeric genes in the haploid strain expressing the two mating-types (Figure 3). In our search for *trans*-acting elements accounting for this difference, we found that several genes encoding proteins playing a direct or indirect role in chromatin structure displayed differences in expression in haploid and diploid cells. The *RSC6*, *ELP3* and *SMC3* genes, encoding proteins involved in chromatin remodeling, were constitutively and more strongly expressed in diploids. The *TEL2* and *EST2* genes, encoding proteins involved in telomere length regulation, were also more strongly expressed in basal conditions in diploids than in haploids. *TBF1* was also expressed more strongly in the diploid. Tbf1 displays insulating capacity, and binds to promoters and in subtelomeric anti-silencing regions (STARs) throughout the yeast genome (48,49). The interaction between Tbf1p and telomeres leads to a loss of silencing at these chromosomal loci (49). This observation is correlated with weaker telomeric silencing in the diploid strain than in haploid cells. The low level of gene silencing observed in diploids was also found in diploids expressing only one mating-type factor. However, unlike diploids heterozygous for mating-type, these strains displayed significant derepression after irradiation, like the haploid parent. Conversely, microarray analysis indicated that haploids expressing both mating-types showed no significant change in subtelomeric gene expression after irradiation (Figure 4). Thus, mating-type heterozygosity prevents chromosome remodeling after irradiation. Most studies on the expression of telomere-proximal genes have been carried out in a haploid background (50–52). Such a difference in the silencing status of subtelomeric regions between the haploid and diploid states has never before been described. Affecting silencing by inactivating Sir2 protein or by inducing chromatin remodeling by irradiation would have the direct consequence to suppress the HML and HMR loci repression leading to expression of both mating-type cassettes as in pseudo-diploids. Thus, based on our data and published results, it is difficult to determine the respective role of mating-type and silencing in the HS-IR response regulation. We propose that irradiation disturbs the silencing chromatin all over the chromosomes leading to transient expression of most of the genes under its control, including cassettes at HML and HMR loci. The expression of both mating-type in the irradiated haploid would decrease general silencing as observed for subtelomeric genes silencing in non-irradiated diploids and a(α) pseudo-diploid, and thereby delay silencing restoration and extend the HS-IR response. Actually whereas genes induced in haploids and diploids show a very rapid and transient induction of expression, most of the HS-IR genes remain overexpressed during all the cell division arrest.

The loss of TPE for artificial telomere-proximal genes has been shown to be concomitant with checkpoint-dependent delocalization of the heterochromatin structural proteins Sir1-4, Rap1 and Ku following DNA damage induced by various agents (EcoRI or HO endonuclease, MMS or bleomycin treatment) (53,39). Martin also showed, by chromatin immunoprecipitation (ChIP), that Sir3, Sir4 and Ku80 were redistributed from telomeric DNA to damaged sites. Thus, the local loss of telomeric proteins due to DNA damage may lead to the derepression of subtelomeric genes. The loss of heterochromatic structure at telomeres therefore appears to be a response to DNA damage. This response seems to be partially controlled by the kinases (Mec1 and Rad53) playing key roles in the checkpoint response to DNA-damaging treatments. For example, the redistribution of Sir3 after DNA-damaging treatment depends on Mec1, but not the Rad53 or Tel1 checkpoint proteins (39,53,54). However, Rad53 contributes to genome stability independently of Mec1, by preventing the damaging effects of excess histones, both during normal cell cycle progression and in response to DNA damage (55). Analysis of our data and those of Gasch *et al*. indicated that Sir2 and Hdf1 controlled the response to irradiation of HS-IR genes, whereas Mec1 did not. The mechanism underlying the dependence of this response on mating-type factors remains to be demonstrated.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS

We would like to thank the 'Consensus' research group and F. Fabre for stimulating discussions, and the Genopole® and the DRIDF. We also thank S. Makhzami and M. Pierre for technical assistance. This work was supported by *l'Institut National de Recherche et de Sécurité* (convention no. 5011888), the CNRS, the Institut Curie, the *Association pour la Recherche sur le Cancer* (#5659) and the *Ligue Nationale Contre le Cancer*. Funding to pay the Open Access publication charges for this article was provided by Institut Curie.

Conflict of interest statement. None declared.

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