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Large-scale screening of yeast mutants for sensitivity to the IMP dehydrogenase inhibitor 6-azauracil

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Abstract

Mutations in several genes encoding components of the RNA polymerase II elongation machinery render *S. cerevisiae* cells sensitive to the drug 6-azauracil (6AU), an inhibitor of IMP dehydrogenase and orotidylate decarboxylase. It is thought that a reduction in nucleotide levels following drug treatment causes transcriptional elongation to be more dependent on a fully functional RNA polymerase. To gain insight into the basis of the 6AU-sensitive phenotype and discern its specificity, we screened almost 3000 deletion mutants for growth in the presence of drug; 42 (1.5%) were reproducibly sensitive to the drug. The sensitive mutants included several missing known transcription elongation factors, but the majority were in genes involved in other cellular processes. Not all of the 6AU-sensitive strains displayed cross-sensitivity to mycophenolic acid (MPA), another drug that inhibits IMP dehydrogenase and has been employed as a screening agent for elongation mutants, showing that these two drugs are mechanistically distinct. Several of the mutants were tested for the ability to induce transcription of IMP dehydrogenase-encoding genes, in response to 6-AU and MPA treatment. As expected, mutants defective in transcriptional elongation factors were unable to fully induce IMPDH expression. However, most of the 6AU-sensitive strains had normal levels of IMPDH expression. Thus, although 6AU-sensitivity often results from defects in the elongation machinery, mutations that compromise processes other than transcription and induction of IMPDH also lead to sensitivity to this drug.

Keywords

azauracil; mycophenolic acid; transcription elongation; yeast deletants

Introduction

S. cerevisiae strains bearing mutations in genes encoding RNA polymerase II subunits and its accessory elongation factors are often sensitive to the drugs 6-azauracil (6AU) and mycophenolic acid (MPA). Indeed, sensitivity to 6AU was the first phenotype identified for cells with defective RNA polymerase II elongation factors, and it has been widely considered to be diagnostic for proteins involved in transcriptional elongation (Hubert *et al.*, 1983; Hampsey, 1997). The basis for this sensitivity appears to be the drug's inhibition of IMP dehydrogenase, leading to a reduction in ribonucleotide levels and a concomitant increased dependence upon fully functional transcriptional elongation factors for efficient transcription (Archambault *et al.*, 1992; reviewed in Wind and Reines, 2000). The gene encoding IMP dehydrogenase, *IMD2*, is transcriptionally induced in response to nucleotide

depletion by these inhibitors and this response is dependent upon an optimally functioning elongation machinery (Shaw and Reines, 2000; Shaw *et al.*, 2001; Escobar-Henriques and Daignan-Fornier, 2001).

The availability of a nearly complete set of gene deletion mutants of yeast (Giaever *et al.*, 2002) enables large-scale screening of null mutants for a variety of phenotypes. Screening the yeast ‘disruptome’ has proved to be a high-throughput functional genomics tool to identify genes involved in Ty element function (Griffith *et al.*, 2003) and responsiveness to the drugs wortmannin, rapamycin, and MPA (Chan *et al.*, 2000; Desmoucelles *et al.*, 2002; Zewail *et al.*, 2003), as well as other phenotypes. Although mutations in genes encoding components of the transcriptional elongation machinery render cells sensitive to 6AU, it is clear that there is more than one mechanism to achieve this sensitivity, e.g. by mutation of a regulator of pyrimidine biosynthesis (Hubert *et al.*, 1983; Shaw and Reines, 2000). Using the deletion collection, we set out to determine the spectrum of genes whose deletion alters growth rates in the presence of 6AU. This study complements a similar recent analysis on MPA, a biochemically distinct inhibitor of IMP dehydrogenase.

Materials and methods

The homozygous diploid yeast gene knockout (YKO) mutants were transformed to Ura⁺ with pBM2636 (1.2 kb *HindIII*–*EcoRI* fragment of the *HXT1* promoter fused to *lacZ* on the *URA3*-containing multicopy plasmid YE357R; Ozcan and Johnston, 1995). Transformants were replicated to YM agar plates lacking uracil [1.7 g/l Yeast Nitrogen Base (Difco), 5 g/l ammonium sulphate, 20 g/l dextrose, 1.0 g/l drop-out mix without uracil (US Biological)] and containing either 200 µg/ml 6AU or 100 µg/ml MPA. Mutants that appeared more or less sensitive than wild-type were re-tested by plating several dilutions of the cells on the same plates. The most sensitive strains (scored ‘3’) showed little or no growth after 3 days on medium containing 6AU or 2 days for MPA; moderately sensitive strains (scored ‘2’) exhibited limited growth after 3 and 2 days for 6AU and MPA, respectively; we are not confident of results scored ‘1’.

For Northern analysis, strains were grown in YPD to an optical density at 600 nm of ≈ 0.5 . MPA (15 µg/ml w/v; Sigma, St. Louis, MO) or 6AU (100 µg/ml w/v; Sigma, St. Louis, MO) were added and cells were collected and frozen after 2 h at 30 °C. Total RNA was isolated from thawed cell pellets by phenol extraction and quantitated by measuring absorbance at 260 nm. RNA (15 µg) was resolved on a 1% formaldehyde/agarose gel and blotted onto Zeta-probe GT nylon membrane (Bio-Rad; Hercules CA). Filters were baked at 80 °C for 2 h, pre-hybridized for a minimum of 3 h at 42 °C in 5× SSC (1 × SSC = 0.15 M NaCl, 0.015 sodium citrate), 5× Denhardt’s solution, 50% v/v formamide, 1% w/v SDS, and 100 µg/ml salmon sperm DNA. Filters were hybridized under the same conditions with $\approx 10^8$ cpm [³²P]-labelled DNA probe for 15–18 h. Filters were washed twice at 22 °C in 2% SSC/0.1% SDS, for 5 min each, twice in 0.2% SSC/0.1% SDS for 5 min each, twice in 0.2% SSC/0.1% SDS at 42 °C for 20 min each, exposed to XOMAT film. *IMD2* DNA probe (which cross-hybridizes to *IMD3*; Hyle *et al.*, 2003) was prepared using the polymerase chain reaction with a wild-type yeast genomic DNA template (S288C; Research Genetics, Huntsville, AL) and primers complementary to the gene; 5′-GTGGTATGTTGGCCGGTACTACCG-3′ and 5′-TCAGTTATGTAAACGCTTTTCGTA-3′. Probes were labelled to a specific activity of approximately 10^7 – 10^8 cpm/µg with Klenow DNA polymerase (Promega Life Sciences, Madison WI), random hexamer primers (Gibco BRL, Rockville MD), and [α -³²P]dATP (Amersham Pharmacia Biotech, Piscataway, NJ).

Results and discussion

We screened 2804 mutants from the YKO Collection (Giaever *et al.*, 2002) for their sensitivity to 6AU. This screen required that YKO strains be transformed to Ura⁺, since uracil in the medium competes with drug and abrogates its effect (list of mutants screened available at <http://www.genetics.wustl.edu/6AZAURACIL>). About 200 candidates from the first screen were re-tested several times for growth on agar plates containing the drug, yielding 44 mutants that were reproducibly more sensitive or resistant than wild-type cells (Table 1, Figure 1). Each of these mutants was also tested for its ability to induce expression of IMPDH in response to 6AU and MPA (Figure 2). An example of a time course of induction for five sensitive strains compared to an isogenic wild-type control is shown in Figure 3.

As expected, deletion of several genes, including *DST1*, *POP2* (also called *CAF1*) and *RTF1*, which have been implicated in transcriptional elongation (Archambault *et al.*, 1992; Lennon *et al.*, 1998; Denis *et al.*, 2001; Costa and Arndt, 2000; Squazzo *et al.*, 2002), were identified in the screen (Table 1, lines 1–7). The observed sensitivity of strains deleted for genes known to be involved in transcriptional elongation validates this screen. As in a previously reported screen of the YKO collection for MPA-sensitive strains, we found that the most sensitive mutants are those missing genes involved in transcription (Desmoucelles *et al.*, 2002). A survey of IMPDH induction in the 6AU-sensitive strains confirmed prior work showing that induction was compromised when *DST1*, the gene encoding elongation factor SII (also called TFIIS) was deleted (Figure 2). Deletion of an ORF (YGL042C) considered ‘dubious’ that overlaps *DST1* also conferred 6AU-sensitivity and rendered IMPDH uninducible (Table 1, line 44).

Another gene necessary for resistance to 6AU identified by this screen was *SDT1* (Table 1, line 27, and Figure 1), the deletion of which is known to render cells 6AU-sensitive (Shimoaraiso *et al.*, 2000). *SDT1* was initially identified as a multi-copy suppressor of the 6AU-sensitivity of a *dst1* Δ strain, and encodes a nucleotidase that detoxifies pyrimidine nucleotide derivatives (Nakanishi and Sekimizu, 2002). It was also of interest that deletion of *NOT3*, which encodes subunits of the *Ccr4–Not* complex implicated in transcriptional elongation (Denis *et al.*, 2001), causes resistance to both drugs (Table 1, line 8). A new 6AU-sensitive mutant identified here is *gal11* Δ (also called *SPT13*; Table 1, line 6). Gal11p is a component of the RNA polymerase II holoenzyme that stimulates the TFIIF protein kinase and is implicated in the transition from transcriptional initiation to elongation (Kim *et al.*, 1994; Sakurai and Fukasawa, 1998; Badi and Barberis, 2001). Other members of the *SPT* gene family also have a demonstrated role in elongation (Yamaguchi *et al.* 2001). Another new 6AU-sensitive mutant found here was *thp1* Δ (Table 1, line 7). The phenotype is not unexpected, since *THP1* has been linked to transcription elongation and mRNA transport (Gallardo and Aguilera, 2001; Gallardo *et al.*, 2003; Fischer *et al.*, 2002).

Importantly, and perhaps surprisingly, a majority of the mutants are defective in processes seemingly unrelated to transcription. These include genes involved in fatty acid metabolism (Table 1, lines 9–12), amino acid transport (lines 13–15), protein ubiquitinylation (lines 16–18), stress and cell cycle progression (lines 19–20), protein trafficking (lines 21–22), RNA processing (lines 23–25) and individual mutants defective in several diverse processes. Since most of these mutants exhibit near-normal levels of IMPDH expression in the presence of the drugs, we conclude that there are multiple mechanisms of attaining drug sensitivity, only one of which is a transcriptional elongation defect. We can imagine plausible explanations for the drug sensitivity of some of these mutants. For example, *tfp3* Δ is defective in vacuole function, which might affect the strain’s ability to sequester the drugs. At high concentrations, 6AU has been reported to poison amino acid metabolism

(Tamaki *et al.*, 1989). The mutations in amino acid transport and synthesis we found (Table 1, lines 13–15, 26) may exacerbate the 6AU-induced accumulation of a toxic intermediate. For many of the strains, however, it is difficult to understand the mechanistic basis for the phenotype, e.g. how do perturbations in protein degradation or trafficking lead to drug sensitivity (lines 17, 18, 21, 22)? It remains possible that some of these genes may eventually be linked to transcriptional elongation. In any case, it seems clear that sensitivity to 6AU or MPA is not a reliable diagnostic criterion for defects in transcriptional elongation, and that caution should be exercised in interpreting the 6AU-sensitive phenotype.

This report is useful in light of the recent analysis of a comprehensive identification of genes required for MPA resistance from the YKO collection (Desmoucelles *et al.*, 2002). We found that 1.5% of the mutants we screened were sensitive to 6AU, a value similar to the fraction of mutants sensitive to MPA (2%) (Desmoucelles *et al.*, 2002). While much is known about the mechanism of uncompetitive inhibition of IMP dehydrogenase by MPA, including a co-crystal of enzyme and inhibitor, the metabolism and mechanism of action of 6AU is less well understood (Exinger and Lacroute, 1992; Sintchak *et al.*, 1996; Hedstrom, 1999). Nevertheless, it has become a popular test for yeast mutants because of the extensive characterization of 6AU-sensitive strains with well-demonstrated transcriptional elongation defects. Early work suggested that the sensitivity to either MPA or 6AU could serve as a biomarker for transcriptional elongation factor mutants (Exinger and Lacroute, 1992). Indeed, both we and Desmoucelles *et al.* (2002) observe a large degree of cross-sensitivity to 6AU and MPA and both screens independently identified several genes encoding proteins known or likely to be involved in transcriptional elongation [*RTF1*, *POP2* (*CAF1*), *SPT3*, and *DST1*]. On the other hand, both studies show that not all mutants are equally affected by 6AU and MPA, e.g. this work and that of Desmoucelles *et al.* (2002) show that the *mot3* mutant is relatively more sensitive to 6AU than MPA. We observe a similar phenotype for *sdt1* Δ , which was previously shown to be 6AU-sensitive (Nakanishi and Sekimizu, 2002). *SDT1* encodes a nucleotidase which was identified as a suppressor of the 6AU-sensitivity of *dst1* Δ and is thought to act by hydrolysing toxic nucleotide derivatives such as 6AU (Nakanishi and Sekimizu, 2002). Since it would not be expected to affect the metabolism of MPA, which is not a nucleotide, and the *sdt1* Δ strain is not impaired in IMPDH induction, it is easy to see why the strain is not MPA-sensitive and was not detected in the screen of Desmoucelles *et al.* (2002). This is a good example of a non-transcription-related pathway that, when mutated, confers 6AU-sensitivity.

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References

- Archambault J, Lacroute F, Ruet A, Friesen JD. Genetic interaction between transcription elongation factor TFIIS and RNA polymerase II. *Mol Cell Biol.* 1992; 12:4142–4152. [PubMed: 1508210]
- Badi L, Barberis A. Proteins that genetically interact with the *Saccharomyces cerevisiae* transcription factor Gal11p emphasize its role in the initiation–elongation transition. *Mol Genet Genom.* 2001; 265:1076–1086.
- Chan TF, Carvalho J, Riles L, Zheng XF. A chemical genomics approach toward understanding the global functions of the target of rapamycin protein (TOR). *Proc Natl Acad Sci USA.* 2000; 97:13 227–13 232.
- Costa PJ, Arndt KM. Synthetic lethal interactions suggest a role for the *Saccharomyces cerevisiae* Rtf1 protein in transcription elongation. *Genetics.* 2000; 156:535–547. [PubMed: 11014804]

- Denis CL, Chiang YC, Cui Y, Chen J. Genetic evidence supports a role for the yeast CCR4–NOT complex in transcriptional elongation. *Genetics*. 2001; 158:627–634. [PubMed: 11404327]
- Desmoucelles C, Pinson B, Saint-Marc C, Daignan-Fornier B. Screening the yeast ‘disruptome’ for mutants affecting resistance to the immunosuppressive drug, mycophenolic acid. *J Biol Chem*. 2002; 277:27 036–27 044.
- Escobar-Henriques M, Daignan-Fornier B. Transcriptional regulation of the yeast GMP synthesis pathway by its end products. *J Biol Chem*. 2001; 276:1523–1530. [PubMed: 11035032]
- Exinger F, Lacroute F. 6-Azauracil inhibition of GTP biosynthesis in *Saccharomyces cerevisiae*. *Curr Genet*. 1992; 22:9–11. [PubMed: 1611672]
- Fischer T, Strasser K, Racz A, et al. The mRNA export machinery requires the novel Sac3p–Thp1p complex to dock at the nucleoplasmic entrance of the nuclear pores. *EMBO J*. 2002; 21:5843–5852. [PubMed: 12411502]
- Gallardo M, Aguilera A. A new hyperrecombination mutation identifies a novel yeast gene, THP1, connecting transcription elongation with mitotic recombination. *Genetics*. 2001; 157:79–89. [PubMed: 11139493]
- Gallardo M, Luna R, Erdjument-Bromage H, Tempst P, Aguilera A. Nab2p and the Thp1p–Sac3p complex functionally interact at the interface between transcription and mRNA metabolism. *J Biol Chem*. 2003; 278:24 225–24 232.
- Giaever G, Chu AM, Ni L, et al. Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature*. 2002; 418:387–391. [PubMed: 12140549]
- Griffith JL, Coleman LE, Raymond AS, et al. Functional genomics reveals relationships between the retrovirus-like Ty1 element and its host *Saccharomyces cerevisiae*. *Genetics*. 2003; 164:867–879. [PubMed: 12871900]
- Hampsey M. A review of phenotypes in *Saccharomyces cerevisiae*. *Yeast*. 1997; 13:1099–1133. [PubMed: 9301019]
- Hedstrom L. IMP dehydrogenase: mechanism of action and inhibition. *Curr Med Chem*. 1999; 6:545–560. [PubMed: 10390600]
- Hyle JW, Shaw RJ, Reines D. Functional distinctions between IMP dehydrogenase genes in providing mycophenolate resistance and guanine prototrophy to yeast. *J Biol Chem*. 2003; 278:28 470–28 478.
- Hubert JC, Guyonvarch A, Kammerer B, et al. Complete sequence of a eukaryotic regulatory gene. *EMBO J*. 1983; 2:2071–2073. [PubMed: 6139279]
- Kim YJ, Bjorklund S, Li Y, Sayre MH, Kornberg RD. A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. *Cell*. 1994; 77:599–608. [PubMed: 8187178]
- Lennon JC III, Wind M, Saunders L, Hock MB, Reines D. Mutations in RNA polymerase II and elongation factor SII severely reduce mRNA levels in *Saccharomyces cerevisiae*. *Mol Cell Biol*. 1998; 18:5771–5779. [PubMed: 9742094]
- Nakanishi T, Sekimizu K. SDT1/SSM1, a multicopy suppressor of S-II null mutant, encodes a novel pyrimidine 5′-nucleotidase. *J Biol Chem*. 2002; 277:22 103–22 106.
- Ozcan S, Johnston M. Three different regulatory mechanisms enable yeast hexose transporter (HXT) genes to be induced by different levels of glucose. *Mol Cell Biol*. 1995; 15:1564–1572. [PubMed: 7862149]
- Sakurai H, Fukasawa T. Functional correlation among Gal11, transcription factor (TF) IIE, and TFIH in *Saccharomyces cerevisiae*. Gal11 and TFIIE cooperatively enhance TFIH-mediated phosphorylation of RNA polymerase II carboxyl-terminal domain sequences. *J Biol Chem*. 1998; 273:9534–9538. [PubMed: 9545282]
- Shaw RJ, Reines D. *Saccharomyces cerevisiae* transcription elongation mutants are defective in PUR5 induction in response to nucleotide depletion. *Mol Cell Biol*. 2000; 20:7427–7437. [PubMed: 11003640]
- Shaw RJ, Wilson JL, Smith KT, Reines D. Regulation of an IMP dehydrogenase gene and its overexpression in drug-sensitive transcription elongation mutants of yeast. *J Biol Chem*. 2001; 276:32 905–32 916.

- Shimoaraiso M, Nakanishi T, Kubo T, Natori S. Transcription elongation factor S-II confers yeast resistance to 6-azauracil by enhancing expression of the SSM1 gene. *J Biol Chem.* 2000; 275:29 623–29 627. [PubMed: 10617581]
- Sintchak MD, Fleming MA, Futer O, et al. Structure and mechanism of inosine monophosphate dehydrogenase in complex with the immunosuppressant mycophenolic acid. *Cell.* 1996; 85:921–930. [PubMed: 8681386]
- Squazzo SL, Costa PJ, Lindstrom DL, et al. The Paf1 complex physically and functionally associates with transcription elongation factors *in vivo*. *EMBO J.* 2002; 21:1764–1774. [PubMed: 11927560]
- Tamaki N, Fujimoto S, Mizota C, Kaneko M, Kikugawa M. Inhibitory effect of 6-azauracil on β -alanine metabolism in rat. *J Nutr Sci Vitaminol.* 1989; 35:451–461. [PubMed: 2632679]
- Wind M, Reines D. Transcription elongation factor SII. *Bioessays.* 2000; 22:327–336. [PubMed: 10723030]
- Yamaguchi Y, Narita T, Inukai N, Wada T, Handa H. SPT genes: key players in the regulation of transcription, chromatin structure and other cellular processes. *J Biochem.* 2001; 129:185–191. [PubMed: 11173517]
- Zewail A, Xie MW, Xing Y, et al. Novel functions of the phosphatidylinositol metabolic pathway discovered by a chemical genomics screen with wortmannin. *Proc Natl Acad Sci USA.* 2003; 100:3345–3350. [PubMed: 12615994]

Figure 1.

Drug-sensitive growth phenotype for selected deletion strains. Two-fold serial dilutions of the indicated deletion strains were spotted onto solid media in the presence of 6AU (200 μ g/ml) or MPA (100 μ g/ml) and incubated at 30 $^{\circ}$ C for 3 days and 2 days, respectively

Figure 2. IMPDH induction in selected deletion strains. The indicated deletion strains were grown in liquid media in the presence or absence of 6AU (75 µg/ml) or MPA (15 µg/ml) and RNA was isolated for Northern blot analysis. Filters were probed with a PCR product corresponding to *IMD2* sequences, as indicated in Materials and methods

Figure 3.

Time course of induction of IMPDH in five deletants sensitive to 6AU/MPA. Strains were inoculated into YPD, grown at 30 °C until they reached $OD_{600} = 0.5$. RNA was prepared from a sample ('0 time') and MPA (15 $\mu\text{g/ml}$) was added. Aliquots of cells were withdrawn at the indicated times and RNA was isolated for Northern blot analysis. Filters were probed with a PCR product corresponding to *IMD2* sequences, as indicated in Materials and methods. Signals were quantitated by phosphorimaging and plotted in phosphorimager units on the y axis

Table 1

Strains with altered growth properties in the presence of 6AU and MPA

ORF	Gene name	wild-type	Process	Function	6AU sensitivity	MPA sensitivity
YGL043W	DST1	Transcription	Transcription	Transcription elongation factor S-II	3	3
YMR070W	MOT3	Transcription	Transcription	Transcriptional repressor and activator	3	1
YNR052C	POP2	Transcription	Transcription	Transcription factor	3	3
YGL244W	RTF1	Transcription	Transcription	Transcription elongation factor	3	3
YDR392W	SPT3	Transcription	Transcription	SAGA histone acetyltransferase complex member	3	3
YOL051W	GAL11	Transcription	Transcription	RNA polymerase II holoenzyme component	2	2
YOL072W	THP1	Transcription/recomb	Transcription	Transcription elongation factor	3	1
YIL038C	NOT3	Transcription	Transcription	3'-5' exoribonuclease	Resistant	Resistant
YDR315C	IPK1	Fatty acid metabolism	Fatty acid metabolism	Inositol/phosphatidylinositol kinase	3	Resistant
YFL018C	LPD1	Fatty acid metabolism	Fatty acid metabolism	Dihydroliipoamide dehydrogenase	1	3
YOR221C	MCT1	Fatty acid metabolism	Fatty acid metabolism	Malonyl-CoA:ACP transferase	3	1
YMR272C	SCS7	Fatty acid metabolism	Fatty acid metabolism	Very long chain fatty acid hydroxylase	1	3
YKL176C	LST4	Amino acid transport	Amino acid transport	Golgi to plasma membrane transport	2	2
YGR057C	LST7	Amino acid transport	Amino acid transport	Golgi to plasma membrane transport	3	3
YBR069C	TAT1	Amino acid transport	Amino acid transport	Amino acid transporter activity	3	3
YMR275C	BUL1	Protein ubiquitinylation	Protein ubiquitinylation	Involved in ubiquitination	Resistant	0
YER151C	UBP 3	Protein ubiquitinylation	Protein ubiquitinylation	Ubiquitin-specific protease	2	3
YMR304W	UBP15	Protein ubiquitinylation	Protein ubiquitinylation	Putative deubiquitinating enzyme	3	3
YML006C	GIS4	Stress	Stress	Unknown	3	0
YKR072C	SIS2	Cell cycle, stress	Cell cycle, stress	Protein phosphatase inhibitor	3	3
YGL223C	COD3	Protein traffic	Protein traffic	Unknown	3	2
YMR060C	TOM37	Protein traffic	Protein traffic	Mitochondrial import receptor protein	3	0
YDR463W	STP1	RNA processing	RNA processing	Pre-tRNA splicing/uptake of branched-chain amino acids	3	3
YMR125W	STO1	RNA processing	RNA processing	Large subunit of the nuclear cap-binding protein complex	3	2
YNL147W	LSM7	RNA processing	RNA processing	snRNP protein	2	1
YCL009C	ILV6	Amino acid metabolism	Amino acid metabolism	Acetolactate synthase activity	2	0
YGL224C	SDT1	Nucleotide metabolism	Nucleotide metabolism	Nucleotidase	3	0
YNL220W	ADE12	Nucleotide metabolism	Nucleotide metabolism	Adenylosuccinate synthetase	2	1
YDL101C	DUN1	DNA damage	DNA damage	DNA damage checkpoint protein kinase	1	2

ORF	Gene name wild-type	Process	Function	6AU sensitivity	MPA sensitivity
YGL167C	PMR1	Ion homeostasis	Member of the SERCA family of CaATPases	2	3
YIL097W	FYV10	Killer toxin resistance	Unknown	3	3
YPL234C	TFP3	Vacuole	Vacuolar ATPase Y0 domain subunit c'	0	3
YGL020C	MDM39	Mito biogenesis	Unknown	3	3
YMR126C	DLT1	Unknown	Unknown	3	3
YGL168W	HUR1	Unknown	Unknown	3	3
YHR151C	ORF	Unknown	Unknown	3	3
YGR064W	ORF (dubious)	Unknown	Unknown	2	3
YKR073C	ORF (dubious)	Unknown	Unknown	3	2
YML107C	ORF	Unknown	Unknown	3	2
YDL172C	ORF (dubious)	Unknown	Unknown	2	3
YDL173W	ORF	Unknown	Unknown	2	3
YNL136W	ORF	Unknown	Unknown	3	3
YML036W	ORF	Unknown	Unknown	3	3
YGL042C	ORF (dubious)	Unknown	Unknown	3	1