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A DNA Microarray-Based Approach to Elucidate the Effects of the Immunosuppressant SR31747A on Gene Expression in *Saccharomyces cerevisiae*

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SR31747A is an immunosuppressive agent that arrests cell proliferation in the yeast *Saccharomyces cerevisiae.* In this microorganism, SR31747A was shown to inhibit the *ERG2* gene product, namely the Δ8-Δ7 sterol isomerase, involved in the ergosterol biosynthesis pathway. Although previous genetic experiments pointed to this enzyme as the target for SR31747A in yeast, the existence of other potential targets could not be ruled out. To enlighten this issue, we undertook a DNA microarray-based approach in which the expression profile of SR31747A-treated wild-type cells defining the "drug signature" was compared with the "mutant signature," the expression profile of the corresponding *ERG2*-deleted strain. We observed that treatment of *ERG2*-positive cells with SR31747A resulted in the modulation of mRNA levels of numerous genes. Among them, 121 were also affected in untreated *ERG2*-disrupted cells compared with wild-type cells. By contrast, drug exposure did not induce any significant transcriptional change in the *ERG2* null mutant. These results were consistent with SR31747A being an inhibitor of the sterol isomerase and demonstrated the absence of any additional SR31747A target. The detailed analysis of the observed 121 modulated genes provides new insights into the cellular response to ergosterol deprivation induced by SR31747A through inhibition of the *ERG2* gene product.

SR31747 DNA chips Antiproliferative Sterol isomerase Ergosterol pathway

SR31747A is a selective ligand of the peripheral sigma binding sites exhibiting immunosuppressive and antitumoral properties. Immunomodulation by SR31747A was first demonstrated by the inhibition of mitogen-induced T-cell proliferation (2,5). SR31747A was also shown to modulate the proinflammatory and anti-inflammatory cytokine responses (2,12). In addition, this drug displays potent antiproliferative effects on a variety of tumor cell lines, suggesting potentialities in cancer therapy (15,27). To date two high-affinity SR31747A binding proteins have been identified in human cells: the SR31747A binding protein 1 (SRBP1), also known as sigma 1 receptor (21,24), and the human sterol isomerase (HIS). SRBP1 is structurally and pharmacologically related to the fungal C8-C7 sterol isomerase encoded by the *ERG2* gene in yeast (18,21). HSI, originally described as the emopamil binding protein (EBP), shares no structural similarities with SRBP1 or ERG2 (18,42). SRBP1 and HSI colocalize at the endoplasmic reticulum and

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at the outer and inner membranes of the nuclear envelope (15).

The yeast *Saccharomyces cerevisiae* has been used as a model to study the mechanism of the antiproliferative effect of SR31747A (42). In yeast, SR31747A blocks cell proliferation by inhibiting the sterol biosynthesis pathway, chiefly at the sterol isomerase step (41). Such inhibition has also been shown to occur in mammalian cells (27). Although previous genetic studies clearly identified sterol isomerase as the target of SR31747A in yeast, the existence of additional targets could not be ruled out. To further examine this question, we used a global gene expression approach to analyze transcriptomic changes induced by SR31747A. DNA microarrays that contain essentially every open reading frame (ORF) in the *Saccharomyces cerevisiae* genome have been used previously to explore the transcriptional modulations accompanying large changes in cellular metabolism, biological pathways, or cell cycle progression (8,22,28,33, 38,51). Recently, Bammert et al. (1) used a microarray approach to examine the effects of altering ergosterol biosynthesis in *Saccharomyces cerevisiae*. Their data showed a good correlation between the effects obtained by sterol biosynthesis inhibitors and by deletion of the genes encoding the corresponding target proteins, suggesting that this approach could be used to predict the mode of action of new compounds. The use of expression profile as a comprehensive molecular phenotype has also been recently exemplified by Hughes et al. (19), who produced a reference database of expression profiles corresponding to 300 mutations or chemical treatments in the yeast *Saccharomyces cerevisiae*.

The aim of our report was to validate the ERG2 gene product as the primary target of SR31747A mediating the drug-induced proliferation arrest. In parallel, we addressed the issue of whether the effect of SR31747A was sterol isomerase selective or could affect additional pathways. DNA microarrays were used to compare changes in gene expression in drug-treated and untreated wild-type cells as well as in *ERG2*-disrupted cells.

MATERIALS AND METHODS

Strains, Media, Growth Conditions, and SR31747A Treatment

EMY27 (*MATα, ura3, trp1, leu2 fen1::LEU2*), EMY47 (*MATα, ura3, trp1, leu2 fen1::LEU2 erg2::TRP1*), and EMY27 pFL39 were isogenic derivatives of *Saccharomyces cerevisiae* FL100 (ATCC 28383). EMY27 pFL39 was obtained by transforming EMY27 with pFL39, a centromeric plasmid carrying the *TRP1* selection marker. *FEN1 (GCN1/ELO2)* null alleles allow *ERG2* gene-disrupted cells to proliferate in the absence of ergosterol (41,42). The *FEN1* gene was disrupted by deletion-insertion of *LEU2* and the *ERG2* gene by deletion-insertion of *TRP1* in the strain EMY47, as described in (41,42). EMY27 strain was cultured in rich medium (RM) (YPD medium) whereas EMY47 and EMY27 pFL39 were cultured in minimal medium (MM) (synthetic minimal, SD) containing 2% glucose and adequately supplemented with uracil (17). Cultures were grown aerobically at 30°C. Cell growth was monitored by measuring the optical density at 600 nm (OD_{600nm}). An OD unit of 1 corresponds to 3×10^7 cells/ml. Cells were grown to a density corresponding to an OD_{600nm} of 0.9–1.1. SR31747A was prepared as a 10-mM solution in ethanol and added to culture to a final concentration of 2 μM. Control cultures were supplemented with ethanol.

Sterol Analysis

An aliquot from each liquid culture used for RNA extraction was analyzed for sterol content using gas chromatography-mass spectrometry (GC-MS). Sterols were extracted from lyophilized cells in the presence of a constant amount of cholesterol (25 μg/50 mg dry weight) in the indicated samples as previously described (32). Briefly, saponification was performed at 95°C in the presence of KOH, pyrogallol, and methanol. The extraction was performed with heptane as a solvent and desiccated on a Na_2SO_4 column. Samples were analyzed under UV and the percentage of ergosterol was calculated as follows: $OD_{280nm} \times$ heptane volume (ml) $\times$ 396/11500/(dry weight in mg). Sterols were analyzed by GC-MS on a VG ZAB-2E instrument linked to a Hewlett Packard chromatograph in electron impact mode (EI). The sterols were separated by GC on a 50-m CPSil 5 CB column. They were quantified by the area method in comparison with cholesterol added as internal standard (25 μg/50 mg dry weight) (32). Experimental mass spectra of the different peaks were automatically compared with reference spectra included in the NIST and NISTREP spectral banks.

Total RNA Extraction and poly(A)$^+$ RNA Preparation

Cells were broken by standard procedures with the following modifications. Yeast cell pellets were resuspended in breaking buffer (50 mM Tris-Cl, pH 8, 100 mM NaCl, 10 mM EDTA, 5% SDS) and vortexed for 5 min on a multitube vortexer at maximum setting in the presence of 30% glass beads (425–600 μm mesh; Sigma) and 30% phenol/chlorophorm/iso-

amylalcohol (25:25:1, v/v/v). After centrifugation the aqueous phase was reextracted and ethanol precipitated. The nucleic acid pellets were resuspended in guanidine isothiocyanate lysis buffer. Total RNA was extracted by cesium chloride ultracentrifugation. The poly(A)$^+$ RNA was prepared using the FastTrack 2.0 Kit (Invitrogen, Leek, Netherlands). The absence of mRNA degradation was assessed by visualization of residual ribosomal 28S and 18S mRNAs on a denaturing agarose gel.

Complex Probe Preparation and Hybridization to Affymetrix Oligonucleotide Chips

Oligonucleotide arrays (subA, subB, subC, and subD chips of the Ye6100 Set™ from Affymetrix, Santa Clara, CA) allowing the characterization of genome-wide expression levels of approximately 6100 yeast genes were used. Double-stranded cDNA was prepared from 4 μg poly(A)$^+$ RNA using Life Technologies superscript choice system and an oligo(dT)$_{24}$ anchored T7 primer. Biotinylated RNA was synthesized using the T7 megascript system (Ambion, Inc., Texas, USA) with biotin-11-CTP and biotin-16-UTP for 4 h at 37°C. In vitro transcription products (IVT) were purified using microspin S200 HR columns (Amersham-Pharmacia LKB, Orsay, France). Labeled RNA concentration was determined from the OD at 260 nm. Biotinylated RNA was then treated for 35 min at 94°C in a buffer composed of 40 mM Tris acetate, pH 8.1, 100 mM potassium acetate, 30 mM magnesium acetate to produce fragmented labeled RNA of 50–200 bases in length. Affymetrix Ye6100 Set™ array were hybridized with biotinylated IVT products (10 μg/chip) for 16 h at 45°C using the manufacturer's hybridization buffer containing internal standard RNAs. The latter were bacterial- and phage-labeled RNAs prepared as described above from clones containing the T7 RNA polymerase promoter site, and introduced into each sample at a known amount. Each RNA sample was hybridized onto duplicate DNA arrays. After hybridization, the fluidic station 400 from Affymetrix was used for washing and staining the arrays. They were washed with 6× SSPE, 0.01% Tween 20, 0.005% Antifoam (Sigma) at 25°C, and then with 100 mM MES, 0.1 M NaCl, and 0.01% Tween 20 at 50°C. Staining was performed twice for 10 min at 25°C with a 10 μg/ml streptavidin-phycoerythrin solution (Molecular Probes, Eugene, OR, USA) in a 100 mM MES, 1 M NaCl, 0.05% Tween 20, and 0.005% antifoam buffer. A subsequent step using an anti-streptavidin goat biotinylated antibody (Vector Laboratories, Burlingame, CA, USA) at a 3 μg/ml concentration, in the presence of 0.1 mg/ml of normal goat IgG (Sigma), was performed at 25°C for 10 min, followed by another staining with streptavidin-phycoerythrin. The antibody staining protocol was used to amplify the hybridization signals. The DNA chips were then scanned using a specific scanner (Hewlett Packard). The excitation source was an argon ion laser and emission was detected by a photomultiplier tube through a 570-nm-long pass filter. Digitized image data were then processed for gene expression analysis.

Data Analysis

Data analysis was performed by running absolute analyses on each array and comparative analyses between two arrays using the Genechip Software™ package (Affymetrix Inc). Absolute analyses provide for each gene an estimate of its expression intensity as well as a parameter, the "absolute call," defining whether the gene can be considered as expressed ("P": present, "M": marginal) or not ("A": absent). Comparative analyses determine modulation of expression between the two samples as the "difference call" parameter, set to "I" (increased or "MI": marginal increased) for upregulation and to "D" (decreased or "MD": marginal decreased) for downregulation. Only modulations of expression reproduced in comparative analyses of duplicate arrays were considered significant.

The comparative analyses performed by the GeneChip software also provide an estimate of the expression intensity ratio between the two samples being compared. It is called the "fold-change" (FC) and is calculated as the ratio between the higher and the lower intensities of expression. In the case of a decrease in expression relative to the control sample, the fold-change is set to the opposite of the calculated ratio (and therefore given as a negative value). Expression intensity ratios were calculated as equal to the fold-change for positive fold-change values, and as the opposite inverse of the fold-change for negative fold-changes values. Average fold-change values between duplicates were calculated from the average of the logarithm of the intensity ratios.

Expression intensities measured on all arrays were multiplied by a normalization factor in a way that the 75th percentile of the intensity distribution of all genes measured on each array was equal to 200.

Cluster analysis was performed after selecting genes whose expression was significantly affected in the different conditions studied. Only genes that met the following criteria in at least one of the comparisons were retained. Modulation of expression (as determined by the "difference call") had to be reproduced in duplicate comparative analyses, and the fold-change (average value from duplicate analyses)

had to be superior to 1.5 or inferior to −1.5. In addition, only genes whose normalized expression intensity was at least 100 in one sample were considered. Cluster analysis was performed on the base 2 logarithm of the expression intensity ratios. Hierarchical clustering was obtained by calculating similarity as the Euclidian distance and grouping following the average linkage algorithm (UPGMA) using the GenExplore software (Applied Maths Inc.).

Northern Blot Experiments

Northern blot containing poly(A)$^+$ RNA extracted from WT (EMY27 or EMY27 pFL39) or mutant (EMY47) yeast cells cultured in the presence or absence of 2 μM SR31747A for 8 and 16 h (EMY27) in rich medium, 16 (EMY27 pFL39) and 18 h (EMY47) in minimal medium, were prehybridized for 1 h at 65°C in hybridization solution (Church buffer: BSA 1%, SDS 7%, NaH_2PO_4 0.5 M, EDTA 1 mM). They were then hybridized overnight at 65°C using radiolabeled *ERG2* or *YSR3* probe (4.10^5 cpm/μl) in hybridization solution containing 100 μg/ml salmon sperm DNA. Blots were washed twice with WBA (BSA 0.5%, SDS 5%, NaH_2PO_4 40 mM, EDTA 1 mM) for 5 min at 65°C, and once with WBB (SDS 1%, NaH_2PO_4 40 mM, EDTA 1 mM) for 10 min at 65°C. Finally blots were autoradiographed using Kodak X Ray film for 24 h at −70°C. The *ERG2* (Accession number YMR202W) and *YSR3* (Accession number YKR053C) probes were prepared by PCR from the corresponding cDNA and radiolabeled with [α-^{32}P]dCTP with RTS Radprime labeling Kit (Gibco, Life Technologies) according to the manufacturer's instructions.

The signal intensities were quantified using the STORM 860 (ImageQuant 5.0, Molecular Dynamics) and represented as a percentage of the highest signal intensity observed.

RESULTS

Validation of the Experimental Procedures

Analysis of the Cellular Sterol Content Under Different Culture Conditions. We first determined the culture conditions under which the sterol biosynthesis pathway was impaired by SR31747A. As proliferation is arrested by SR31747A treatment or by the *ERG2* gene deletion in *Saccharomyces cerevisiae* cells, we used *fen1* mutants, which do not require ergosterol for growth (41). Treatment with the drug was performed at a final concentration of 2 μM as this concentration has been shown sufficient for inhibiting cell growth (41). The drug impact was analyzed under three different conditions: culture in minimal medium during 16 h for EMY27 pFL39 WT strain and in rich medium during 8 and 16 h for EMY27 WT strain; the strain carrying the *ERG2* gene deletion, EMY47, was grown in minimal medium for 18 h. These incubation times were sufficient for all the cultures to reach mid-logarithmic phase ($OD_{600nm} = 0.9–1.1$). The sterol content of the cells was analyzed after culture in each experimental condition. Sterols were extracted, quantified under UV, and analyzed by GC-MS (Fig. 1, Table 1). As expected, Δ8-sterols were accumulated at the expense of ergosterol in SR31747A-treated cells as in the *ERG2* null mutant. In minimal medium, SR31747A did not completely eliminate cellular ergosterol in the wild-type strain, although inhibition of synthesis was clear. In rich medium inhibition was already almost complete after an 8-h treatment and total after a 16-h treatment (Table 1). Unusual sterols were detected instead of ergosterol (Fig. 1). The different peaks were identified as follows: peak 1: ergosta-5,7,22-trien-3β-ol (ergosterol); peak 2: ergosta-5,8,22-trien-3βol; peak 3: ergost-8 (14)-en-3βol; peak 4: ergosta-8-14-dien-3β-ol (ignosterol); peak 5: cholesta-8,24dien-3βol (zymosterol); peak 6: lanosterol.

Evaluation of the DNA Chips Procedure. SR31747A-induced changes in gene expression were monitored using the GeneChip methodology developed by Affymetrix. By this approach, we compared the RNA expression patterns in yeast cells grown in the presence or in the absence of SR31747A. As reproducibility is one of the predominant criteria used to validate DNA chip analyses, we examined the reproducibility of our experiments by hybridizing the same RNA complex probes to two independent sets of arrays. As shown in Figure 2, expression ratios obtained in duplicate comparisons for modulated genes were found to be highly reproducible (correlation coefficient $r = 0.94$). All the data reported here and in the subsequent analyses were obtained from duplicate experiments.

Comparison of the Drug Signature and the Mutant Signature

As SR31747A was shown to inhibit sterol isomerase, one may expect the changes in gene expression induced by SR31747A (drug signature) to be similar to those resulting from *ERG2* deletion (mutant signature). The drug signature was examined in EMY27 pFL39 cells grown in minimal medium for 16 h and in EMY27 cells grown in rich medium for 8 and 16 h in the presence or absence of SR31747A. The mutant signature corresponded to the difference in gene expression between *ERG2* gene-deleted EMY47 cells

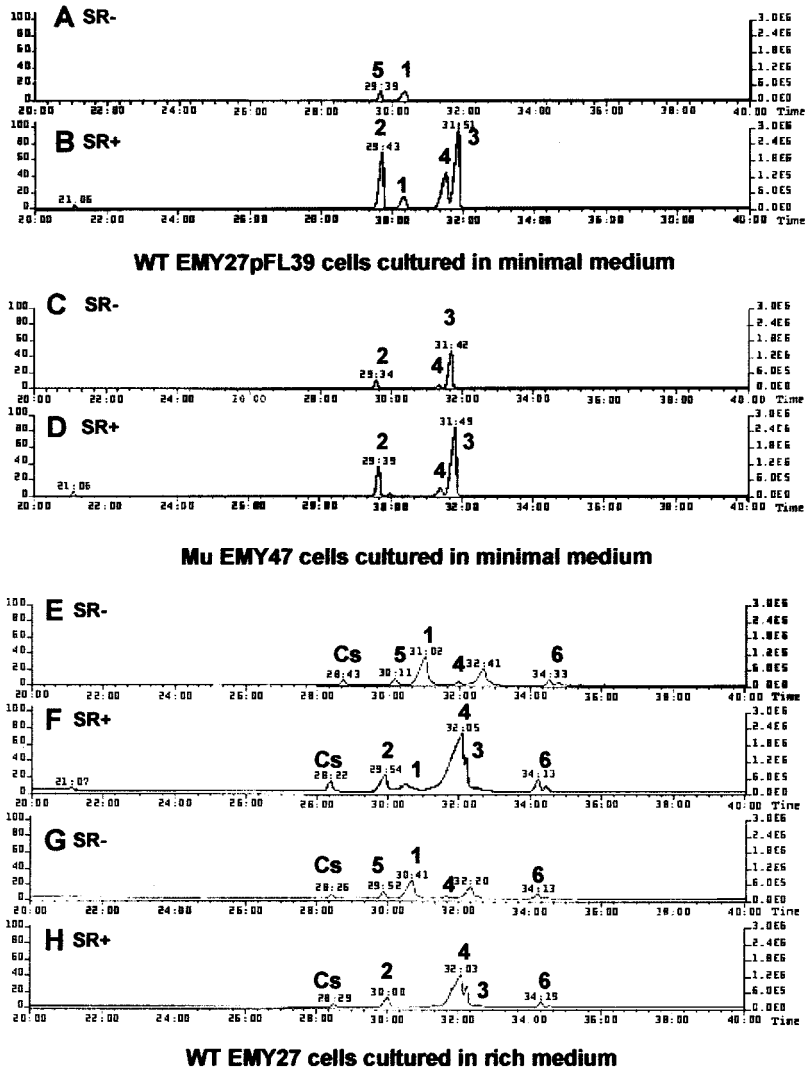

Figure 1. GC-MS analysis of membrane sterols extracted from wild-type EMY27 or EMY27 pFL39 and mutant EMY47 yeast strain. Comparative GC-MS profiles of membrane sterols extracted from the cells cultured in the absence (A, C, E, G) or in the presence (B, D, F, H) of 2 μM SR31747A for 16 h (A, B) or 18 h (C, D) in minimal medium (MM) or for 8 h (E, F) or 16 h (G, H) in rich medium (RM). Cholesterol (Cs) added as internal standard (25 μg/50 mg dry weight) in samples (E, F, G, H). Peaks in each assay were recognized by their mass spectra as (1) ergosta-5,7,22-trien-3β-ol (ergosterol); (2) ergosta-5,8,22-trien-3β-ol; (3) ergost-8(14)-en-3β-ol; (4) ergosta-8-14-dien-3β-ol (ignosterol); (5) cholesta-8,24-dien3β-ol (zymosterol); (6) lanosterol.

(18-h culture) and wild-type EMY27 pFL39 cells (16-h culture) grown in minimal medium.

Treatment with SR31747A affected more genes when wild-type cells were grown in rich medium than in minimal medium (Table 2). This could reflect the fact that ergosterol was still present in treated cells grown in minimal medium, whereas it was totally absent in cells treated for 16 h in rich medium (Table 1). Many of the genes that were modulated by the drug were also modulated by the *ERG2* gene deletion. However, the converse was not true: many genes whose expression was affected by the *ERG2* gene deletion were not modulated by SR31747A in *ERG2*-positive cells. Table 2 presents the 121 genes whose expression was modulated, in duplicate comparisons, by SR31747A at least in one experimental condition and by the mutation. The genes are classified on the basis of their known function or predicted structure. The majority of these genes were modulated in the same way in the mutant and in the SR31747A-treated sample and most of them were upregulated; only a few genes were downregulated in both signatures. Fold-changes (FC) are expressed as the average values obtained from duplicate comparisons. In most cases, modulations ranged between 1.6- and 4-fold. The FC value for a given gene was usually higher in the mutant than in the drug signature.

As expected, several genes modulated upon ergos-

TABLE 1
QUANTIFICATION OF ERGOSTEROL IN EMY27, EMY27 pFL39, AND EMY47 YEAST STRAIN IN CONTROL CULTURES OR FOLLOWING TREATMENT WITH 2μM SR31747A

Samples	Weight (dry, mg)	OD 280 nm	Ergosterol Content (mg)	% Ergosterol
ERG2 WT yeast cells				
EMY27 pFL39				
Untreated: 16 h, MM	28.5	1.773	0.3	1.07
SR treated: 16 h, MM	25.2	0.298	0.05	0.2
EMY 27				
Untreated: 8 h, RM	50.4	2.022	0.35	0.69
SR treated: 8 h, RM	50.6	0.225	0.04	0.08
Untreated: 16 h, RM	50.5	2.243	0.39	0.76
SR treated: 16 h, RM	50.6	0	0	0
ERG2 mutant yeast cells				
EMY47				
Untreated: 18 h, MM	17.9	0.058	0.01	0.06
SR treated: 18 h, MM	19.6	0	0	0

EMY27 were cultured in rich medium (RM) for 8 or 16 h. EMY27 pFL39 and EMY47 were cultured in minimal medium (MM) for 16 and 18 h, respectively. The percentage of ergosterol was calculated as follows (OD_{280nm} × vol heptane × 396/11500/mg dry weight).

terol starvation encoded proteins involved in the ergosterol biosynthetic pathway (13 genes) or more broadly in phospholipid and sphingolipid metabolism (6 genes) (Table 2, Fig. 3). The 13 genes belonging to the ergosterol pathway showed an increase in expression ranging between 1.6- and 4.5-fold. Among them, the *ERG2* gene was stimulated by a factor of 1.8 or 2.7 upon SR31747A treatment for 8 or 16 h, respectively, in rich medium, which is in agreement with previous reports (1,45). The *ERG2* gene modulation was confirmed by Northern blotting experiments that evidenced an increase in *ERG2* gene expression very similar to that measured on DNA chips (Fig. 4A). Among the genes involved in lipid metabolism, *YSR3* was found to have elevated FC (ranging from +6 to +18) under the conditions tested: this gene encodes a phosphosphingoid-base phosphatase that is thought to play a key role in sphingolipid metabolism and possibly in stress response. The induction of *YSR3* upon SR31747A treatment was also confirmed by Northern blot analysis (Fig. 4B). The range of *YSR3* modulations observed in Northern Blot was smaller than that obtained from DNA chips data, probably reflecting the general squeezing of signal intensities in Northern blot experiments or during scanning for the evaluation of the differences in signal intensities.

In addition, we have identified other families of genes having properties and roles already known to be related to sterol effects: membrane fluidity and trafficking [cell wall maintenance (11 genes), membrane transport (7 genes), protein degradation and vesicle trafficking (4 genes)], stress response (11

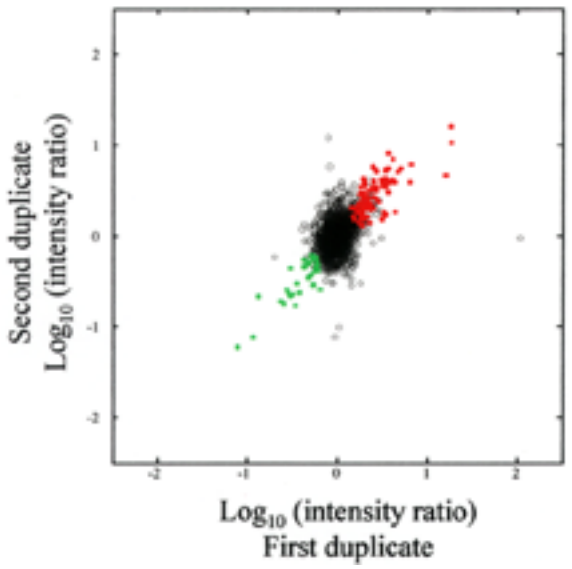

Figure 2. Reproducibility of the fold-change parameter defining the quantitative variations in gene expression profiles. EMY27 cells were cultured in rich medium for 16 h. The expression profile of cells treated with 2 μM SR31747 was compared with the expression profile of untreated cells. The scatter plot compares the $\log_{10}$ (intensity ratio) in duplicate comparisons for all genes for which the calculated normalized expression intensity was superior to 100 and the absolute call was "P" (Present) or "M" (Marginal) in at least one of the two conditions in each comparison (2575 genes). Genes showing reproducible modulation are depicted in red (stimulation) and green (inhibition). The correlation coefficient between values obtained in both comparisons for modulated genes is 0.94.

TABLE 2
CLASSES OF DIFFERENTIALLY EXPRESSED GENES

Gene (Acc. Number)	Function	Fold-Change: ERG2⁻ Cells (18 h, MM)	Fold-Change: WT Cells SR31747A (16 h, MM)	Fold-Change: WT Cells SR31747A (8 h, RM)	Fold-Change: WT Cells SR31747A (16 h, RM)
Ergosterol biosynthesis					
ERG8 (YMR220W)	Phosphomevalonate kinase	+3.6			+2.1
ERG9 (YHR190W)	Squalene synthase (farnesyl-diphosphate farnesyl-transferase)	+1.7	+1.6	+1.6	+2.3
NCP1 (YHR042W)	NADP-cytochrome P450 reductase, possibly involved in ERG11 reaction	+2.4			+1.6
CYB5 (YNL111C)	Cytochrome B5, possibly involved in ERG11 reaction, involved in fatty acid desaturation	+2.7		+2.1	+2.6
ERG24 (YNL280C)	Sterol C-14 reductase	+2.4			+1.9
ERG25 (YGR060W)	Sterol C-4 methyloxidase	+2.0	+1.6	+1.9	+2.2
ERG26 (YGL001C)	Sterol C-3 dehydrogenase	+2.0		+2.4	+2.7
ERG6 (YML008C)	Sterol C-24 methyltransferase	+2.9		+1.9	+1.8
***ERG2* (YMR202W)**	**Sterol C8-C7 isomerase**	**−82.9**		**+1.8**	**+2.7**
ERG3 (YLR05056W)	Sterol C-5 desaturase	+1.6	+1.6		
ERG5 (YMR015C)	Sterol C-22 desaturase	+3.0		+3.3	+2.3
ERG4 (YGL012W)	Sterol C-24 (28) reductase	+2.0		+2.2	+2.4
ERG28 (YER044C)	Unknown [Hughes et al. (19)]	+2.2	+2.0	+3.5	+4.5
Lipid metabolish—sterol related					
YSR3 (YKR053C)	Sphingoid base-phosphate phosphatase, a putative regulator of sphingolipid metabolism and stress response	+8.2	+6.0	+18.4	+17
YDR213W (YDR213W)	Transcription factor that inhibits uptake and intracellular esterification of sterol	+7.7			+2.2
HES1 (YOR237W)	Protein implicated in ergosterol biosynthesis	+6.7		+8.3	
INO1 (YJL153C)	Inositol-1-phosphate synthase, functions in the inositol biosynthesis pathway	+6.6	−2.4		
ATF2 (YGR177C)	Sterol *O*-acetyltransferase, acetylates certain toxic steroids	+3.1		+2.5	
PLB1 (YMR008C)	Phospholipate B, preferentially deacylates phosphatidyl-choline and phosphatidyl-ethanolamine	+1.7		+1.6	
Energy generation					
NCA3 (YJL116C)	Protein involved in regulation of synthesis of ATP6 and ATP8 (subunits 6 and 8 of the ATP synthase)	+5.4		−5.5	
HAP1 (YLR256W)	Transcription factor with heme-dependent DNA binding activity, responsible for the heme-dependent activation of many genes, essential for anaerobic growth	+2.3		+3.0	+2.3
YHR039C (YHR039C)	Protein with strong similarity to aldehyde dehydrogenase	+2.0		+1.9	
YPL088W (YPL088W)	Putative aryl alcohol dehydrogenase, may participate in late steps of degradation of aromatic compounds that arise from the degradation of lignocellulose	+1.6	+1.7		
DIC1 (YLR348C)	Mitochondrial dicarboxylate transport protein, member of the mitochondrial carrier family	−3.8		+1.9	+1.7
Membrane transport					
SIT1 (YEL065W)	Ferrioxamine B permease, member of the yeast-specific multidrug resistance (MFS-MDR) family of the major facilitator superfamily (MFS)	+5.2			+4.3
PMC1 (YGL006W)	Vacuolar Ca^{2+}-transporting P-type ATPase, member of the cation transporting (E1-E2) P-type ATPase superfamily, functions to pump Ca^{2+} into the vacuole	+3.4			+2.2

TABLE 2
(*CONTINUED*)

Gene (Acc. Number)	Function	Fold-Change: ERG2⁻ Cells (18 h, MM)	WT Cells SR31747A (16 h, MM)	WT Cells SR31747A (8 h, RM)	WT Cells SR31747A (16 h, RM)
YDL025C (YDL025C)	Ser/Thr protein kinase with similarity to members of the NPR1 subfamily that regulates transport systems for nitrogen source nutrients	+2.7		+2.1	+3.0
DAL5 (YJR152W)	Allantoate and ureidosuccinate permease, member of the allantoate family of the major facilitator superfamily, nitrogen utilization gene	+2.5		+2.0	
POM152 (YMR129W)	Nuclear pore membrane glycoprotein	+2.3		+1.6	
FTR1 (YER145C)	Iron permease that mediates high-affinity iron uptake	+2.2			+2.9
ZRT2 (YLR130C)	Low-affinity zinc transport protein, member of the ZIP family of metal ion transporters	−1.6		+2.2	
Stress response/ detoxification					
HSP12 (YFL014W)	Heat shock protein induced by heat, osmotic stress, oxidative stress and in stationary protein	+16.4	+2.0	−2.5	+2.8
YDR453C (YDR453C)	Protein with high similarity to TSA1 (thioredoxin peroxidase, thiol-specific antioxidant protein)	+6.5	+2.0	−2.5	+2.8
GTT1 (YIR038C)	Glutathione transferase	+5.4			+2.5
TSL1 (YML100W)	Component of the trehalose-6-phosphate synthase/phosphatase complex	+5.2		+1.7	
HOR2 (YER062C)	DL-glycerol phosphate phosphatase	+4.5			+2.1
HSP42 (YDR171W)	Heat shock protein involved in the restoration of the cytoskeleton	+2.9			+1.9
SGE1 (YPR198W)	Crystal violet resistance protein, member of the multi-drug-resistance 14-spanner family of the major facilitator superfamily (MFS-MDR)	+2.6		+2.9	
RTA1 (YGR213C)	Protein involved in 7-aminocholesterol resistance	+2.6		+3.6	+5.2
CUP2 (YGL166W)	Copper-dependent transcription factor inducing copper resistance genes (CUP1A, CUP1B, CRS5, and SOPD1)	+2.0			+1.8
DDR48 (YMR173W)	Protein induced by heat shock, DNA damage or osmotic stress	+1.8			+2.4
ZEO1 (YOL109W)	Protein that leads to resistance to zeocin when overproduced	−1.7		−1.6	−2.5
Amino acid metabolism					
GCV2 (YMR189W)	Glycine decarboxylase pyridoxal phosphate containing P subunit	+2.3		+2.2	+2.3
ARG3 (YJL088W)	Ornithine carbamyltransferase, arginine biosynthesis pathway	−2.5		+7.9	+6.3
YFR055W (YFR055W)	Protein with similarity to *E. coli* cystathionine α lyase	−1.9			
Protein degradation— vesicle trafficking					
SRO77 (YBL106C)	Docking protein that functions together with SEC9 in exocytosis downstream of the RHO3 GTPase (positive regulator of exocytosis)	+3.9		+2.4	+2.3
YPS3 (YLR121C)	GPI-anchored aspartyl protease	+2.8	+2.7		+1.9
YHR138C (YHR138C)	Protein involved in vacuolar fusion	+2.4			+2.1
APG5(YPL149W)	Protein involved in autophagy and nutrient starvation	+1.9		+2.2	+1.7

TABLE 2
(*CONTINUED*)

Gene (Acc. Number)	Function	Fold-Change: ERG2⁻ Cells (18 h, MM)	Fold-Change: WT Cells SR31747A (16 h, MM)	Fold-Change: WT Cells SR31747A (8 h, RM)	Fold-Change: WT Cells SR31747A (16 h, RM)
Carbohydrate metabolism					
MTH1 (YDR277C)	Transcription factor that represses hexose transport genes	+11.9		−3.5	−3.2
AMS1 (YGL156W)	α-mannosidase	+4.2		+2.2	+2.8
HXK1 (YFR053C)	Hexokinase I	+3.5		−1.9	−1.9
HXT4 (YHR092C)	Moderate to low-affinity hexose transporter	+2.7		−10.1	−5.7
ARA1 (YBR149W)	Subunit of $NADP^+$-dependent D-arabinase dehydrogenase	+2.6		+2.2	
HXT1 (YHR094C)	Low-affinity hexose transporter	+2.3			+2
HXT7 (YDR342C)	High-affinity hexose transporter	+1.8		−1.6	
HXT6 (YDR343C)	High-affinity hexose transporter	+1.6		−1.4	
Cell cycle control					
SCM4 (YGR049W)	Protein that suppresses temperature-sensitive allele of CDC4 when overexpressed	+4.4	+2.1	+2.3	+1.7
PCL1 (YNL289W)	G_1/S-specific cyclin that can interact with the CDC28-like kinase PHO85 (repressed by a factor)	+2.0			+3.0
TFS1 (YLR178C)	CDC25-dependent nutrient- and ammonia-response cell cycle regulator, similar to lipid binding proteins of higher eucaryotes	+2.0			+1.7
Mating response					
CMK2 (YOL016C)	Ca^{2+}/calmodulin-dependent ser/thr protein kinase type II, promotes survival of pheromon-induced growth arrest	+2.2		+2.0	+3.8
MFALPHA2 (YGL089C)	Mating pheromone α2 factor, arrests cell division	−52.2	−2.7	−3.7	−4.6
SST2 (YLR452C)	Protein involved in desensitization to α-factor pheromone/negative regulator of GPA1 that is a protein subunit of pheromone response pathway	−7.1	−1.9		
ALPHA1 (YCR040C)	Transcription factor that activates α-specific genes	−5.9			−1.9
KAR4 (YCL055W)	Transcription factor required for pheromone induction of karyogamy genes	−3.5			−2.1
A2 (YCR096C)	Regulatory protein	−2.3			−1.7
SAG1 (YJR004C)	α-Agglutinin involved in cell–cell interactions during mating	−1.9	−1.6	−5.5	−3.7
RNA processing					
STP4 (YDL048C)	Transcription factor with strong similarity to STP1 that is involved in tRNA splicing and branched-chain amino acid uptake	+3.2		−3.0	−2.0
TAD3 (YLR316C)	Subunit of tRNA-specific adenosine-34 deaminase	−2.7		+1.4	
SEN2 (YLR105C)	tRNA splicing endonuclease, α subunit	−2.0		+2.3	
PRP19 (YLL036C)	Non-snRNP spliceosome component, pre-mRNA splicing	−1.7		+1.9	
Cell wall maintenance—members of the PAU family					
DAN1 (YJR150C)	Cell wall mannoprotein induced during anaerobic growth, similar to members of the PAU family (seripauperin)	+47.9	+26.4	+4.2	+2
YIL011W (YIL011W)	Protein with similarity to the PAU1 family	+6.3	+1.8	+2.1	+1.5
GSC2 (YGR032W)	Component of α1,3-gulcan synthase, essential for sporulation	+6.3			+1.8
YJR151C (YJR151C)	Member of the PAU family	+4.8		+22.5	+8.6
PIR3 (YKL163W)	Cell wall protein	+2.6	+2.2	−1.9	

TABLE 2
(*CONTINUED*)

Gene (Acc. Number)	Function	Fold-Change: ERG2⁻ Cells (18 h, MM)	WT Cells SR31747A (16 h, MM)	WT Cells SR31747A (8 h, RM)	WT Cells SR31747A (16 h, RM)
PAU5 (YFL020C)	Member of the PAU family	+2.5			+2.0
YOR009W (YOR009W)	Member of the PAU family	+2			+1.7
CIS3 (YJL158C)	Cell wall protein with similarity to members of the PIR1/HSP150/PIR3 family	+1.8		−2.8	
TIP1 (YBR067C)	Cold- and heat shock-induced mannoprotein of the cell wall, member of the PAU family	−2.3	−2.1	−4.8	−10.5
ECM7 (YLR443W)	Protein possibly involved in cell wall structure or biosynthesis	−2.2		+1.9	
EXG1 (YLR300W)	Exo α-1,3-glucanase, sporulation specific	−1.8		−1.8	
Protein synthesis and modification					
IRS4 (YKR019C)	Protein involved in silencing of ribosomal DNA	+3.4			+1.9
MRPL36 (YBR122C)	Mitochondrial ribosomal protein of the large subunit	+2.4			−1.8
RPS10B (YMR230W)	Ribosomal protein S10	+1.9	+1.5		
GCD7 (YLR291C)	Translation initiation factor elF2B	−1.9		+1.7	
ANB1 (YJR047C)	Translation initiation factor elF5A, hypoxic gene repressed by heme	−1.9			−1.9
NMT1 (YLR195C)	*N*-myristoyltransferase, catalyzes transfer of myristate to certain nascent proteins such as GPA1, proteins in the secretory pathway, INO2, and INO4	−1.7		+1.9	
Chromatin structure—subtelomerically encoded proteins					
YLL067C (YLL067C)	Protein with possible helicase activity	+3.3		+3.3	+2.0
YPR202C (YPR202C)	Protein with similarity to other subtelomerically encoded proteins	+3.0	+2.9		
YML133C (YML133C)	Protein with similarity to other subtelomerically encoded proteins	+2.6			+2.4
YLR464W (YLR464W)	Protein with similarity to other subtelomerically encoded proteins	+2.5		+1.9	+2.0
YPR204W (YPR204W)	DNA, helicase, subtelomerically encoded	+2.4	+2.1		
Unknown—unclassified					
YPL272C (YPL272C)	Protein of unknown function	+20.4	+6.5	+24.9	+14.0
YGR131W (YGR131W)	Protein of unknown function	+5.6		+3.4	
YJL213W (YJL213W)	Protein with weak similarity to Nocardia aryldialkylphosphatase	+4.9		+3.1	+2.2
YMR316W (YMR316W)	Protein of unknown function	+4.0	+2.4	+2.6	+3.7
YIR007W (YIR007W)	Protein with similarity to endoglucanase	+3.6			+2.2
YAR068W (YAR068W)	Protein with similarity to ICWP protein	+3.5	+2.0	+2.2	+2.3
YOR220W (YOR220W)	Protein of unknown function	+3.3		+2.4	+3.8
SRL3 (YKR091W)	Protein of unknown function	+3.3	+3.3		
YHR214W-A (YHR214W6A)	Protein of unknown function	+3.2	+2.0	+1.7	+2.3
YMR087W (YMR087W)	Protein of unknown function	+3.1		+1.9	
YPL170W (YPL170W)	Protein of unknown function	+2.8	+2.0	+2.0	
YNL278W (YNL278W)	Protein of unknown function	+2.8		+1.9	+2.6
YLL058W (YLL058W)	Protein with similarity to *Neurospora crassa* *O*-succinyl-homoserine (thiol)-lyase	+2.7	+2.7	+2.9	+2.5
YGR161C (YGR161C)	Protein of unknown function	+2.7		+1.9	
BOP1 (YPL221W)	Protein of unknown function	+2.4	+1.8		
YBR005W (YBR005W)	Protein of unknown function	+2.1	+1.7	3.0	+4.1
YLR194C (YLR194C)	Protein of unknown function	+2.0	+2.9	+1.7	+3.6
YGR035W (YGR035W)	Protein of unknown function	+1.9			−1.8
YLL012W (YLL012W)	Protein with similarity to human triacylglycerol lipase	+1.8	+1.7	+2.1	

TABLE 2
(*CONTINUED*)

Gene (Acc. Number)	Function	Fold-Change ERG2⁻ Cells (18 h, MM)	WT Cells SR31747A (16 h, MM)	WT Cells SR31747A (8 h, RM)	WT Cells SR31747A (16 h, RM)
YLR068W (YLR068W)	Protein of unknown function	–4.9		+1.4	
YLR154C (YLR154C)	Protein of unknown function	–2.2		+1.4	
YOR203W (YOR203W)	Protein of unknown function	–2.1		+1.6	+2.8
YLR019W (YLR019W)	Protein of unknown function	–2.1		+1.6	
Others					
TY4B (YJL113W)	Ty4 transposon B protein	+3.1	+3.1	+5.5	+6.7
RIM101 (YHL027W)	Transcription factor involved in induction of IME1, IME2, DIT, and DIT2 transcription (sporulation-specific genes, meiosis)	+2.3			+1.9
SOK2 (YMR016C)	Transcription factor that plays a general regulatory role in the PKA signal transduction pathway (growth and cell cycle progression)	+1.9		+1.6	
ADE17 (YMR120C)	5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase/IMP cyclohydrolase (de novo purine biosynthesis pathway)	+1.7		+1.6	+2.1
TY2A (YCL020W)	Ty2 transposon B protein	–2.7			–1.8

Gene expression levels measured in either *erg2* disruptant (ERG2⁻ cells) or wild-type SR31747A-treated cells (WT cells SR31747A) were compared with wild-type untreated cells. SR31747A treatment (2 μM) was performed for the indicated time period as described in Materials and Methods. Modulated genes were classified according to their known function and their fold-change is indicated. Increased and decreased gene expressions are shown by + and –, respectively. MM, minimal medium; RM, rich medium.

genes), cell cycle control (3 genes), energy source utilization [carbohydrate metabolism (8 genes) and energy generation (5 genes)]. We also highlighted other functionally related gene families that encode proteins involved in: chromatin structure (5 genes), mating response (7 genes), RNA processing (4 genes), protein synthesis (6 genes), and amino acid metabolism (3 genes). A few modulated genes could not be classified (5 genes) and a number of other genes have not yet been functionally characterized (23 genes). In

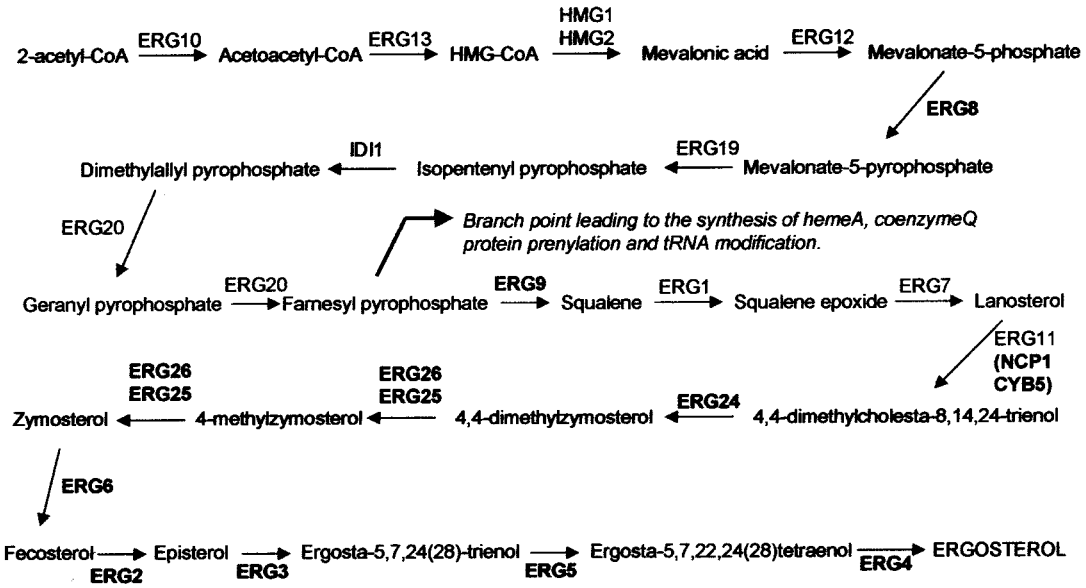

Figure 3. The ergosterol biosynthesis pathway from acetyl-CoA to ergosterol in the yeast *Saccharomyces cerevisiae*. The gene names encoding the various enzymatic steps are shown. Genes modulated upon SR31747A treatment or whose expression is affected by *ERG2* deletion are indicated in bold letters. Only the well-characterized genes are indicated.

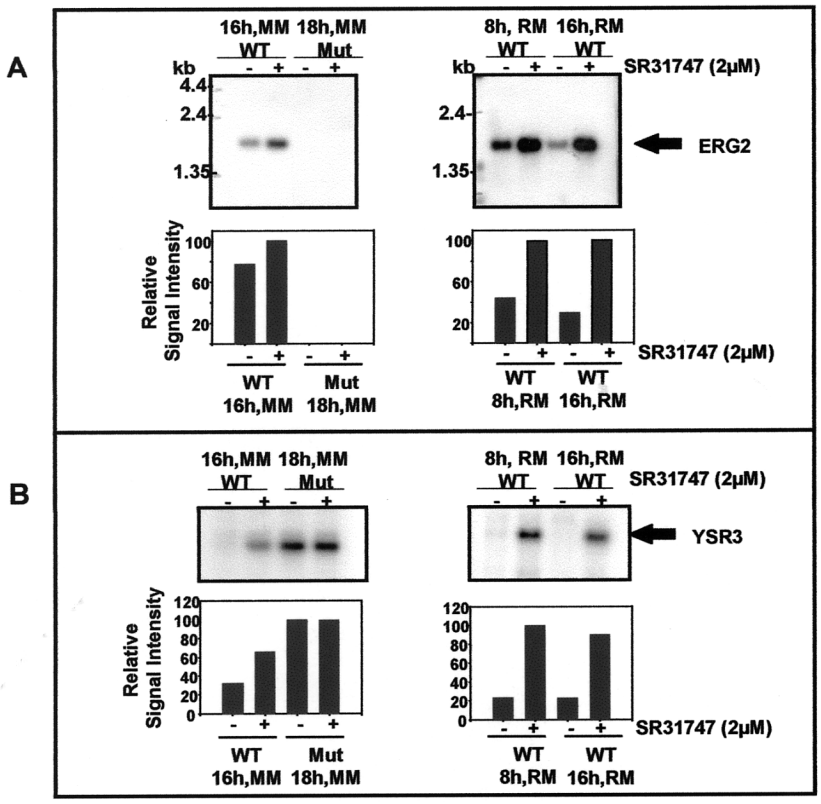

Figure 4. Northern Blot analysis of *ERG2* (A) and *YSR3* (B) mRNA expression in WT (EMY27 or EMY27 pFL39) or mutant (EMY47) yeast cells cultured in the presence or absence of SR31747A (2 µM) for the indicated time periods. Signal intensities were measured and relative expression is represented as a percentage of the maximal intensity observed. RM, rich medium; MM, minimal medium.

particular, the transcript level of the uncharacterized YPL272C gene is strongly increased in all conditions studied, exhibiting fold-changes ranging from 6.5 to 24.9.

Specificity of the Response to SR31747A

To investigate the specificity of SR31747A for the ergosterol biosynthesis pathway, we examined whether the modulation of expression by SR31747A treatment was lost in *ERG2* mutant cells compared with that observed in wild-type cells.

We analyzed the expression pattern of wild-type cells (EMY27 pFL39 cells grown in minimal medium, EMY27 cells grown in rich medium) treated with SR31747A for 16 h compared with untreated cells (i.e., the drug signature of the WT cells). In parallel, the drug signature of the *ERG2*-disrupted EMY47 cells was determined after 18 h of SR31747A treatment in minimal medium compared with untreated mutant cells.

Cluster analysis was performed on the average expression ratios between the treated and untreated samples calculated in duplicate comparisons by the GeneChip software. As shown in Figure 5, SR31747A had virtually no effect in the *ERG2* mutant in contrast to what was observed in ERG2-positive cells. No modulated gene was found in common between the drug signature in the mutant and in the wild-type cells on the basis of our reproducibility criteria. These results are consistent with the sterol isomerase being the only target of SR31747A.

DISCUSSION

The analysis of global gene expression we performed in the yeast *Saccharomyces cerevisiae* model allowed us to identify genes that were modulated upon SR31747A treatment and to better understand the antiproliferative effect elicited by SR31747A.

Most importantly, the transcriptomic analysis of the *ERG2* mutant grown in the presence and in the absence of the drug shows that SR31747A is not effective at modulating any gene in the absence of sterol isomerase in that model. This strongly suggests that sterol isomerase is the single primary target of SR31747A.

In addition, we observed that sterol isomerase targeting by SR31747A elicited multiple biological ef-

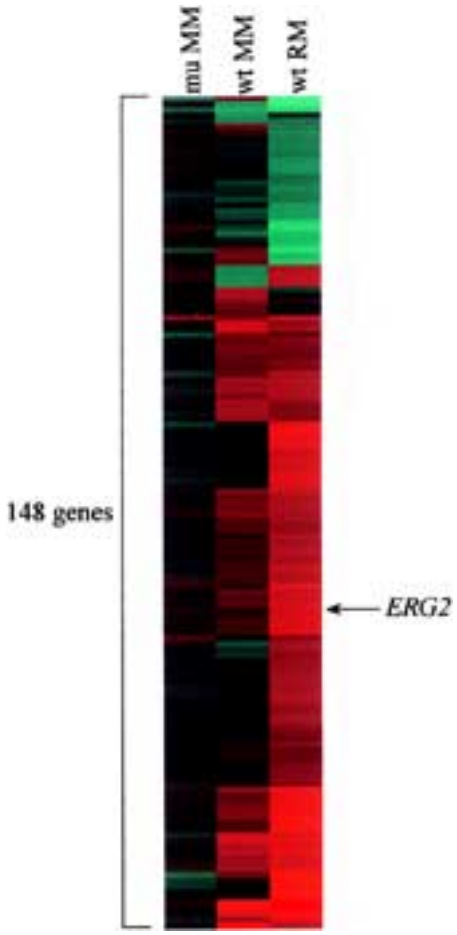

Figure 5. Hierarchical clustering analysis of the effect on gene expression exerted by SR31747A in wild-type (wt) and ERG2 mutant (mu) cells. To evaluate the wild-type drug signature, EMY27 pFL39 cells were grown with or without SR31747A for 16 h in minimal medium (MM), and EMY27 cells were grown with or without SR31747A for 16 h in rich medium (RM). Drug signature of the ERG2 mutant EMY47 cells was determined after 18 h of treatment with SR31747A in minimal medium compared with untreated mutant cells. Red indicates stimulation, green indicates inhibition, and black indicates no change in expression. Red and green colors are saturated for eightfold modulation.

fects that are evidenced by numerous changes in gene expression. The treatment with SR31747A performed in rich medium was found to modulate more genes than treatment in minimal medium, which likely reflects the fact that there is residual ergosterol in minimal medium (Fig. 1, Table 1). Comparison of the drug signature with the *ERG2* mutant signature showed that there were 121 modulated genes in common. However, modulated genes in the mutant are much more abundant than drug-affected genes. This discrepancy may result from the fact that the genetic deletion is a stable, drastic situation where there is no sterol isomerase expressed at all. By contrast, drug treatment only temporarily blocks the enzyme and one cannot exclude some residual protein expression, especially because the *ERG2* gene expression is upregulated upon SR31747A treatment. In this context, higher concentrations of SR31747A or longer duration of treatment would probably better reproduce the mutant phenotype. Signature of two unrelated deletion mutants used as controls in the same procedure displayed completely different sets of modulated genes (data not shown), indicating the specificity of the signature observed for the *ERG2*-disrupted strain.

Definition of the Drug Signature

The substantial overlap between the drug and the mutant signatures proves that the yeast *ERG2*-encoded sterol isomerase is the primary target of the drug SR31747A and is definitely required to generate the drug signature. The functional classification of the 121 identified genes provides new insights into the mechanism by which SR31747A acts through the inhibition of the ERG2 gene product.

Responsive Genes Encoding Products Acting in the Ergosterol Pathway. Among the genes modulated both in the drug and the mutant signature 13 genes, all induced (except in the mutant where ERG2 is absent) encode enzymes belonging to the ergosterol biosynthesis pathway. In yeast, ergosterol is the major sterol product of the isoprenoid pathway and isoprenoid intermediates play a role in a vast array of cellular processes, including structural composition of the lipid bilayer, electron transport during respiration, cell cycle completion, protein and tRNA modification. In addition, the sterol biosynthetic pathway is the major target for antifungal compounds. Yeast cells are indeed dependent on ergosterol biosynthesis to proliferate aerobically, because they are unable to take up exogenous ergosterol in the presence of oxygen (42).

It was recently shown that *ERG2* expression is regulated at the transcriptional level by ergosterol starvation (45). In agreement with this observation, we evidenced an increase by 1.8- to 2.7-fold in *ERG2* expression following SR31747A treatment. *ERG8*, whose expression was found to be induced by a factor of 2.1 upon SR31747A treatment, encodes an enzyme acting much earlier in the isoprenoid biosynthesis pathway, prior to the split into several branches leading to synthesis of sterols, hemeA, coenzymeQ, protein prenylation, or tRNA modification (14). The first enzyme in the linear branch leading to ergosterol, ERG9, called squalene synthase, is a crucial enzyme known to be subject to regulation (25); indeed, we observed an induction by SR31747A with a FC of 1.6 to 2.3. All other ERG-related proteins

that were found to be upregulated act late in the pathway, just upstream (NCP1, CYB5, ERG24, ERG25, ERG26, and ERG6) or downstream (ERG3, ERG4, and ERG5) of ERG2, showing FC values ranging from +1.6 to +4.5. These modulations reflect the dynamic regulation of the pathway in response to accumulation or depletion of intermediates induced by *ERG2* targeting.

Through the inhibition of ERG2, SR31747A treatment affects other cellular functions. We will focus on lipid and oxidative metabolism, energy source utilization, and cell cycle.

Responsive Genes Encoding Products Playing a Role in Lipid Metabolism. Six genes related to lipid metabolism were modulated in *ERG2* mutant cells as well as in SR31747A-treated cells. Three of them suggest an interaction between ergosterol, sphingolipid, and phospholipid metabolism. *YSR3/LBP2* is one of the genes displaying the strongest upregulation in all conditions studied and encodes a sphingoid base-phosphate phosphatase (31). Phospholipase B (*PLB1*), whose expression was increased in the mutant and in one treated condition, deacylates phosphatidylcholine and phosphatidylethanolamine, the two main phospholipids (29). Inositol-1-phosphate synthase (*INO1*), involved in the synthesis and regulation of inositol, is a transcriptional regulator of phospholipid formation (26); it had increased expression in the mutant but decreased expression in one SR-treated condition. The modulation of expression of the two latter genes may indicate compensatory regulatory mechanisms leading to increased phospholipid and inositol content in response to limited sterols as a result of the ergosterol pathway perturbation.

The three other modulated genes related to lipid metabolism were all induced upon treatment and *ERG2* deletion. The *HES1* product, homologous to the human oxysterol binding protein (OSBP), is likely to be involved in ergosterol biosynthesis. The product of the *YDR213W* gene is a transcription factor inhibiting uptake and intracellular esterification of sterol. Its induction likely results from the lack of available ergosterol to be esterified (10). Finally, the protein encoded by the *ATF2* gene is a sterol *O*-acetyltransferase, which acetylates some toxic steroids to render them excretable (6). Its induction may be a response to an overproduction of toxic aberrant sterols formed during inhibition of the ergosterol pathway.

Responsive Genes Encoding Products Acting in Oxidative Metabolism and Energy Generation. The transcriptional regulation (stimulation in most cases) of genes involved in oxidative metabolism (*YHR039C, YPL088W*) and energy generation (*NCA3, DIC1*) (36) was also observed in response to inhibition of the ergosterol pathway. In addition, levels of transcripts from two permeases mediating iron uptake, encoded by the *SIT1* and *FTR1* genes (30,46), were increased in mutant and in SR-treated cells ("membrane transport" group). Iron being the metal present in heme and cytochomes, the induction of those genes can be related to the increase of oxidative metabolism and energy production. In this context it is interesting to note that the *NCP1* gene, also observed to be induced ("ergosterol biosynthesis" group), encodes a NADPH-dependent ferrireductase known to lead to an accumulation of iron (30).

In the course of normal catalysis, reactive oxygen species may form through reduction of molecular oxygen along the mitochondrial respiratory chain. In accordance with the observed upregulation of genes whose products increase oxidative metabolism, four genes involved in oxidative stress response were also transcriptionally stimulated both in the mutant and the SR-treated cells: *GTT1*, encoding the glutathione transferase, the main intracellular antioxidant (7); *HSP12*, encoding the heat shock protein 12; *YDR453C*, encoding a protein similar to TSA1, the thioredoxin peroxidase; *CUP2*, encoding an inducer of the SOD1 superoxide dismutase (4).

Furthermore, of particular interest is our observation that the HAP1 gene, encoding a transcriptional activator with heme-dependent activity, was upregulated; this suggests elevated intracellular heme levels following *ERG2* disruption and SR treatment. Together with heme, HAP1 exerts a coordinated control of both mitochondrial and nonmitochondrial hemoproteins (like catalase T, cytochrome C1, b2, etc.) (50). Heme is known to play a central role in sterol synthesis and to regulate the transcription of several genes involved in this process (52); it is required for the enzymatic activities of ERG3 and ERG5 (34); in addition, the *ERG11* gene, encoding the lanosterol C-14 demethylase, was described as transcriptionally regulated by HAP1 (11). Heme plays also a role in sensing intracellular oxygen levels. Thus, the perturbation of ergosterol levels within the cell may result in an increase of intracellular oxygen levels as this perturbation leads to the modulation of genes indicative of an increased oxidative stress.

Responsive Genes Encoding Products Playing a Role in Energy Source Utilization. Membrane sterol modification in yeast has also been shown to affect energy source utilization. Such effect was also evidenced here. In our study we identified five modulated genes whose products are related to nitrogen source nutrients utilization. Two genes are related to nutrient transport: the *DAL5* gene, encoding the allan-

toate and ureidosuccinate permease (37), and the *YDL025* gene, encoding a serine/threonine kinase similar to the NPR1 family of proteins that regulate transport systems for nitrogen source nutrients ("membrane transport" group), (47); the ornithine carbamyltransferase *ARG3* gene product (9), acting in the arginine biosynthesis pathway, arginine being a source of nitrogen ("amino acid metabolism" group); the *APG5* gene ("protein degradation" group), involved in autophagy and nutrient starvation; and the *TFS1* gene ("cell cycle control" group), coding for a nutrient and ammonia response cell cycle regulator (3). The expression of these five genes was increased in mutant as well as in SR-treated cells, excepted for *ARG3*, which showed opposite modulation in the mutant and SR-treated cells. Thus, a block in ERG2 function appears to lead to a state of poor or limiting nitrogen sources and to a need of increasing nitrogen source utilization.

In parallel, we observed the modulation of genes involved in the control of the glycolytic flux, known to be induced during nitrogen starvation (49). The modulated genes include those for four hexose transporters that function as glucose sensors, allowing uptake of sugar as substrate for glycolysis (*HXT1, HXT4, HXT6, HXT7*); the *MTH1* gene, coding for a transcription factor that represses hexose transport genes (40); the *HXK1* gene, encoding hexokinaseI, a key glycolytic enzyme. It is intriguing to note that, except *HXT1*, these genes were upregulated in *ERG2*-disrupted cells, whereas they were downregulated in SR31747A-treated cultures. Mutant cells, as an adaptation to the permanent deletion of *ERG2* gene, appear to need to reprogram their metabolism in order to increase the availability of sugars as nutrient source. On the contrary, SR-treated cells, where ERG2 function is temporarily inhibited, appear to reduce sugar source and glycolytic flux. We cannot rule out the possibility that these opposite gene modulations in mutant compared with SR-treated cells resulted from the different culture media used.

In addition, it is interesting to note that we also observed an increase in the expression of sporulation-specific genes, sporulation being a process occurring during nutritional starvation: *RIM101* ("others" group), which encodes a transcription factor involved in the induction of various sporulation-specific genes (23); *GSC2* encoding the beta-1,3-glucan synthase (20); and *EXG1* encoding the exo-beta-1,3-glucanase (48) (both are in "cell wall maintenance").

Responsive Genes Encoding Products Playing a Role in the Cell Cycle At nanomolar concentrations, ergosterol has been suggested to function as a signal known as the "sparking effect" required to exit the G_1 phase of the cell cycle (44). Recent reports have indicated that the sparking function may be related to the presence or absence of heme; ergosterol has been suggested to act in conjunction with heme as an additional intracellular signal indicating the presence of oxygen. In our study, three induced genes were involved in cell cycle control (*TFS1, SCM4*, and *PCL1*). Proteins encoded by *SCM4* and *PCL1* are required at the G_1/S boundary (16,43).

Other Responsive Genes. Several modulated genes were involved in the mating response (7 genes) and were generally inhibited. This repression may originate indirectly from the inhibition of isoprenoid biosynthesis, because two components of the mating pathway are subject to protein prenylation (a-factor and the gamma subunit of the heterotrimeric G protein STE18 (39). Furthermore, levels of transcripts from three of the four genes listed in the "RNA processing" group are involved in tRNA processing. This is intriguing because the process of tRNA modification is directly connected (through the MOD5 enzyme) to the isoprenoid biosynthetic pathway (see Fig. 3) (13).

As a summary, we identified the modulation of a set of genes that may be characteristic of an "ergosterol response" following inhibition of the ERG2 gene product. Some genes that are modulated upon treatment encoded products acting in the ergosterol biosynthetic pathway or in lipid metabolism. In addition, we observed gene expression changes that were suggestive of perturbations in energy generation, energy source utilization, heme biosynthesis, and increased intracellular oxygen tension. The other responsive genes that have been concomitantly identified provide insight into the relation of ergosterol biosynthesis to other important physiological changes in the cell such as cell cycle regulation, mating response, and sporulation. All these modulations contribute to the definition of the drug signature.

Two recent studies reported the analysis of global expression patterns measured in *Saccharomyces cerevisiae* cells exposed to drug treatments or genetic mutations that affect the ergosterol biosynthesis pathway. Bammert et al. (1) used the DNA chip strategy to analyze the expression profiles of yeast cells exposed to seven antifungal agents and compared them with those obtained from strains containing deletions of *ERG2, ERG5*, or *ERG6*. Dimster-Denk et al. (14) undertook a different approach based upon promoter fusions as a readout of transcriptional changes induced by eight inhibitors targeting different enzymatic steps of the isoprenoid pathway. The comparison of our data with theirs reinforces our analysis in that some genes and pathways modulated following

ergosterol biosynthesis pathway disturbance were common in the three studies. A few discrepancies do exist, likely reflecting methodological differences such as timing of the treatments, genetic background differences of the yeast strains, or criteria applied for considering a gene as modulated.

In conclusion, in this study genome-wide transcriptional changes observed in response to treatment with SR31747A do correlate with responses induced by the genetic alteration of the *ERG2* gene. Thanks to this convergent pattern we conclude that the *ERG2*-encoded sterol isomerase is the primary target of the drug and mediates the global cellular response to the drug in that model. Furthermore, the total absence of the SR31747A characteristic signature in the SR-treated *ERG2* mutant indicates that the enzyme is very likely to be the only target of the drug in this model. Our analysis also allowed us to describe a profile of the global transcriptional effect due to ergosterol deprivation. These results reinforce SR31747A as a very interesting compound whose antiproliferative properties are produced by a single-target mechanism in yeast. SR31747A is known to have immunosuppressive and anti-inflammatory properties as well as antiproliferative effects in mammals (35). Therefore, it would be now highly interesting to test whether this drug has a similar mode of action in human cells.

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