Chemistry & Biology, Vol. 9, 607–618, May, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S1074-5521(02)00137-0

Transcriptional Response Pathways in a Yeast Strain Sensitive to Saframycin A and a More Potent Analog: Evidence for a Common Basis of Activity

Alleyn T. Plowright, Scott E. Schaus, and Andrew G. Myers[1]
Department of Chemistry and Chemical Biology
Harvard University
12 Oxford Street
Cambridge, Massachusetts 02138

Summary

Saframycin A (SafA) is a natural product that inhibits human cancer cell proliferation. Its synthetic analog, QAD, is a more potent inhibitor of these cells. SafA does not affect wild-type yeast, but it does inhibit growth of the strain CCY333 (Δ*PDR1*/*PDR3*/*ERG6*) (IC_{50} = 0.9 μM). QAD is also a more effective inhibitor of CCY333 growth (IC_{50} = 0.4 μM). Transcription profiling of SafA- and QAD-treated CCY333 cultures showed that both drugs generated nearly identical profiles, with altered expression levels (≥2-fold) of more than 240 genes. Both agents induced the overexpression of genes involved in glycolysis, oxidative stress, and protein degradation and repressed genes encoding histones, biosynthetic enzymes, and the cellular import machinery. Significantly, neither drug affected the expression of known DNA-damage repair genes, as might have been expected if their primary mechanism of action involved the covalent modification of DNA.

Introduction

Saframycin A (SafA) is a structurally complex natural product ([1, 2]; Figure 1) that inhibits the growth of human cancer cells at extremely low doses in culture (mean GI_{50} values, 0.3–12 nM; NCI human tumor cell line screen, four data sets) [3, 4]. The basis for the antiproliferative activity of SafA has not been established, although important experiments directed toward an understanding of its mechanism of action have been conducted. In early work, Ishiguro et al. [5] showed that treatment of cultured L1210 leukemia cells with SafA (IC_{50}, 5.6 nM [6]) led to diminished incorporation of [^{3}H]-uridine into nucleolar and nucleoplasmic RNA. In addition, newly synthesized (^{3}H-labeled) RNA from both fractions was found to be of shorter length. Both effects were amplified as the concentration of SafA was increased. SafA was also shown to inhibit the template activity of DNA in the presence of a reducing agent such as dithiothreitol; such an activity is associated with the ability of SafA to alkylate the exocyclic amino group of guanine residues in double-stranded DNA after reductive activation [5, 7–11]. The DNA-alkylation reaction has been demonstrated to be reversible and specific for duplex DNA; single-stranded DNA does not form a stable SafA alkylation product. The structurally related alkaloid ecteinascidin-743 (Et-743) [12, 13], currently in advanced clinical trials for cancer therapy [14–17], has also been shown to alkylate G residues of duplex DNA [18]. For both natural products, compelling evidence has been offered to support the involvement of an iminium ion intermediate in this process (this intermediate is formed by the expulsion of cyanide in the case of SafA and water in the case of Et-743) [19]. Proposals concerning the mechanism of action of SafA have focused on DNA as a primary target, perhaps in association with one or more cellular proteins. Experiments designed to evaluate the mechanism of action of Et-743 have investigated the possible involvement of ternary complexes of drug, DNA, and transcription factors [20–22], as well as drug, DNA, and an excision-repair protein [23, 24]. Recently, Et-743 and the equipotent synthetic analog Pt-650 [25] have been subjected to comparative analysis by transcription-profiling experiments in human cancer cells [26]. It has not been established that SafA and Et-743 have a common basis of activity.

As a result of an extensive program leading to the development of an efficient laboratory synthetic route to SafA [27], we have synthesized a large number of SafA structural analogs, many of which were more potent than SafA itself [28]. The analog QAD, which is modified by replacement of the pyruvamide side chain of SafA with a quinaldic acid amide residue and by transformation of the two quinone rings of SafA to hydroquinone methyl ethers, is one of the more potent compounds we have discovered. The compound QAD showed 30-fold greater activity versus SafA in a lung carcinoma cell line (A549) and 4-fold greater activity in a melanoma cell line (A375) [28]. QAD has been reported to show 100-fold greater potency versus Et-743 in three human sarcoma cell lines (Joseph Bertino and Wei Wei Li, Memorial Sloan-Kettering Cancer Center, personal communication). In light of the substantial structural modification of QAD relative to SafA and its greater potency, the question arose as to whether activity-directed lead optimization had produced an agent that acted by the same mechanism as the natural product. Whole-genome transcription profiling of yeast provides a powerful tool for monitoring changes in expression levels of genes in a simple eukaryotic organism in response to a given stimulus [29–34], and it potentially offers a means for the comprehensive comparison of the effects of two different small-molecule agents in vivo and provides insight into the pathways that are affected [35, 36]. The yeast genome has been fully sequenced, and a large number of yeast gene products have been characterized with regard to function. Although wild-type yeast is not susceptible to growth inhibition by SafA, an *ERG6*/*PDR1*/*PDR3* deletant (strain CCY333; yeast mutants bearing these deletions were previously prepared in the laboratories of Professor Julian Simon, Fred Hutchinson Cancer Research Center. The *CCY333* yeast strain was constructed by Dr. Christina Cuomo [Harvard University] in the laboratories of Professor Andrew Murray [Christina Cuomo, personal communication]; [37]) was found to be sensitive to growth inhibition

[1]Correspondence: myers@chemistry.harvard.edu

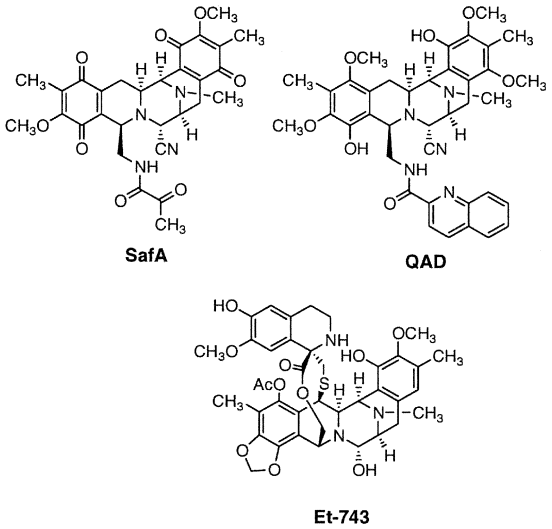

Figure 1. Structures of Saframycin A, the More Potent Quinaldic Acid Derivative, and Ecteinascidin-743.

Saframycin A, SafA; quinaldic acid derivative, QAD; ecteinascidin-743, Et-743.

by both SafA and QAD and was therefore selected for whole-genome transcription profiling experiments.

Results

Identification of a Yeast Strain Sensitive to SafA and QAD and Growth-Inhibition Studies

Growth of *S. cerevisiae* strain BY4743 was found to be unaffected in the presence of SafA or QAD at concentrations as high as 1 mM, confirming previous reports [1]. In contrast, growth of the weakened strain CCY333 was greatly inhibited in the presence of either SafA or QAD. This strain lacks *ERG6*, a gene required for the biosynthesis of ergosterol (affecting membrane permeability) [38], and the genes *PDR1* and *PDR3*, encoding transcription factors that regulate the expression of genes associated with multidrug resistance [39]. Control experiments showed that in the absence of drug, cultures of the weakened strain grew well, with a doubling time of ~2 hr. Growth-inhibition studies conducted in the presence of SafA and QAD established IC_{50} values of 0.9 μM and 0.4 μM, respectively. Thus, the sensitivity to drug treatment in the weakened strain is at least 1000-fold greater than the laboratory strain BY4743. In light of its greater sensitivity to both SafA and QAD, all subsequent experiments were conducted with the yeast strain CCY333.

Drug Treatment Leads to mRNA Depletion; cDNA Preparation for Microarray Analysis

Quantification by UV absorption at 260 nm of mRNA isolated by the use of an oligo(dT) resin from cultures of yeast strain CCY333 treated with SafA or QAD (10 μM) showed that rapid diminution in mRNA levels occurred over the course of the 2 hr incubation (three independent experiments). At 1 hr, a point at which growth inhibition was not yet evident, sufficient mRNA was isolated to synthesize fluorescently labeled cDNA (by the use of random hexamer DNA primers) for subsequent hybridization to a DNA microarray. At 2 hr, a point at which 30% inhibition of growth was observed, insufficient mRNA was isolated by this method for meaningful hybridization experiments.

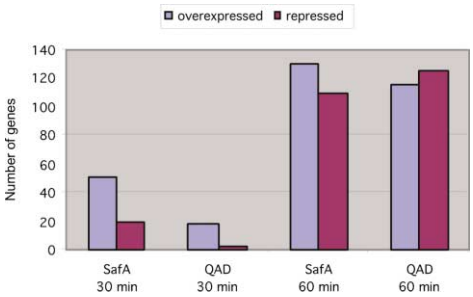

Figure 2. Number of Genes Overexpressed or Repressed ≥2-Fold Relative to Controls at 30 and 60 Minute Time Points after Treatment with SafA or QAD

A greater transcriptional response to drug treatment was detected in hybridizations to DNA microarrays with cDNA synthesized by an alternative technique that did not require the isolation of mRNA [40]. In this procedure, cDNA was prepared from a sample of total RNA by reverse transcription with oligo(dT) primers to selectively hybridize to the polyA tail of mRNA. As in the prior method, no meaningful hybridization data were obtained after 60 min, presumably because of diminished mRNA levels. Because a greater transcriptional response was detected, all subsequent microarray hybridizations were performed with fluorescently labeled cDNAs prepared from total RNA, and analysis was restricted to time points early in the incubation (≤60 min). Except as noted, the analysis below refers to experiments conducted with 60 min drug exposure.

Transcriptional Responses of Yeast Strain CCY333 to SafA and QAD: A Genome-Wide Analysis

Exposure of the mutant yeast strain CCY333 to either SafA or QAD produced a large transcriptional response; expression levels of more than 240 genes were modified by ≥2-fold. After 60 min, treatment with SafA led to ≥2-fold-enhanced expression of 130 genes (33 of unknown function) and decreased the expression of 110 genes ≥2-fold (27 of unknown function) relative to nontreated controls, whereas treatment with QAD led to ≥2-fold-enhanced expression of 115 genes (31 of unknown function) and decreased the expression of 125 genes ≥2-fold (28 of unknown function, Figure 2). Genes overexpressed ≥2-fold in both the SafA and QAD expression profiles and those repressed ≥2-fold in both profiles were strongly correlated (Figure 3A, P-values $<1.0 \times 10^{-40}$). There was virtually no overlap of genes overexpressed by one agent (SafA or QAD, ≥1.7-fold) and repressed by the other (≥1.0-fold, Figure 3B). The statistical correlation between the gene expression data sets at 60 min was 0.87 (Figure 4). At 30 min, fewer genes were transcriptionally modified by drug treatment, and the statistical correlation between the data sets was lower (0.72).

Analysis of Genes Overexpressed in Both SafA and QAD Expression Profiles

Genes overexpressed ≥2-fold after exposure of the yeast strain CCY333 to SafA or QAD (60 min) can be

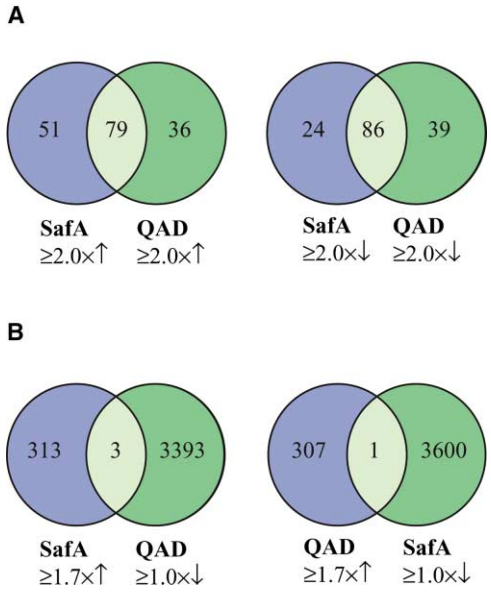

Figure 3. Venn Diagrams Comparing Gene Overexpression by SafA and QAD

Venn diagrams comparing (A) sets of genes overexpressed or repressed (≥2-fold) in both the SafA and QAD expression profiles and (B) genes overexpressed (≥1.7-fold by one agent (SafA or QAD) and repressed (≥1.0-fold) by the other.

separated into functional categories, as listed in Table 1. The principal categories are identified and discussed briefly below.

Proteins Involved in Glycolysis

With the exception of the gene encoding triosephosphate isomerase (*TPI1*, overexpressed 1.6- and 1.5-fold for SafA and QAD, respectively, at 60 min, and 2.0- and 1.9-fold, respectively, at 30 min), each of the genes associated with the conversion of fructose-6-phosphate to pyruvate was overexpressed 1.8-fold or greater upon treatment with SafA at the 60 min time point. Three of the genes that code for components of pyruvate dehydrogenase (*PDA1, PDA2,* and *PDB1*), an enzyme that converts pyruvate to acetyl CoA, also showed enhanced expression.

Proteins Involved with Protein Degradation

The expression of genes encoding proteins involved in two different protein degradation pathways was enhanced. Genes encoding proteins, and components of the proteasome, in the ubiquitin-mediated proteolytic pathway were overexpressed, as were genes encoding proteins involved in the transport of intracellular material to the vacuole (autophagocytosis) and proteases involved in vacuolar protein degradation.

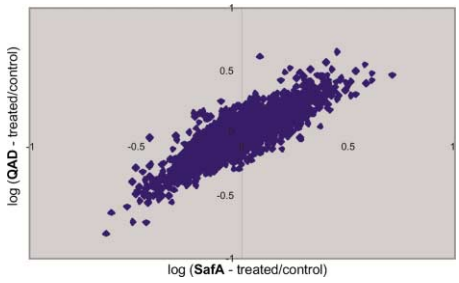

Figure 4. A Logarithmic Plot of the Ratios of Gene Expression Levels for Each Gene in the Expression Profiles of SafA and QAD

Proteins Associated with Oxidative-Stress Response

In this category, genes overexpressed included those encoding Cu/Zn and Mn superoxide dismutase (*SOD1* and *SOD2*), thioperoxidases (*TSA1*, *AHP1*, and *YBL064C*), thioredoxins (*TRX1* and *TRX2*), and glutathione peroxidase (*HYR1*).

Proteins Associated with Other Stress Responses

In addition to genes associated with oxidative-stress response, genes encoding heat-shock proteins, and genes encoding proteins required for protein refolding (cyclophilins *CPR3, CPR6, CYP5, CYP2*, and nuclear isomerases *FPR1* and *FPR3*), were overexpressed.

Proteins Involved in the Synthesis and Processing of RNA

Genes overexpressed in this category were *HRP1*, encoding a protein involved in mRNA processing [41], and *SBP1*, encoding a protein that is essential for ribosomal RNA processing [42].

DNA Damage-Responsive Genes

Expression of *RNR2* and *RNR4*, components of ribonucleotide reductase, was enhanced (but see *RNR1*, Table 2, and discussion below). No other genes known to be involved in DNA damage repair were upregulated.

Analysis of Genes Repressed in Both SafA and QAD Expression Profiles

Genes repressed ≥2-fold after exposure of the yeast strain CCY333 to SafA or QAD (60 min) can be grouped into functional categories, as listed in Table 2. The principal categories are identified and discussed briefly below.

Proteins Involved in the Transport of Nutrients into the Cell

Many genes that encode transporter proteins were repressed. These included transporters for amino acids, metals, hexoses, ammonia, purines, and oligopeptides.

Subunits of the Histone Octamer

Genes encoding all eight of the histone proteins were repressed ≥2-fold.

Proteins Required for the Synthesis of Fatty Acids from AcetylCoA

Genes encoding protein components of the fatty-acid synthesis pathway (*FAS1, FAA3, FAA4*) were repressed upon treatment with SafA or QAD (1.8–3.6-fold).

Proteins Involved in Glycolysis

Phosphofructokinase (*PFK1*) was unique among glycolytic proteins in that both SafA and QAD strongly repressed transcript levels of the gene encoding this enzyme (≥2-fold, 60 min).

Analysis of Genes Expressed to Different Degrees upon Exposure of the Yeast Strain CCY333 to SafA or QAD

Although the gene expression profiles obtained after exposure of the yeast strain CCY333 to SafA or QAD for 60 min were almost identical, analysis of the data sets did reveal genes that were overexpressed or repressed to different degrees (Table 3). Among these are genes (such as *CUP1*, a metallothionein) that encode

Table 1. Genes Overexpressed in Both SafA and QAD Profiles

Gene Name	Fold Change		Protein Function
	SafA	QAD	
			Glycolysis
FBA1	2.7	2.3	Fructose bisphosphate aldolase II
TDH1	2.5	1.7	Glyceraldehyde-3-phosphate dehydrogenase 1
TDH2	3.8	2.5	Glyceraldehyde-3-phosphate dehydrogenase 2
TDH3	3.2	2.6	Glyceraldehyde-3-phosphate dehydrogenase 3
PGK1	1.8	1.7	Phosphoglycerate kinase
GPM1	2.4	2.1	Phosphoglycerate mutase
ENO1	2.5	2.1	Enolase 1 (2-phosphoglycerate dehydratase)
ENO2	2.5	2.2	Enolase 2 (2-phosphoglycerate dehydratase)
PYK1	2.1	1.6	Pyruvate kinase, catalyzes final step in glycolysis
PDA1	2.1	2.3	Pyruvate dehydrogenase complex E1-alpha subunit
PDA2	1.9	1.8	Pyruvate dehydrogenase complex, E2 component
PDB1	2.0	1.8	Pyruvate dehydrogenase complex, E1-beta subunit
			Pentose Phosphate Pathway
RPE1	2.0	1.8	Ribulose-5-phosphate 3-epimerase
			Protein Degradation
UBC4	3.0	2.5	Ubiquitin-conjugating (E2) enzyme
UBC1	1.9	2.0	Ubiquitin-conjugating (E2) enzyme
PRE7	2.1	2.2	Proteasome subunit beta6
PRE9	2.0	1.8	Proteasome subunit alpha3
RPN8	2.0	2.2	Non-ATPase subunit of the 26S proteasome complex
AUT7	3.7	3.5	Required for delivery of autophagosomes to vacuole
PEP4	2.0	2.5	Proteinase A
LAP3	1.9	2.2	Aminopeptidase of cysteine protease family
LAP4	1.9	2.7	Aminopeptidase I of the vacuole
PRC1	2.1	2.4	Carboxypeptidase Y, a vacuolar serine protease
CPS1	2.0	1.8	Gly-X carboxypeptidase
			Vacuolar H^+-ATPases
VMA2	2.1	2.0	Vacuolar H^+-ATPase regulatory subunit
VMA4	2.2	2.2	Vacuolar H^+-ATPase hydrophilic subunit
			Oxidative-Stress Response
SOD1	1.9	1.8	Copper-zinc superoxide dismutase
SOD2	2.3	2.0	Manganese superoxide dismutase
TRX1	2.5	2.3	Thioredoxin I
TRX2	2.4	2.3	Thioredoxin II
HYR1	2.5	2.9	Glutathione peroxidase
TSA1	2.3	2.4	Thioredoxin peroxidase
PRX1	2.9	2.7	Mitochondrial thiol peroxidase
			Heat Shock
HSP42	4.1	3.0	Heat shock protein
HSP12	2.8	4.5	Heat shock protein of 12 kDa
HSP10	2.5	2.5	Mitochondrial chaperonin
HSP26	2.4	2.9	Heat shock protein of 26 kDa
SSE1	2.1	1.8	Heat shock protein of the Hsp70 family
SBA1	2.5	2.5	Hsp90 associated cochaperone
			Proline Isomerases
CPR6	5.0	2.9	Cyclophilin
CPR3	2.4	2.2	Cyclophilin
CYP2	2.1	3.3	Cyclophilin
CYP5	2.3	1.7	Cyclophilin
FPR1	2.4	2.3	Peptidylprolyl *cis-trans* isomerase
FPR3	2.2	2.1	Peptidylprolyl *cis-trans* isomerase
			Other Cell-Stress Response
GRE3	2.2	2.3	Aldose reductase with NADPH specificity
GTT1	2.6	3.3	Glutathione transferase
GTT2	1.9	2	Glutathione transferase
PNC1	2.5	2.7	Pyrazinamidase and nicotinamidase
PHO88	2.1	2.2	Inorganic phosphate transport

(*continued*)

Table 1. Continued

Gene Name	Fold Change SafA	Fold Change QAD	Protein Function
			RNA Processing
HRP1	2.4	2.4	Nuclear polyadenylated RNA-binding protein
SBP1	2.1	2.1	Important for rRNA processing
			DNA-Damage Response
RNR2	2.0	2.6	Component of ribonucleotide reductase
RNR4	2.3	2.9	Component of ribonucleotide reductase
			Amino Acid Metabolism
CYS3	2.2	2.2	Cystathionine gamma-lyase
PRO3	2.1	2.0	Delta-1-pyrroline-5-carboxylate reductase
ARO8	2.1	2.0	Aromatic amino acid aminotransferase I
GCV3	1.9	2.9	Glycine decarboxylase hydrogen carrier
HOM6	2.0	1.8	Homoserine dehydrogenase
ILV6	2.7	2.2	Acetolactate synthase regulatory subunit
			Structural Proteins
ARP3	1.9	2.4	Actin-related protein
TPM1	2.1	2.0	Tropomyosin, localized to actin cables
TUB1	2.3	1.9	Tubulin alpha-1 chain
			Vesicular Transport
YGR284C	2.7	2.5	Component of COPII-coated vesicles
COF1	2.4	2.4	Cofilin, actin binding and severing protein
			Protein Synthesis
TEF2	2.6	2.1	Translation elongation factor EF-1 alpha
MRP8	2.1	2.4	Mitochondrial ribosomal protein
			Miscellaneous
CMD1	2.5	2.1	Calmodulin, calcium-binding protein
TFS1	1.9	2.4	Nutrient- and ammonia-response cell cycle regulator
YLR179C	2.1	2.1	Protein with similarity to Tfs1p
BTN2	2.2	2.0	Role in cellular pH homeostasis
IPP1	2.3	2.1	Inorganic pyrophosphatase, cytoplasmic
NCE102	2.4	2.0	Export that lacks classical secretory signal sequences
GUK1	2.2	2.1	Guanylate kinase
ARA1	2	2.2	Subunit D-arabinose dehydrogenase
NTF2	2.6	2.0	Nuclear transport factor
YDL100C	2.0	2.0	Protein with similarity to E. coli ArsA

Time points were taken after 60 min.

proteins associated with the sequestering of metal ions and genes (such as *AHP1*, alkylhydroperoxide reductase) that encode proteins involved in oxidative-stress response. No genes overexpressed $\geq$1.7-fold after treatment with either agent (60 min) were repressed $\geq$1.7-fold in the profile of the other.

Statistical Comparison of the SafA Expression Profiles with Profiles of Known DNA-Modifying Agents and the Natural Product Rapamycin

Comparison of the expression profile (logarithmic plot of the ratios of gene expression levels, treated:control) from SafA treatment with profiles obtained from treatment of the same yeast strain (CCY333) with the known DNA-modifying agents mitomycin C (10 μM, 1 hr, ρ = 0.34) and MMS (50 μM, 1 hr, ρ = 0.24) (S.E.S., unpublished data) showed little statistical correlation. Similarly, comparison of the SafA profile with profiles obtained from treatment of the yeast strain BY4743 with the known DNA-damaging agent NCS (50 μM, 1 hr) [34] and the natural product rapamycin (100 nM, 1 hr) [32], shown to affect nutrient-sensing in yeast, also showed little statistical correlation (ρ = 0.03 and 0.10, respectively).

Discussion

Deletion of the ergosterol biosynthesis gene *ERG6*, as well as the transcription factors *PDR1* and *PDR3*, which regulate the expression of a network of genes that are involved in multidrug resistance, renders yeast susceptible to growth inhibition by SafA and QAD (IC_{50} = 0.9 and 0.4 μM, respectively), suggesting that the resistance of wild-type yeast to these small molecules is at least in part due to drug permeability/export issues. The availability of a viable yeast mutant that is sensitive to SafA and QAD has allowed us to conduct DNA microarray experiments to monitor the effects of drug treatment at the level of transcription in a simple eukaryotic organ-

Table 2. Genes Repressed in Both SafA and QAD Profiles

Gene Name	Fold Change		Protein Function
	SafA	QAD	
			Transporters
DIP5	−3.3	−2.4	Dicarboxylic amino acid permease
VAP1	−2.3	−2.4	Amino acid permease
BAP2	−2.9	−2.3	Branched-chain amino acid permease
GNP1	−2.5	−2.6	High-affinity glutamine permease
PHO84	−2.7	−2.6	High-affinity inorganic phosphate/H^+ symporter
PTR2	−2.7	−2.4	Di- and tri-peptide permease
HXT1	−2.0	−2.3	Low-affinity hexose transporter
HXT4	−1.8	−2.0	High-affinity glucose transporter
PMA1	−1.9	−2.5	Plasma membrane H^+-ATPase
PMA2	−2.1	−2.8	H^+-transporting ATPase of the plasma membrane
FCY2	−2.9	−3.1	Cytosine/purine permease
SSU1	−2.7	−2.2	Plasma membrane transporter involved in sulfite efflux
TAT2	−2.3	−2.5	High affinity tryptophan permease
BAP3	−2.1	−2.3	Valine transporter
FRE1	−3.1	−2.6	Membrane-associated flavocytochrome
FRE7	−2.9	−2.0	Protein with weak similarity to Fre1p and Fre2p
STE6	−2.0	−1.9	ABC transporter
SNQ2	−1.9	−2.0	Putative ATP-dependent permease
TPO2	−1.8	−2.6	Polyamine transport protein
TPO3	−1.8	−2.3	Polyamine transport protein
OPT1	−2.2	−1.9	Protein member of the oligopeptide transporter family
AQY2	−2.7	−1.7	Aquaporin water channel protein
MEP3	−1.9	−1.7	Ammonia permease
			Histones
HTA1	−2.6	−3.1	Histone H2A
HTA2	−2.5	−2.9	Histone H2A
HTB1	−2.0	−2.4	Histone H2B
HTB2	−2.1	−2.5	Histone H2B
HHT1	−2.1	−2.2	Histone H3
HHT2	−2.2	−2.3	Histone H3
HHF1	−2.0	−2.3	Histone H4
HHF2	−2.3	−2.2	Histone H4
			Fatty Acid, Steroid and Amino Acid Metabolism
FAS1	−2.8	−3.6	Fatty acyl-CoA synthase
FAA3	−2.6	−2.3	Acyl-CoA synthase
FAA4	−1.8	−2.1	Long-chain fatty acid-CoA ligase and synthetase 4
GLN1	−4.4	−6.4	Glutamine synthetase
CPA2	−2.8	−2.2	Carbamoylphosphate synthetase
ILV5	−2.7	−3.1	Ketol-acid reductoisomerase
GLY1	−2.8	−3.5	Threonine aldolase
ASN1	−2.0	−2.0	Asparagine synthetase
HMG1	−2.5	−2.7	3-Hydroxy-3-methylglutaryl-coenzyme A reductase
ERG1	−2.2	−2.2	Squalene monoxygenase
ERG5	−2.3	−2.6	Cytochrome P450
GPD2	−1.8	−2.1	Glycerol-3-phosphate dehydrogenase (NAD^+)
			Cell Cycle Control and Cyclins
EGT2	−2.2	−3.1	Involved in correct timing of cell separation
CLN1	−2.4	−2.7	G1/S-specific cyclin
CLN3	−1.8	−2.1	G(sub)1 cyclin
CLB1	−2.8	−2.6	G2/M-phase-specific cyclin
			DNA-Damage Response
RNR1	−2.2	−2.5	Ribonucleotide reductase large subunit
			Glycolysis
PFK2	−2.1	−2.3	Phosphofructokinase
			Protein Synthesis
RPL3	−3.2	−3.0	Ribosomal protein L3
RPL15A	−2.2	−2.3	Ribosomal protein L15
RPL15B	−2.6	−2.7	Ribosomal protein L15

(*continued*)

Table 2. Continued

Gene Name	Fold Change SafA	Fold Change QAD	Protein Function
			Protein Synthesis
RPL4A	−2.4	−2.3	Ribosomal protein L4
RPL4B	−2.5	−2.4	Ribosomal protein L4
RPL12A	−1.9	−2.0	Ribosomal protein L12
DED1	−3.0	−3.4	ATP-dependent RNA helicase
TIF4631	−2.2	−2.3	mRNA cap-binding protein (eIF4F)
MSS116	−2.0	−1.7	Mitochondrial RNA helicase
			Cell Wall Maintenance
PIR1	−2.4	−3.2	Protein required for tolerance to heat shock
PIR3	−2.2	−3.5	Similar to Pir1
SCW11	−3.5	−3.8	Putative cell wall protein
CHS1	−2.0	−2.3	Chitin synthase 1
			Miscellaneous
BDF1	−2.4	−2.3	Protein required for sporulation
ALD5	−2.6	−2.3	Mitochondrial aldehyde dehydrogenase
ALD6	−2.9	−2.8	Cytosolic acetaldehyde dehydrogenase
FKS1	−2.6	−3	Component of beta-1,3-glucan synthase
YDL037C	−3.3	−5.1	Similarity to glucan 1,4-alpha-glucosidase
RPM2	−2.0	−2.0	Subunit of mitochondrial RNase P
YFR055W	−2.7	−2.2	Similarity to E. coli cystathionine beta-lyase
HPT1	−2.3	−2.2	Hypoxanthine Phosphoribosyltransferase
MCD4	−2.5	−2.4	Glycosylphosphatidylinositol anchor synthesis
GPI13	−1.7	−2.0	Glycosylphosphatidylinositol biosynthesis
ASH1	−2.3	−2.2	GATA-type transcription factor
CDC39	−2.2	−2.5	Negatively regulates basal transcription
NUP100	−2.2	−2.5	Nuclear pore complex protein
ROX1	−2.2	−2.0	Site-specific DNA binding protein, repressor
YNR053C	−2.0	−2.1	Export of 6OS ribosomal subunit from nucleus
SUR1	−2.2	−2.3	Mannosylated sphingolipids synthesis
RHR2	−2.2	−2.6	DL-glycerol-3-phosphatase
NOG1	−2.2	−2.2	Putative nucleolar GTP-binding protein
MNN1	−2.1	−2.1	Alpha-1,3-mannosyltransferase
MNN2	−2.1	−2.0	Probable type II membrane protein
HOS3	−1.9	−2.0	Member of the histone deacetylase family
VID27	−2.0	−1.8	Fructose-1,6-bisphosphatase import into Vid vesicles

Time points were taken after 60 min.

ism. In order to minimize potential temporal effects and maximize the probability that the profiling data would represent primary responses to drug treatment, we conducted incubations for only 1 hr time periods.

The results of whole-genome transcription-profiling experiments conducted with the *ERG6/PDR1/PDR3*-deletant (strain CCY333) reveal that extensive modification of the expression levels of large numbers of yeast genes occurs in response to treatment with SafA and QAD. By comparing the transcription profiles, we were able to establish that there was a high degree of similarity in the response of yeast to each agent (0.87 statistical correlation at 60 min). Differences in the degrees of overexpression or repression of genes between the profiles were few (Table 3), and almost no genes were overexpressed in one profile and repressed in the other (Figure 3B). Those genes that did display differing degrees of expression in profile comparisons point toward altered redox properties of SafA and QAD, which is not unexpected in light of their quinone/hydroquinone relationship, and may also reflect a modification of metal-ion transport in response to drug treatment. Variations in transcript levels of the great majority of genes relative to controls, however, were the same for both agents, suggesting that the primary effects of the two compounds are the same. This conclusion is strengthened by comparison of the expression profiles from SafA treatment with those obtained from the known DNA-modifying agents mitomycin C and MMS (S.E.S., unpublished data) as well as with profiles obtained from the DNA-damaging agent NCS [34] and the mechanistically unrelated natural product rapamycin [32]. In all cases, statistical correlations (ρ-values) fell below 0.35. The data may be taken to support the reasonable but unproven assertion that transcription-profiling experiments can be used as a tool for analyzing similarities or differences among cellular response pathways induced by different small molecules and, in a more general sense, for evaluating structure-activity relationships among bioactive compounds.

Analysis of genes that were overexpressed or repressed to similar degrees in SafA and QAD expression profiles (Tables 1 and 2) provides a wealth of information concerning the pathways that are influenced by drug treatment. The largest group of genes with modified transcript levels, both in terms of the number of repre-

Table 3. Genes Expressed to Different Degrees in SafA and QAD Profiles

Gene Name	Fold Change		Protein Function
	SafA	QAD	
			Metal Ion Uptake and Sequestering
CUP1	1.9	1.2	Metallothionein (copper chelatin)
FIT2	2.6	1.5	Possibly involved in iron uptake
FIT3	3.2	2.0	Possibly involved in iron uptake
CTR1	−3.2	−1.9	Required for high-affinity uptake of copper
			Oxidative Stress
AHP1	2.8	1.9	Alkyl hydroperoxide reductase
YBR101C	2.0	1.6	Involved in resistance to H_2O_2
UTH1	2.3	1.7	Involved in the aging process
			Additional Stress Response
DDR2	1.6	2.1	Induced by stresses such as DNA damage
GRE2	1.5	2.0	Induced by osmotic stress
FKB2	1.4	2.0	FKBP (FK506 binding protein), heat shock
SSA2	2.1	1.5	Member of 70 kDa heat shock protein family
SSA4	1.7	1.0	Chaperone of the HSP70 family
			Protein Synthesis
HYP2	2.1	1.4	Translation initiation factor elf-5A
TEF4	2.2	1.5	Translation elongation factor EF-1gamma
RPS25A	2.0	1.5	Ribosomal protein S25A
RPLA0	2.5	1.8	Acidic ribosomal protein A0
YKL056C	2.1	1.4	Possibly involved in cytoplasmic ribosome function
			Nonclassical Export
NCE103	1.5	2.7	Export that lacks classical secretory signal sequences
			Unknown Function
YKL136W	2.3	1.1	Unknown function
YHL005C	1.2	4.1	Unknown function
YJL182C	1.7	3.3	Unknown function
YJL217W	1.8	3.1	Unknown function
YDL124W	1.6	3.1	Unknown function

Time points were taken after 60 min.

sentatives and the magnitude of expression modification, were those involved in glycolysis. Glycolysis transforms glucose into two molecules of pyruvate and provides much of the energy for yeast growing in glucose-rich media [43]. Interestingly, although the majority of the genes of the glycolytic pathway were overexpressed, with the three isoforms of glyceraldehyde-3-phosphate dehydrogenase upregulated to the greatest degree, phosphofructokinase, the gene coding for the principal rate-limiting enzyme in glycolysis, was substantially downregulated (*PFK2*, 2.1- and 2.3-fold for SafA and QAD, respectively) [44]. This discontinuity in expression modification is intriguing and may signal the occurrence of a primary interaction between SafA (or QAD) with an enzyme or enzymes of the glycolytic pathway. It may also represent a secondary response, perhaps the diversion of glucose metabolism toward the pentose phosphate pathway, although ribulose-5-phosphate 3-epimerase (*RPE1*) was the only gene in this pathway that was overexpressed to a notable degree in both profiles (SafA and QAD, 2.0- and 1.8-fold, respectively, at 60 min). In addition to genes of the glycolytic pathway, three of the genes encoding pyruvate dehydrogenase, catalyzing the transformation of pyruvate into acetyl CoA, were overexpressed (*PDA1*, *PDA2*, and *PDB*; see Table 1). Transcript levels of genes of the tricarboxylic acid cycle and the glyoxylate cycle, pathways utilizing acetyl CoA, were not modified, however, and several genes directing the synthesis of fatty acids from acetyl CoA were repressed (*FAS1*, *FAA3, FAA4*, Table 2). Unlike treatment with hydrogen peroxide [45] or rapamycin [32], treatment with SafA and QAD does not appear to induce the diauxic shift [29] in the time period examined.

Both SafA and QAD induced a series of genes associated with protein degradation (Table 1). Expression of several genes encoding subunits of the proteasome (*PRE7*, *PRE9*, *RPN8*) was enhanced, as was the expression of genes encoding enzymes of the ubiquitin-mediated proteolytic pathway (*UBI4, UBC4*, Table 1) [46, 47]. The expression profiles also provided evidence of an increased role in treated cells of the process of autophagy, the nonspecific bulk flow transport pathway that delivers cytosolic material and even whole organelles to the vacuole for degradation [48, 49]. The gene *AUT7*, encoding a protein that attaches the vesicles known as autophagosomes to the microtubules for delivery to the vacuole [50], is greatly upregulated (3.7- and 3.5-fold for SafA and QAD, respectively). Members of the heat-shock protein family have also been shown to be in-

volved in the uptake of cytosolic proteins into the vacuole; expression of two of these genes (*SSE1* and *SSA2*) was enhanced (Tables 1 and 3) [51]. Genes encoding several different proteases involved in the degradation of proteins in the vacuole also exhibited enhanced expression levels (*LAP4*, *PEP4*, *CPS1*, and *PRC1*; Table 1). In addition, we observed increased expression of genes encoding vacuolar H^+-ATPases, required to maintain the acidity of the vacuole (*VMA3*, *VMA4*, and *VMA13*; Table 1) [52]. Although we see little evidence of transcriptional activation of the carbon starvation [29] and nitrogen discrimination pathways (glutamine synthetase, *GLN1*, is actually downregulated 4.3- and 6.4-fold in the SafA and QAD profiles, respectively, at 60 min) [53], more than 20 genes encoding proteins for nutrient transport were significantly repressed (Table 2). Among these are permeases for amino acids (*DIP5*, *BAP2*, *BAP3*, *GNP1*, *SCM2*, and *VAP1*), ammonia (*MEP3*), protons (*PMA1*, *PMA2*), purines (*FCY2*), water (*AQY2*), and inorganic phosphate (*PHO84*). It is possible that this global repression of transporter proteins may represent an attempt to prevent further import of a toxic drug substance. As a response to a reduced influx of nutrients, cellular contents may then be redistributed through the process of autophagy, which is often caused by nutrient starvation, as well as oxidative stress. The drug efflux proteins *SNQ1*, *SNQ2*, and *YPR156C,* other genes associated with small-molecule import and export, were repressed in both profiles (somewhat surprisingly), whereas overexpression of genes encoding the nonclassical export proteins *NCE102* and *NCE103* was enhanced significantly.

In addition to the functional categories described, several genes associated with oxidative-stress response were upregulated to similar degrees in profiles of both SafA and QAD (Table 1), and a few genes of this category were more strongly upregulated in the SafA profile (Table 3). SafA and QAD are nominally at different ends of a redox spectrum (SafA being more oxidizing), although this distinction may be obfuscated by the possible interconversion of quinone and hydroquinone forms within the cell by redox processes. Although overexpression of genes associated with oxidative stress was one of the most extensive transcriptional responses in terms of the number of genes that were upregulated, it was not the only form of stress response evident upon drug treatment. Many of the heat-shock proteins, as well as proline isomerases and *GRE3*, a gene induced by osmotic stress, were overexpressed [54, 55].

One of the more surprising findings from analysis of the profiling data, in light of prior work showing that SafA can reversibly alkylate guanine residues of duplex DNA in vitro, was the lack of transcriptional upregulation of any known DNA-damage repair proteins [56]. Although enhanced expression was observed for *RNR2* and *RNR4*, genes encoding components of ribonucleotide reductase (required for deoxyribonucleotide pool maintenance during DNA-damage repair and DNA replication), *RNR1*, the gene encoding the major subunit of ribonucleotide reductase, was significantly repressed (2.2- and 2.5-fold for SafA and QAD, respectively, at 60 min) [57]. Peaking in G1 to S phase, the expression of *RNR1* is more strongly regulated by the cell cycle than that of *RNR2* and *RNR4*, which suggests that drug treatment leads to a lower population of cells in this phase of the cell cycle with *RNR1* relative to controls [40]. In this regard, it is significant that genes encoding the eight histone proteins were also strongly repressed in both profiles. These genes have been shown to be tightly regulated by the cell cycle and to display high levels only during S phase; their mRNA abundance is very low during G1 and G2 phases [58]. These observations suggest that SafA and QAD may influence the cell cycle after S phase.

In view of fact that treatment of the mutant yeast strain with SafA and QAD led to rapid depletion of mRNA levels, it is noteworthy that the gene *HRP1*, encoding a protein responsible for mRNA processing, was upregulated in both profiles (2.4-fold), as was *SBP1*, a gene important in rRNA processing. It is not clear from the data at hand if mRNA depletion is a primary or secondary consequence of drug treatment, but it is clearly a significant component of the drug-induced cellular response.

Significance

Identification of an *S. cerevisiae* strain (CCY333) that is sensitive to treatment with SafA and QAD has allowed us to evaluate and compare the genetic responses to drug treatment by whole-genome transcription profiling (DNA microarray analysis). In this way we have identified many of the significant genetic response pathways (upregulation of genes involved in glycolysis, protein degradation pathways, and oxidative-stress response; downregulation of genes encoding transporter proteins, histones, proteins involved in fatty-acid synthesis, two RNA-processing enzymes, and *PFK2*, the principal rate-limiting enzyme in glycolysis). Perhaps of equal importance, we have found that known DNA-damage response pathways are not transcriptionally modified, suggesting that the primary mechanism of action of SafA (and QAD) may not involve known DNA-damage pathways. In addition to providing these insights into the effects of treatment of a simple eukaryotic organism with the natural product saframycin A (SafA), the profiling data have allowed us to conclude that activity-directed synthesis optimization produced an agent (QAD) that not only showed greater antiproliferative activity but also affected the same biochemical pathways as the lead structure, showing that transcriptional profiling experiments can provide a valuable tool in evaluating structure-activity relationships in a bioactive-compound series [26].

Experimental Procedures

Yeast Strains and Growth of Cultures

Saccharomyces cerevisiae strains BY4743 (diploid; BY4741/BY4742 [*MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*]) and CCY333 (*MATa ura3-1 leu2-3,11 his3-11 trp1-1 can1-100 ade2-1 bar1-1 erg6Δ::TRP1 pdr3Δ::HIS5 pdr1Δ::KAN* [isogenic with W303 strain background (W303-1a; R. Rothstein, Columbia University, New York)]) were used for these studies. For growth-inhibition and expression-profiling experiments, 5 ml of YPD medium (2% glucose/2% peptone/1% yeast extract) was inoculated with a single colony of the specified yeast strain. The cells were grown at 30°C on a shaker (275 rpm) for 24 hr, and the growth medium was diluted 100-fold in YPD medium to give two separate cultures, each with an absorbance (A_{600}) of 0.07.

Incubation continued until the absorbance (A_{600}) of the culture was 0.3. The cells were then treated with solutions of SafA or QAD in DMSO to give a final concentration of drug equal to 10 μM (the maximum concentration of DMSO in the total culture was 1% v:v). An equal volume of DMSO was added to the control cultures. Aliquots of the culture medium (40 ml) were harvested at the indicated times. Cells from each aliquot were pelleted by centrifugation (2500 × g) for 5 min at 30°C, then were flash-frozen in liquid nitrogen and stored at −80°C for later extraction of RNA. Each experiment with SafA or QAD was carried out in duplicate.

Growth Inhibition Studies

Exponentially growing yeast cultures were diluted with YPD medium (2% glucose/2% peptone/1% yeast extract) to give a solution with an absorbance (A_{660}) of 0.06. Aliquots (135 μl) of the diluted cell cultures were then dispensed into each well of flat-bottomed 96-well plates [37]. Solutions of SafA and QAD (50 mM DMSO) were diluted serially with YPD medium and DMSO to afford solutions of each drug varying incrementally over a range of concentrations (0.056–370 μM, total concentration of DMSO, 20% v:v). Wells containing yeast cultures were treated with 15 μl aliquots of the drug solutions; 15 μl aliquots of YPD medium (20% v:v DMSO) were added to the control wells. Each concentration value for each drug was represented in triplicate on the plate, and each experiment was conducted twice. Plates were incubated for 22 hr at 30°C. The absorbance (A_{660}) of each well was read with a SPECTRAmax PLUS 384 spectrophotometer (Molecular Devices, Palatine, IL). Control wells gave a final absorbance (A_{660}) of 0.8. Inhibition (%) was calculated according to the following formula: % = 100 × (C − T)/C, where T is the absorbance of the well containing the test compound minus the absorbance of a sample of YPD medium (containing 20% v:v DMSO) alone and C is the absorbance of the control well minus the absorbance of a sample of pure YPD medium (20% DMSO v:v). IC_{50} values were calculated by plotting percentage inhibition against log (concentration) and using the least-squares method.

DNA Microarrays

DNA microarrays were constructed as previously described [29, 32, 59].

RNA Extraction and Isolation of mRNA

Total RNA was extracted from flash-frozen pellets of cultured yeast cells by the acidic phenol method [60] and further purified by the use of an RNeasy Mini Kit (Qiagen, Chatsworth, CA). Poly-A RNA was isolated by the use of an oligo(dT) resin (Oligotex, Qiagen, Chatsworth, CA).

Preparation of Fluorescently Labeled cDNA and Hybridizations to DNA Microarrays

Fluorescently labeled cDNA probes were prepared from samples of total RNA (drug-treated or control) by the use of an oligo(dT)-primed reverse transcriptase (GIBCO BRL, Life Technologies, Rockville, MD). Reverse transcription reactions employed 5-(3′-aminoallyl)-dUTP (0.8 μl, 16 mM, Sigma Aldrich, St Louis, MO), oligo(dT′) (5 μg, GIBCO BRL, Life Technologies, Rockville, MD), and total RNA (16 μg) and were otherwise conducted essentially as described [29, 40]. Fluorescent dyes were subsequently incorporated into cDNA prepared from control and drug-treated yeast populations by coupling with Cy3-*N*-hydroxysuccinimide or Cy5-*N*-hydroxysuccinimide esters (Amersham Pharmacia Biotech, Piscataway, NJ), respectively. Competitive hybridizations were performed in duplicate for each drug at each time point.

Data Acquisition and Analysis

Fluorescently labeled DNA bound to the microarray was detected with a GenePix 4000A array scanner (Axon Instruments, Foster City, CA) with the GenePix 3.0 software package to locate individual spots, quantitate the Cy3- and Cy5-fluorescence intensity at each spot, and determine background signal intensities. Data from spots that were determined to be the result of hybridization anomalies or microarray errors were excluded from further analysis. Fluorescence intensity values were determined by subtraction of the local background from the foreground. Only those signal intensities greater than three standard deviations above the average background intensity (calculated over the entire array) were considered for further analysis in order to avoid errors due to low signal intensity. The ratio (total signal from all Cy3 channels)/(total signal from all Cy5 channels) was calculated to provide a scaling factor for normalization between the channels; this factor was then applied uniformly to each spot. Only genes with reproducible (≥2 hybridizations) expression differences of 1.7-fold or more were considered in our analysis. The Yeast Protein Database (YPD) [61] and the GeneSpring software package (Silicon Genetics, Redwood City, CA) were used for data analysis. Data sets from profiling experiments can be obtained at http://www.chem.harvard.edu/groups/myers/myers_research_group.htm.

Statistical Analysis

The correlation coefficient was calculated from the following formula:

$$\rho_{(X,Y)} = \frac{\mathrm{cov}(X, Y)}{\sigma_Y \cdot \sigma_X}$$

where,

$$\sigma_X^2 = \frac{1}{n} \Sigma (X_i - \mu_x)^2$$

$$\sigma_Y^2 = \frac{1}{n} \Sigma (Y_i - \mu_y)^2$$

The GeneSpring software package (Silicon Genetics, Redwood City, CA) was used for P value determination.

Acknowledgments

We thank Dr. Christina Cuomo (Harvard University) for kindly providing the yeast strain CCY333 (constructed in the laboratories of Professor Andrew Murray) and for helpful discussions. We are indebted to the staff of the Harvard University Center for Genomics Research for their technical expertise and advice. We would like to thank Dr. Bradley Bernstein (Harvard University) for critical reading of the manuscript. Financial support from the National Institutes of Health is gratefully acknowledged.

Received: December 21, 2001
Revised: February 19, 2002
Accepted: February 26, 2002

References

1. Arai, T., Takahashi, K., and Kubo, A. (1977). New antibiotics, saframycins A, B, C, D and E. J. Antibiot. *30*, 1015–1018.
2. Arai, T., Takahashi, K., Nakahara, S., and Kubo, A. (1980). The structure of a novel antitumor antibiotic, saframycin A. Experientia *36*, 1025–1027.
3. Arai, T., Takahashi, K., Ishiguro, K., and Mikami, Y. (1980). Some chemotherapeutic properties of two new antitumor antibiotics, saframycins A and C. Gann *71*, 790–796.
4. Developmental Therapeutics Program, National Cancer Institute, National Institutes of Health (2000). http://dtpsearch.ncifcrf.gov/htbin/nsc_search.exe?resform=Individually&searchnsc=325663.
5. Ishiguro, K., Sakiyama, S., Takahashi, K., and Arai, T. (1978). Mode of action of saframycin A, a novel heterocyclic quinone antibiotic. Inhibition of RNA synthesis in vivo and in vitro. Biochemistry *17*, 2545–2550.
6. Kishi, K., Yazawa, K., Takahashi, K., Mikami, Y., and Arai, T. (1984). Structure-activity relationships of saframycins. J. Antibiot. *37*, 847–852.
7. Ishiguro, K., Takahashi, K., Yazawa, K., Sakiyama, S., and Arai, T. (1981). Binding of saframycin A, a heterocyclic quinone antitumor antibiotic to DNA as revealed by the use of the antibiotic labeled with [^{14}C]tyrosine or [^{14}C]cyanide. J. Biol. Chem. *256*, 2162–2167.
8. Lown, J.W., Joshua, A.V., and Lee, J.S. (1982). Molecular mech-

anisms of binding and single- strand scission of deoxyribonucleic acid by the antitumor antibiotics saframycins A and C. Biochemistry *21*, 419–428.

9. Rao, K.E., and Lown, J.W. (1990). Mode of action of saframycin antitumor antibiotics: sequence selectivities in the covalent binding of saframycins A and S to deoxyribonucleic acid. Chem. Res. Toxicol. *3*, 262–267.
10. Rao, K.E., and Lown, J.W. (1992). DNA sequence selectivities in the covalent bonding of antibiotic saframycins Mx1, Mx3, A, and S deduced from MPE•Fe(II) footprinting and exonuclease III stop assays. Biochemistry *31*, 12076–12082.
11. Hill, G.C., and Remers, W.A. (1991). Computer simulation of the binding of saframycin A to d(GATGCATC)$_2$. J. Med. Chem. *34*, 1990–1998.
12. Rinehart, K.L., Holt, T.G., Fregeau, N.L., Keifer, P.A., Wilson, G.R., Perun, T.J., Jr., Sakai, R., Thompson, A.G., Stroh, J.G., Shield, L.S., et al. (1990). Bioactive compounds from aquatic and terrestrial sources. J. Nat. Prod. *53*, 771–792.
13. Sakai, R., Jares-Erijman, E.A., Manzanares, I., Silva Elipe, M.V., and Rinehart, K.L. (1996). Ecteinascidins: putative biosynthetic precursors and absolute stereochemistry. J. Am. Chem. Soc. *118*, 9017–9023.
14. Izbicka, E., Lawrence, R., Raymond, E., Eckhardt, G., Faircloth, G., Jimeno, J., Clark, G., and Von Hoff, D.D. (1998). In vitro antitumor activity of the novel marine agent, ecteinascidin-743 (Et-743, NSC-648766) against human tumors explanted from patients. Ann. Oncol. *9*, 981–987.
15. Valoti, G., Nicoletti, M.I., Pellegrino, A., Jimeno, J., Hendricks, H., D'Incalci, M., Faircloth, G., and Giavazzi, R. (1998). Ecteinascidin-743, a new marine natural product with potent antitumor activity on human ovarian carcinoma xenografts. Clin. Cancer Res. *4*, 1977–1983.
16. Delaloge, S., Yovine, A., Taamma, A., Riofrio, M., Brain, E., Raymond, E., Cottu, P., Goldwasser, F., Jimeno, J., Misset, J.L., et al. (2001). Ecteinascidin-743: a marine-derived compound in advanced, pretreated sarcoma patients—preliminary evidence of activity. J. Clin. Oncol. *19*, 1248–1255.
17. Ryan, D.P., Supko, J.G., Eder, J.P., Seiden, M.V., Demetri, G., Lynch, T.J., Fischman, A.J., Davis, J., Jimeno, J., and Clark, J.W. (2001). Phase I and pharmacokinetic study of ecteinascidin 743 administered as a 72-hour continuous intravenous infusion in patients with solid malignancies. Clin. Cancer Res. *7*, 231–242.
18. Pommier, Y., Kohlhagen, G., Bailly, C., Waring, M., Mazumder, A., and Kohn, K.W. (1996). DNA sequence- and structure-selective alkylation of guanine N2 in the DNA minor groove by ecteinascidin *743*, a potent antitumor compound from the Caribbean tunicate *Ecteinascidia turbinate*. Biochemistry *35*, 13303–13309.
19. Moore, B.M., II, Seaman, F.C., Wheelhouse, R.T., and Hurley, L.H. (1998). Mechanism for the catalytic activation of ecteinascidin 743 and its subsequent alkylation of guanine N2. J. Am. Chem. Soc. *120*, 2490–2491.
20. S. Jin., B. Gorfajn, G. Faircloth, K.W. Scotto (2000).Ecteinascidin *743*, a transcription-targeted chemotherapeutic that inhibits MDR1 activation. Proc. Natl. Acad. Sci. USA *97*, 6775–6779.
21. Minuzzo, M., Marchini, S., Broggini, M., Faircloth, G., D'Incalci, M., and Mantovani, R. (2000). Interference of transcriptional activation by the antineoplastic drug ecteinascidin *743*. Proc. Natl. Acad. Sci. USA *97*, 6780–6784.
22. García-Nieto, R., Manzanares, I., Cuevas, C., and Gago, F. (2000). Increased DNA binding specificity for antitumor ecteinascidin 743 through protein-DNA interactions? J. Med. Chem. *43*, 4367–4369.
23. Takebayashi, Y., Pourquier, P., Zimonjic, D.M., Nakayama, K., Emmert, S., Ueda, T., Urasaki, Y., Kanzaki, A., Akiyama, S.-I., Popescu, N., et al. (2001). Antiproliferative activity of ecteinascidin 743 is dependent upon transcription-coupled nucleotide excision-repair. Nat. Med. *7*, 961–966.
24. Zewail-Foote, M., Li, V.-S., Kohn, H., Bearss, D., Guzman, M., and Hurley, L.H. (2001). The inefficiency of incisions of ecteinascidin 743-DNA adducts by the UvrABC nuclease and the unique structural feature of the DNA adducts can be used to explain the repair-dependent toxicities of this antitumor agent. Chem. Biol. *8*, 1033–1049.
25. Martinez, E.J., Owa, T., Schreiber, S.L., and Corey, E.J. (1999). Phthalascidin, a synthetic antitumor agent with potency and mode of action comparable to ecteinascidin *743*. Proc. Natl. Acad. Sci. USA *96*, 3496–3501.
26. Martinez, E.J., Corey, E.J., and Owa, T. (2001). Antitumor activity- and gene expression-based profiling of ecteinascidin Et 743 and phthalascidin Pt *650*. Chem. Biol. *8*, 1151–1160.
27. Myers, A.G., and Kung, D.W. (1999). A concise, stereocontrolled synthesis of (–)-saframycin A by the directed condensation of α-amino aldehyde precursors. J. Am. Chem. Soc. *121*, 10828–10829.
28. Myers, A.G., and Plowright, A.T. (2001). Synthesis and evaluation of bishydroquinone derivatives of (–)-saframycin A: identification of a versatile molecular template imparting potent antiproliferative activity. J. Am. Chem. Soc. *123*, 5114–5115.
29. DeRisi, J.L., Iyer, V.R., and Brown, P.O. (1997). Exploring the metabolic and genetic control of gene expression on a genomic scale. Science *278*, 680–686.
30. Lashkari, D.A., DeRisi, J.L., McCusker, J.H., Namath, A.F., Gentile, C., Hwang, S.Y., Brown, P.O., and Davis, R.W. (1997). Yeast microarrays for genome wide parallel genetic and gene expression analysis. Proc. Natl. Acad. Sci. USA *94*, 13057–13062.
31. Wodicka, L., Dong, H., Mittman, M., Ho, M.-H., and Lockhart, D.J. (1997). Genome-wide expression monitoring in *Saccharomyces cerevisiae*. Nat. Biotechnol. *15*, 1359–1367.
32. Hardwick, J.S., Kuruvilla, F.G., Tong, J.K., Shamji, A.F., and Schreiber, S.L. (1999). Rapamycin-modulated transcription defines the subset of nutrient-sensitive signaling pathways directly controlled by the Tor proteins. Proc. Natl. Acad. Sci. USA *96*, 14866–14870.
33. Bernstein, B.E., Tong, J.K., and Schreiber, S.L. (2000). Genomewide studies of histone deacetylase function in yeast. Proc. Natl. Acad. Sci. USA *97*, 13708–13713.
34. Schaus, S.E., Cavalieri, D., and Myers, A.G. (2001). Gene transcription analysis of *S. cerevisiae* exposed to neocarzinostatin protein chromophore complex reveals evidence of DNA damage, a potential mechanism of resistance, and consequences of prolonged exposure. Proc. Natl. Acad. Sci. USA *98*, 11075–11080.
35. Marton, M.J., DeRisi, J.L., Bennett, H.A., Iyer, V.R., Meyer, M.R., Roberts, C.J., Stoughton, R., Burchard, J., Slade, D., Dai, H., et al. (1998). Drug target validation and identification of secondary drug target effects using DNA microarrays. Nat. Med. *4*, 1293–1301.
36. Bammert, G.F., and Fostel, J.M. (2000). Genome-wide expression patterns in *Saccharomyces cerevisiae*: comparison of drug treatments and genetic alterations affecting biosynthesis of ergosterol. Antimicrob. Agents Chemother. *44*, 1255–1265.
37. Simon, J.A., Szankasi, P., Nguyen, D.K., Ludlow, C., Dunstan, H.M., Roberts, C.J., Jenson, E.L., Hartwell, L.H., and Friend, S.H. (2000). Differential toxicities of anticancer agents among DNA repair and checkpoint mutants of *Saccharomyces cerevisiae*. Cancer Res. *60*, 328–333.
38. Welihinda, A.A., Beavis, A.D., and Trumbly, R.J. (1994). Mutations in LIS1 (ERG6) gene confer increased sodium and lithium uptake in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta *1193*, 107–117.
39. Balzi, E., and Goffeau, A. (1995). Yeast multidrug resistance: the PDR network. J. Bioenerg. Biomembr. *27*, 71–76.
40. Spellman, P.T., Sherlock, G., Zhang, M.Q., Iyer, V.R., Anders, K., Eisen, M.B., Brown, P.O., Botstein, D., and Futcher, B. (1998). Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hydridization. Mol. Biol. Cell *9*, 3273–3297.
41. Kessler, M.M., Henry, M.F., Shen, E., Zhao, J., Gross, S., Silver, P.A., and Moore, C.L. (1997). Hrp1, a sequence-specific RNA-binding protein that shuttles between the nucleus and the cytoplasm, is required for mRNA 3′-end formation in yeast. Genes Dev. *11*, 1703–1716.
42. Jong, A.Y., Clark, M.W., Gilbert, M., Oehm, A., and Campbell, J.L. (1987). *Saccharomyces cerevisiae* SSB1 protein and its rela-

tionship to nucleolar RNA-binding proteins. Mol. Cell. Biol. *7*, 2947–2955.
43. Goncalves, P., and Planta, R.J. (1998). Starting up yeast glycolysis. Trends Microbiol. *6*, 314–319.
44. Heinisch, J.J., Boles, E., and Timpel, C. (1996). A yeast phosphofructokinase insensitive to the allosteric activator fructose 2,6-bisphosphate. J. Biol. Chem. *271*, 15928–15933.
45. Godon, C., Lagniel, G., Lee, J., Buhler, J.-M., Kieffer, S., Perrot, M., Boucherie, H., Toledano, M.B., and Labarre, J. (1998). The H_2O_2 stimulon in *Saccharomyces cerevisiae*. J. Biol. Chem. *273*, 22480–22489.
46. Seufert, W., and Jentsch, S. (1992). In vivo function of the proteosome in the ubiquitin pathway. EMBO J. *11*, 3077–3080.
47. Hochstrasser, M. (1996). Ubiquitin-dependent protein degradation. Annu. Rev. Genet. *30*, 405–439.
48. Harding, T.M., Hefner-Gravink, A., Thumm, M., and Klionsky, D.J. (1996). Genetic and phenotypic overlap between autophagy and the cytoplasm to vacuole protein targeting pathway. J. Biol. Chem. *271*, 17621–17624.
49. Thumm, M. (2000). Structure and function of the yeast vacuole and its role in autophagy. Microsc. Res. Tech. *51*, 563–572.
50. Lang, T., Schaeffeler, E., Bernreuther, D., Bredschneider, M., Wolf, D.H., and Thumm, M. (1998). Aut2p and Aut7p, two novel microtubule-associated proteins are essential for delivery of autophagic vesicles to the vacuole. EMBO J. *17*, 3597–3607.
51. Satyanarayana, C., Schröder-Köhne, S., Craig, E.A., Schu, P.V., and Horst, M. (2000). Cytosolic Hsp70s are involved in the transport of aminopeptidase I from the cytoplasm into the vacuole. FEBS Lett. *470*, 232–238.
52. Kane, P.M., Tarsio, M., and Liu, J. (1999). Early steps in assembly of the yeast vacuolar H^+- ATPase. J. Biol. Chem. *274*, 17275–17283.
53. Magasanik, B. (1992). The Molecular and Cellular Biology of the Yeast *Saccharomyces*, Vol. 2 (Plainview, NY: Cold Spring Harbor Laboratory), 283–317.
54. Estruch, F. (2000). Stress-controlled transcription factors, stress-induced genes and stress tolerance in budding yeast. FEMS Microbiol. Rev. *24*, 469–486.
55. Causton, H.C., Ren, B., Koh, S.S., Harbison, C.T., Kanin, E., Jennings, E.G., Lee, T.I., True, H.L., Lander, E.S., and Young, R.A. (2001). Remodeling the yeast genome expression in response to environmental changes. Mol. Biol. Cell *12*, 323–337.
56. Brooks, N., McHugh, P.J., Lee, M., and Hartley, J.A. (2000). Alteration in the choice of DNA repair pathway with increasing sequence selective DNA alkylation in the minor groove. Chem. Biol. *7*, 659–668.
57. Nguyen, H.-H.T., Ge, J., Perlstein, D.L., and Stubbe, J.A. (1999). Purification of ribonucleotide reductase subunits Y1, Y2, Y3, and Y4 from yeast: Y4 plays a key role in diiron cluster assembly. Proc. Natl. Acad. Sci. USA *96*, 12339–12344.
58. Hereford, L.M., Osley, M.A., Ludwig, T.R., II, and McLaughlin, C.S. (1981). Cell-cycle regulation of yeast histone mRNA. Cell *24*, 367–375.
59. Eisen, M.B., and Brown, P.O. (1999). Methods Enzymol. *303*, 179–205.
60. Schmitt, M.E., Brown, T.A., and Trumpower, B.L. (1990). Nucleic Acids Res. *18*, 3091–3092.
61. Proteome. http://www.proteome.com.