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Transcriptional Effects of the Potent Enediyne Anti-Cancer Agent Calicheamicin γ_1^I

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Summary

We have investigated the mode of action of calicheamicin in living cells by using oligonucleotide microarrays to monitor its effects on gene expression across the entire yeast genome. Transcriptional effects were observed as early as 2 min into drug exposure. Among these effects were the upregulation of two nuclear proteins encoding a Y′-helicase (a subtelomerically encoded protein whose function is to maintain telomeres) and a suppressor of *rpc10* and *rpb40* mutations (both *rpc10* and *rpb40* encode RNA polymerase subunits). With longer calicheamicin exposure, genes involved in chromatin arrangement, DNA repair and/or oxidative damage, DNA synthesis and cell cycle checkpoint control as well as other nuclear proteins were all differentially expressed. Additionally, ribosomal proteins and a variety of metabolic, biosynthetic, and stress response genes were also altered in their expression.

Introduction

Calicheamicin γ_1^I (CLM) is one of the enediyne family of secondary metabolites, a class of naturally occurring anti-tumor antibiotics that include neocarzinostatin, esperamicin, dynemicin, and kedarcidin. Results of in vitro experiments suggest that members of this family elicit their lethal effects by promoting DNA strand scission through radical mechanisms. However, they differ both in their sequence selectivity and in their ability to invoke either single- or double-strand breaks. Calicheamicin is one of the most sequence selective of this class and has a strong preference reported (in naked DNA) for TCCT and poly-A/T sequences [1–4]. Activation of the drug and DNA cleavage is proposed to result from nucleophilic attack on the allylic methyltrisulfide of CLM by a reductant (presumed to be the thiolate anion of glutathione in vivo), promoting intramolecular addition of thiolate to the dienone with subsequent electrocyclization to give the highly reactive 1,4-didehydrobenzene. Single- and double-stranded cuts are thought to arise when this diradical, positioned within the minor groove of DNA, abstracts hydrogen atoms from the deoxyribose backbone [5]. The resulting DNA radicals scavenge oxygen and initiate a sequence of events that ultimately leads to strand scission.

The interaction of calicheamicin with DNA has been extensively studied in vitro (primarily with DNA fragments containing various prokaryotic and eukaryotic DNA sequences) to reveal the fundamental aspects of DNA recognition, binding, and cleavage [6–9]. Additionally, experiments mimicking in vivo conditions with either isolated nuclei or nucleosome core particles have clearly demonstrated the importance of local DNA conformation and flexibility as well as steric constraints posed by sequence in the recognition and cleavage of DNA by calicheamicin [10–12]. The effects of the drug in vivo have not been well investigated, although the suggestion has been made from experiments with MOLT-4 cells (a human leukemic cell line with a 1000-fold greater susceptibility than that of other cell lines) that cytotoxicity of enediynes might involve additional protein-mediated mechanisms [13]. Here we have investigated the mode of action of calicheamicin in vivo by using oligonucleotide microarrays to profile the effects on gene transcription in wild-type yeast.

Results and Discussion

Saccharomyces cerevisiae was grown at 30°C in liquid culture to an O.D. of 1.0 and subsequently exposed to CLM (10 and 100 ng/ml, in duplicate) for 2 min, 15 min, and 30 min, respectively. The medium was then removed, the cells were washed thoroughly (3×) with distilled water, and total RNA was isolated by hot phenol extraction. Although the external supply of calicheamicin is greatly diminished upon removal of the medium, intracellular calicheamicin can still induce cleavage and invoke transcriptional changes until the RNA is finally extracted with hot phenol. Biotinylated cRNA was then prepared, fragmented, and subsequently hybridized (two independent hybridizations) to yeast (Ye6100) oligonucleotide arrays. On a global level, across the approximately 6200 genes that constitute the entire yeast genome, we detected consistent expression-level changes of ≥2-fold in approximately 60 genes between cells treated with calicheamicin at the high dose and longest time point (100 ng/ml, 30 min.; Table 1) versus untreated cells. A substantial fraction of these genes (approximately 35%) were nuclear-related responses including genes involved in chromatin arrangement, DNA repair and/or oxidative damage, DNA synthesis, and cell cycle control. The remainder included ribosomal proteins and genes involved in stress response, biosynthesis, and metabolism.

Histones were among the genes whose expression changed the most upon calicheamicin treatment. Concurrent downregulation of histones H2A (approximately 5-fold; average), H2B (approximately 4-fold; average), H3 (3–4 fold) and H4 (approximately 3-fold; average) was observed. Histones regulate germination as well as the response to heat shock and nutrient starvation [14]. Both H2A/H2B and H3/H4 mutants have been shown to form reduced-size colonies on plates and to exhibit a 30% increase in doubling time in liquid medium [15]. They

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Table 1. Effect of Calicheamicin on Yeast at 2 min, 15 min, and 30 min of Incubation with 10 ng/ml and 100 ng/ml of Drug

Probe Set	Gene/Description	Fold Change[a,b] (at 2 min)	(at 15 min)	(at 30 min)
Ribosomal Proteins				
YMR230W/_ex1_at	Ribosomal protein S10 (rat S10), nearly identical to Rps10Ap	-- –	*−2* –	-- –
YDL075W/RPL43A_ex1	Ribosomal protein L31 (yeast L34; YL36; YL28; rat L31), nearly identical to Rpl31Bp	-- –	-- **3.6**	-- –
YKL180W/RPL17_ex2	Ribosomal protein L17 (yeast L20; YL17; E. coli L22; rat and human L17), nearly identical to Rpl17Bp	-- –	-- –	-- **2**
YKR057W/RPS25_ex	Ribosomal protein S21 (yeast S26; YS25; rat S21), identical to Rps21Bp	-- –	-- –	-- **2.2**
YMR230W/_ex2_f_at	Ribosomal protein S10 (rat S10), nearly identical to Rps10Ap	-- –	-- –	-- **−2.7**
YLR197W/SIK1_at	Nucleolar protein component of box C/D snoRNPs, which are necessary for 2′-O-methylation of ribosomal RNAs	-- –	-- –	-- **2**
YMR131C/_at	Protein involved in ribosome assembly, member of WD (WD-40) repeat family	-- –	-- –	-- **−3.5**
RNA Polymerases				
YER148wM_at	TATA-binding component of RNA polymerases I, II, and III; part of initiation factors TFIID and TFIIIB	-- –	-- –	-- **−2**
YJL148W/_at	RNA polymerase I subunit, not shared with other RNA polymerases	-- –	-- –	-- **−3**
YJR063W/RPA12_at	RNA polymerase I subunit A 12.2	-- –	-- –	-- −4.5
Chromatin Arrangement				
YBL003c/HTA2_at	Histone H2A	-- –	-- **−3.1**	*−2.3* **−8.1**
YDR225w/HTA1_f_at	Histone H2A	-- –	-- –	-- **−3.4**
YDR225w/HTA1_i_at	Histone H2A	-- –	-- –	-- **−3.3**
YDR224c/HTB1_at	Histone H2B	-- –	-- –	-- **−2.7**
YBL002w/HTB2_at	Histone H2B	-- –	-- **−4.1**	*−2.7* **−10.6**
YBR010w/HHT1_at	Histone H3	-- –	-- **−3.2**	*−2* **−3.8**
YBR009c/HHF1_f_at	Histone H4	-- –	-- **−2.7**	*−2.2* **−3.6**
YNL030W/HHF2_f_at	Histone H4	-- –	-- –	-- **−3.3**
YOL012C/_at	Histone-related protein that can suppress histone H4 point mutation	-- –	-- –	-- **−2**
DNA Repair/Oxidative Damange				
YDL101C/DUN1_at	Protein kinase required for induction for Rnr3p and DNA repair genes after DNA damage	-- –	-- **2.8**	-- **3.2**
DNA Synthesis				
YER070w/RNR1_at	Ribonucleotide reductase large subunit	-- –	-- **6.4**	-- **7.9**
YJL026W/RNR2_at	Component of ribonucleotide reductase small subunit	-- –	-- **2.6**	*2.5* **3.6**
YIL066C/RNR3_at	Ribonucleotide reductase large regulatory subunit	-- –	*2.4* **5.3**	*3.7* **7.6**
YGR180C/RNR4_at	Component of ribonucleotide reductase small subunit	-- –	-- **2.5**	-- **2.2**
YNL141W/_at	Adenine deaminase (adenine aminohydrolase), enzyme of the purine salvage pathway; has similarity to adenosine deaminases	-- –	-- **−2.2**	-- **−2.7**
YBL039C/_at	CTP synthase; final step in pyrimidine biosynthesis pathway	-- –	-- **−2.3**	-- –
YGL037C/_at	Pyrazinamidase and nicotinamidase	-- –	-- **2**	-- **2.3**

(*continued*)

Table 1. Continued.

Probe Set	Gene/Description	Fold Change[a,b] (at 2 min)	(at 15 min)	(at 30 min)
Nuclear				
YBL113C/_i_at	Unknown function, subtelomerically encoded	*6*	--	--
		5	–	–
YBL113/_r_i_at	Unknown function, subtelomerically encoded	*6.9*	--	--
		4	–	–
YKR092C/SRP40_at	Suppressor of rpc40 and rpb10 mutations	*2.1*	--	--
		4.2	–	–
YIL177C/_ex2_f_at	Protein with similarity to subtelomerically-encoded proteins	--	--	*2.1*
		–	–	**2.4**
YBR214w/_at	Nuclear protein with similarity to *S. pombe* sds23.moe1 protein	--	--	*2*
		–	–	**3.4**
YOR185C/GSP2_at	Ran, a GTP-binding protein member of the ras superfamily involved in trafficking through nuclear pores	--	--	--
		–	–	**2.8**
YER002w/_at	Nuclear protein of unknown function	--	--	--
		–	–	**−2.1**
YOR051C/_at	Nuclear protein of unknown function	--	--	*−2.1*
		–	–	**−2.2**
Cell Cycle				
YDL179W/_at	Cyclin that associates with Pho85p	--	--	--
		–	**−4.1**	–
YAR007C/RFA1_at	DNA replication factorA, 69K subunit, binds single-stranded DNA	--	--	--
		–	–	**2.1**
YNL312W/RFA2_ex1_at	DNA replication factorA, 36 subunit; phosphorylated at the G1/S transition and dephosphorylated at mitosis	--	--	--
		–	–	**2.2**
YPL256C/CLN2_at	G1/S-specific cylin, interacts with Cdc28 protein kinase to control events at START	--	--	--
		–	**−2.8**	–
YIL065C/_at	Protein involved in mitochondrial division	--	--	*2.8*
		–	**3**	**3.4**
YNL327W/_at	Cell-cycle regulation protein, may be involved in the correct timing of cell separation after cytokinesis	--	--	--
		–	–	**−3.5**
YOR272W/_at	Microtubule-associated protein essential for the G1/S transition, member of WD (WD-40) repeat family	--	--	--
		–	**−3.6**	–
YGL134W/_at	Cyclin that associates with Pho85p, involved in glycogen accumulation	--	--	*−2*
		–	–	–

[a] Italic = 10 ng/ml of calicheamicin (--).
[b] Bold = 100 ng/ml of caliceamicin (–).

also fail to undergo meiotic division and sporulate, indicating a cell cycle delay in late S, G2, or M phase [14]. Histones were among the earliest to respond to calicheamicin treatment, and delay in the cell cycle may be the first of a cascade of events in the response of yeast cells to insult by DNA damage (Figure 1). In keeping with this methylmethane sulfonate (MMS), a DNA-alkylating agent has also been shown to decrease the expression of histones [16].

Upon calicheamicin exposure, various cell cycle genes, including two cyclins, PCl9 (YDL179W) and PCl10(YGL134W), respectively, were repressed. Both of these cylcins were found to associate with Pho85 (a regulator of cell cycle progression) [17–18]. PCl9 has been shown to form a functional cyclin-Cdk complex that phosphorylates Pho4p, and it is the only cyclin known to act in the late M phase/early G1 [19–20]. Furthermore, EGT2 (YNL327W), which has been shown to be transcriptionally coregulated in the cell cycle with PCL9, was also repressed in its expression [21]. Other genes that were downregulated in their expression included YTM1 (YOR272W), a microtubule-associated protein essential for the G1/S transition, and CLN2 (YPL256C), a G1/S-specific cyclin that interacts with Cdc28 protein kinase to control START-specific processes [22–24]. The cells used in this experiment were not synchronized, and the changes in the expression level of these genes may reflect various cell cycle delays invoked in DNA-damaged cells. We tested the effects of calicheamicin on synchronized cells but did not observe any significant shift in the population of cells to any one stage of the cell cycle (data not shown).

Consistent with the DNA-cleaving properties attributed to calicheamicin in vitro, we observed upregulation of genes involved in DNA repair and protection against oxidative damage, as well as genes involved in DNA synthesis. DNA repair pathways include base and nucleotide excision repair as well as recombinational repair [25]. Calicheamicin treatment appears to activate both pathways to varying degrees, consistent with the single- and double-strand cleaving properties associated with this drug. For example, both RFA1 and RFA2, DNA replication factors that belong to the RF-A complex (Rfa1p-Rfa2p-Rfa3p), were upregulated. The complex acts early in nucleotide excision repair in DNA damage recognition and acts to regulate the expression of DNA repair and DNA metabolism genes [26]. The RFA heterotrimeric complex is also involved in recombination and has been

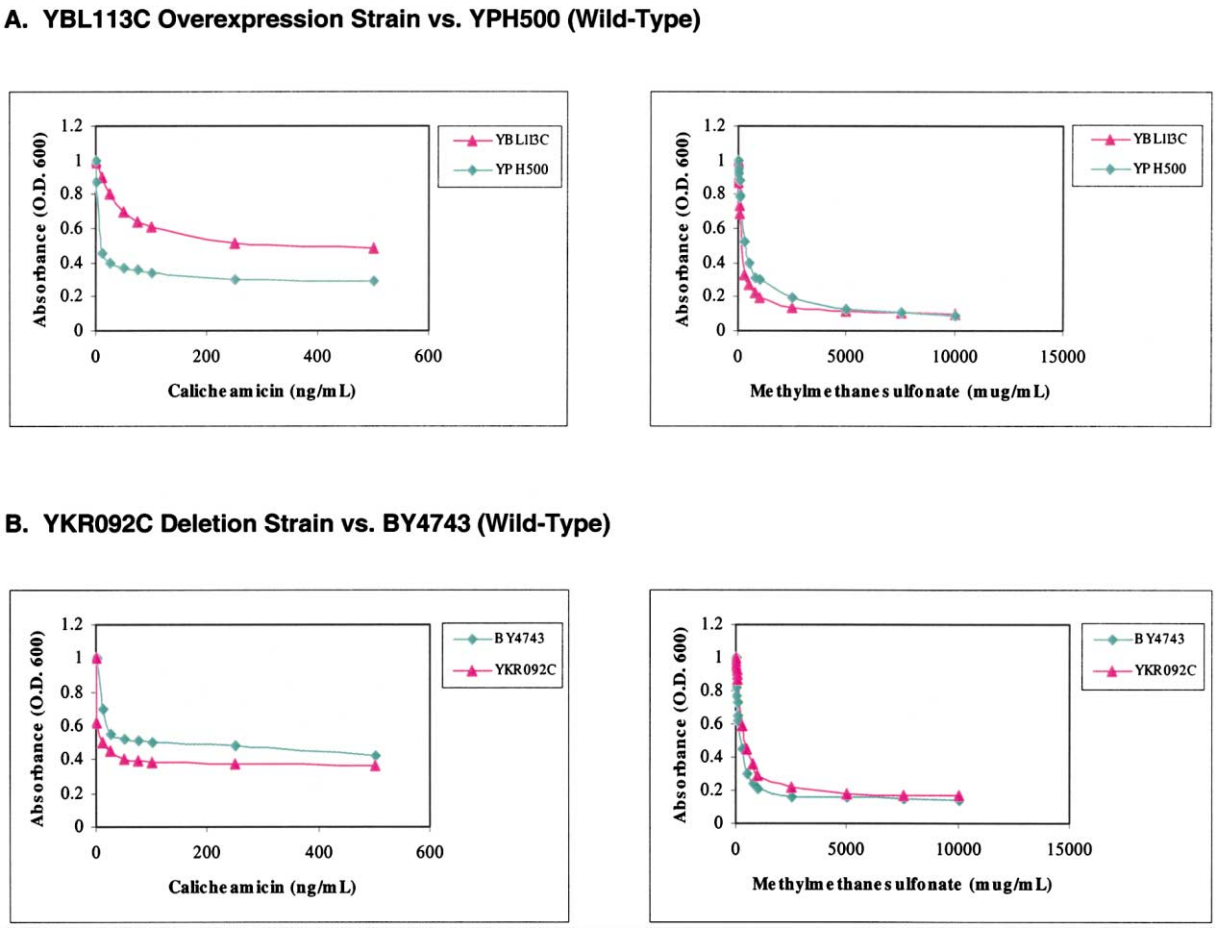

Figure 1. Effect of Calicheamicin and Methylmethanesulfonate on Yeast Growth as Measured by $O.D._{600}$

shown to stimulate DNA strand exchange [27]. mRNA expression levels of these proteins peak at the G1/S phase boundary. They are also increased by methylmethanesulfonate treatment [16].

DUN1, a protein also involved in the DNA damage sensory pathway, was shown to be upregulated by 2–3 fold with calicheamicin treatment. DUN1 is a protein kinase that is required for the induction of ribonucleotide reductase genes and DNA repair genes after DNA damage [28]. Phosphorylation has been shown to increase in response to DNA damage in a Dun1p-dependent manner [29]. mRNA abundance of the protein has been shown to peak in late G1 phase [30]. MMS treatment has also been shown to increase phosphorylation activity by 6-fold [31]. Likewise, all four of the ribonucleotide reductase subunits that compose the $\alpha 2$-$\beta 2$ tetramer required for DNA synthesis were shown to be upregulated upon treatment with calicheamicin. The tetramer consists of two large (Rnr1p and Rnr3p) and two small (Rnr2p and Rnr4p) subunits and is induced by double-strand DNA breaks generated directly by DNA-damaging agents or indirectly by DNA repair processes [32]. Other genes related to DNA synthesis and nucleotide metabolism were also differentially expressed; for example, URA7 and AAH1 were downregulated. URA7 is a CTP synthase that catalyzes the formation of dCTP from dUTP, the final step in pyrimidine biosynthesis, whereas AAH1 is an adenine deaminase (adenine aminohydrolase), an enzyme of the purine salvage pathway, and converts adenosine to inosine [33–36]. Both of these genes have also been shown to be repressed in their expression by treatment with methylmethanesulfonate [16].

Nuclear proteins, with less defined roles, were also affected at the transcriptional level. For example, SDS24 (YBR214W), a protein 62% identical to the spindle pole body protein SDS23, was upregulated by 2-fold in its expression. sds23/moc1 is a multicopy suppressor of protein phosphatase 1 mutants. High osmolarity as well as cell-damaging conditions have been shown to induce mRNA levels of this protein [37]. Moreover, overproduction of this protein in *E. coli* has been shown to elicit an SOS response [38]. Both YOR051C and YER002W were downregulated in their expression by 2-fold and have been shown to purify with proteins associated with the nuclear pore complex [39]. Homozygous diploid mutants of these proteins have each been shown to exhibit a reduced growth rate [40].

Other genes that altered in expression on a global level included a variety of ribosomal proteins, heat shock proteins, multidrug resistance proteins, biosynthetic and metabolic proteins, cell wall proteins, and proteins of unknown function. Although, a complete description of these genes and their functions is beyond the scope

of this article, a complete list of the genes with their fold changes can be found at the following website: ftp://schultzftp.scripps.edu/pub/Coran/CLM_Table_1.xls.

Whether calicheamicin can target DNA or DNA/protein complexes selectively within a cell and favor cleavage of particular chromosomal sites is unknown. Calicheamicin, as well as other enediynes of its class, have been shown to recognize local DNA conformation and deformability and are thought to bind through an induced-fit mechanism [9]. Interestingly, at 2 min of calicheamicin exposure (10 ng/ml, the lowest dose), we observed differential expression of only six genes that changed ≥2-fold. Two nuclear genes (YBL113C and YKR092C) were among these, as well as four other genes that are most likely related to stress response. These four included two heat shock proteins (YCR021C and YBR005W) and two proteins involved in cell wall biosynthesis (YBR078W and YLL025W). YCR021C, or heat shock protein 30 (Hsp30), is a protein located in the plasma membrane and is involved in adaptation to growth under stress conditions [41–42]. YBR005W is a protein that exhibits similarity to HSP30 [43]. YBR078W is involved in cell wall maintenance and has been shown to be one of 127 genes coregulated under 26 cell-damaging conditions, some of which encode proteins involved in sterol metabolism, ion homeostasis, or cell wall, cytoskeleton, or chromatin structure [43–44]. YLL025W, encoding a protein of the seripauperin (PAU) family of possible cell wall mannoproteins, contains two putative stress response elements (STRE) present in the promoter [45–47]. Functional genomic studies have shown that the gene is coregulated among 163 other genes under 26 different cell-damaging conditions [43].

YBL113C, a subtelomerically encoded protein, was upregulated by 4- to 7-fold and corresponds to a putative Y′-helicase (the protein is 84% identical to the Y′-helicase YJL225C) [48]. The protein has been shown to amplify Y′ elements, highly polymorphic repetitive sequences that are present in the subtelomeric regions of many yeast telomeres; such sequences are found to be induced in survivors defective for telomerase [49]. Amplification of Y′ elements has also been shown to be RAD52 dependent, a gene essential for homologous recombination in yeast. On the other hand, YKR092C, or SRP40, which is upregulated by 2- to 4-fold upon 2 min of calicheamicin treatment, is a protein shown to protect against mutations induced in *rpb10* and *rpc40*. *rpb10* encodes a small polypeptide (referred to as ABC10β) that is shared among the three yeast RNA polymerases, whereas *rpc40* encodes the α-like subunit (AC40) of polI and polIII [50]. Interestingly, RNA polymerase genes were actually among those downregulated at 15 min of calicheamicin exposure. However, SRP40 is thought to act specifically on *rpb10* and *rpc40* mutants.

To investigate whether upregulation of these nuclear proteins might make yeast less sensitive to calicheamicin, we treated overexpression or deletion mutant strains of yeast with calicheamicin and compared them to the wild-type strain. To examine the effects of YBL113C, we overexpressed the protein since it is subtelomeric (next to telomeres) and the unavailability of a deletion mutant strain suggested possible lethality. A deletion mutant strain of YKR092C was available, so we chose to use this strain to examine its effects on calicheamicin-induced DNA damage. Employing a range of concentrations, we treated yeast cultures (0.1 O.D.) with the drug and monitored them by UV in a time course analysis (Figures 1A and 1B). Drug treatments were repeated in triplicate, and standard curves were generated to normalize against differences in growth rate.

Overexpression of YBL113C, the Y′-helicase, was shown to be protective against the DNA-damaging effects of calicheamicin, whereas deletion of SRP40, YKR092C, had a slight detrimental effect. (It should be noted that the IC_{50} of calicheamicin on yeast cell growth is approximately 500 ng/ml [in culture]). For comparison, we also tested the effects of another DNA damage agent, methylmethanesulfonate (MMS; DDW as a control), on both yeast strains. The results are depicted in the right panels of Figures 1A and 1B. In both instances the effects of MMS on both yeast strains were minimal. These results may reflect differences in the DNA damage response of the cell or could potentially suggest selectivity of calicheamicin-induced DNA cleavage.

Significance

Calicheamicin γ_1^I isolated from fermentations of *Micromonospora echinospora* ssp. is among a family of unusually potent antitumor agents. In vitro, the drug has been shown to interact with double-helical DNA in the minor groove, where it causes both single- and double-strand breaks. The cleavage chemistry is dependent upon reducing agents and oxygen for activating rearrangement of the enediyne warhead to give the highly reactive 1,4-diylradical species. In vivo, activation of the drug presumably occurs by addition of glutathione to the methyltrisulfide. Transcriptional effects to the drug corroborate the in vitro results and clearly define the role of the drug as a DNA-damaging agent. On a global scale, genes involved in DNA repair and synthesis, chromatin rearrangement, cell cycle checkpoint control, nuclear proteins, ribosomal proteins, metabolic and biosynthetic genes, and proteins involved in stress response are all altered at the transcriptional level. The transcriptional effects of calicheamicin at low concentrations of drug and short exposure times revealed six genes that were affected ≥2-fold. Four of these six are probably involved in stress response, and two are nuclear proteins, including a Y′-helicase involved in telomere maintenance and a suppressor of *rpc10* and *rpb40* mutations (two RNA polymerase subunits). Overexpression of the Y′-helicase was shown to be protective against calicheamicin-induced DNA damage but not against methylmethanesulfonate treatment over a range of concentrations and time points. These results may reflect either differences in the cells' response to various DNA-damaging agents or the possibility that calicheamicin may favor cleavage of certain chromosomal sites.

Experimental Procedures

Organisms and Culture Techniques

The yeast *S. cerevisiae* wild-type strain YPH500α (*MAT*α *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*) was used in this investigation. For preparation of RNA samples for GeneChip hybridization, yeast samples were inoculated into 500 ml YPD medium

(per liter: yeast extract, 10 g; peptone, 20 g; and dextrose [20%, 10× solution, autoclaved separately], 100 ml) and grown to an $O.D._{600}$ of 1 at 30°C, 250 rpm. Cultures were then split into 30 ml aliquots, and for each time point calicheamicin γ_1^I (dissolved in ethanol) was added to one culture while another received ethanol alone. For time course experiments, both YKR092C and BY4743 were grown in standard YPD medium. However, YBL113C was grown in −URA minimal medium, and YPH500α was grown in minimal medium. Minimal medium contains (per 100 ml) 10 ml nitrogen base (6.7%) 3 ml histidine (1 mg/ml), 3 ml trptophan (1 mg/ml), 3 ml leucine (1 mg/ml), 2 ml adenine (1 mg/ml), 2 ml uracil (1 mg/ml), glucose (20%), and casamino acids (1%).

RNA Extraction and GeneChip Hybridization

Total RNA was extracted by an SDS/double phenol extraction method. Yeast cell samples were centrifuged, and the supernatant was decanted. Each cell sample was then washed with 30 ml of water and was subsequently centrifuged (repeated three times). Yeast pellets were then frozen in liquid nitrogen and lysed as follows. To each cell pellet was added 10× high-salt solution (3M NaCl, 200 mM Tris [pH 8.0], and 100 mM EDTA) and deionized distilled water (DDW) to give a final volume of 700 μl. Each sample was then brought to 1% SDS by the addition of 10% SDS (70 ml), at which time 65°C phenol (600 ml) was added. Samples were vortexed, incubated at 65°C for 4 min, and subsequently chilled on ice for 2–4 min. Samples were microfuged for 2 min, and the aqueous phase was transferred to new tubes. The phenol extraction procedure was repeated a second time, followed by extraction with a 25:24:1 phenol:chloroform:isoamyl alcohol. After centrifugation, the aqueous layer of each sample was transferred to new tubes. The RNA was then precipitated by the addition of ethanol to the top of each microfuge tube. Samples were mixed by vortexing, stored at −20°C for 1 hr, and subsequently pelleted by centrifugation. The supernatant was decanted, and the pellet from each sample was washed twice with 70% ethanol. Residual ethanol was removed, and the cell pellet was resuspended in 100 μl of water. The total RNA was then DNase treated by taking 30 μl of total RNA and adding 5 μl of second-strand buffer (Gibco BRL cDNA Superscript Choice Kit) and 1 μl of DNase 1 (RNase-free, Ambion). Samples were incubated at room temperature for 1 hr and then heat inactivated by heating the samples at 75°C for 15 min. As a precaution, samples were also extracted with 25:24:1 phenol:chloroform:isoamyl alcohol. After precipitation and washing, the total RNA was purified with the Qiagen RNeasy Mini Kit, and the total RNA (25 μg) was amplified and biotinylated according to the protocol detailed by Affymetrix (Affymetrix; Santa Clara, California). A set of four oligonucleotide arrays (GeneChip Ye6100 arrays; Affymetrix) representing the yeast genome in its entirety (6218 ORFs) was used for hybridizations.

Cloning and Expression of YBL113C

The YBL113C ORF with the adjacent 991 bp of 5′-nontranslated region was amplified by PCR from yeast chromosomal DNA with the primers FTEL1 (5′-CAGTGTTGCTTGCTAATTGC-3′) and RTEL1 (5′-TTCCTTCACTCTCCAACTTC-3′). The resulting PCR product was then cloned into the 2 μm plasmid pYES2.1/V5-His-TOPO (Invitrogen) to give plasmid pYBL1 and was used for YBL113c overexpression driven by its native promoter. Yeast transfection was carried out with the Yeastmaker transformation system by Clonetech.

Time Course Analyses with YBL113C and YKR092C Overexpression and Deletion Mutant Strains

All yeast strains were grown to an $O.D._{600}$ of 1, diluted to an $O.D._{600}$ of 0.1, and subsequently aliquoted (2 ml). Various concentrations of each drug were then added, and the $O.D._{600}$ values of the cells were then monitored over time. All reactions were carried out in triplicate (three independent runs), averaged, and subsequently plotted to generate standard curves (data not shown).

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