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Tryptophan biosynthesis is important for resistance to replicative stress in *Saccharomyces cerevisiae*

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Abstract

Acute tryptophan depletion is used to induce low levels of serotonin in the brain. This method has been widely used in psychiatric studies to evaluate the effect of low levels of serotonin, and is generally considered a safe and reversible procedure. Here we use the budding yeast *Saccharomyces cerevisiae* to study the effects of tryptophan depletion on growth rate upon exposure to DNA damaging agents. Surprisingly, we find that budding yeast undergoing tryptophan depletion are more sensitive to DNA damaging agents such as methyl methanesulfonate (MMS) and hydroxyurea (HU). We find that this defect is independent of several DNA repair pathways such as homologous recombination, base excision repair, and translesion synthesis, and that this damage sensitivity is not due to impaired S-phase signaling. Upon further analysis, we find that the DNA damage sensitivity of tryptophan depletion is likely due to impaired protein synthesis. These studies describe an important source of variance in budding yeast when using tryptophan as an auxotrophic marker particularly on studies focusing on DNA repair, and suggest that further testing of the effect of tryptophan depletion on DNA repair in mammalian cells is warranted.

INTRODUCTION

Over the past twenty-five years, tryptophan depletion has been widely studied as a method for lowering serotonin levels in the brain (Young, 2013). In humans L-tryptophan is an essential amino acid, obtained entirely from the diet and stored at extremely low levels in the body (Richard, et al., 2009). Since L-tryptophan is the sole precursor for production of serotonin, L-tryptophan depletion is used to lower serotonin levels in otherwise healthy patients (Richard, et al., 2009). This technique is generally considered safe, as the major side-effects of acute L-tryptophan depletion appears to be reversible mood alterations (Richard, et al., 2009; Young, 2013). However, long-term studies of the safety of L-tryptophan depletion are still required as the full consequences of amino acid imbalances on human metabolism are not clear.

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Conflict of interest statement. None declared.

To improve our understanding of the consequences of tryptophan depletion in eukaryotes, we utilized the budding yeast *Saccharomyces cerevisiae* as a model system. In budding yeast, the tryptophan permease is hypomorphic at colder temperatures, and thus growth at room temperature induces tryptophan depletion when tryptophan biosynthesis genes are disrupted (Abe and Horikoshi, 2000). Using this yeast model for tryptophan depletion, we find that disruption of tryptophan metabolism leads to MMS and HU sensitivity at 23°C but not at 30°C on rich medium. The sensitivity observed in tryptophan-limited cells to DNA damaging agents is more severe than mutants of several DNA damage repair and signaling pathways, and may be a novel mechanism for inducing DNA damage sensitivity. To our knowledge, this is the first comprehensive analysis that demonstrates tryptophan depletion can result in sensitivity to DNA damaging agents. Moreover, due to the use of tryptophan depletion in humans, understanding the effect of tryptophan depletion on human DNA repair systems is an important area of further investigation.

MATERIALS AND METHODS

Yeast strains, plasmids, and media

All budding yeast strains are either RAD5 derivatives of the W303 strain W1588 (Thomas and Rothstein, 1989; Zhao, et al., 1998) or are from the BY alpha deletion library (Giaever, et al., 2002) and are listed in Table 1 in the order they appear in the figures and text.

Serial dilutions

The indicated strains were grown to an 0.5 OD₆₀₀ at room temperature (23°C) and then five-fold serial dilutions were spotted onto rich medium (YPD) or YPD with either 0.006% methyl methanesulfonate (MMS) or 100 mM hydroxyurea (HU). YPD Plates were incubated for 2 days at 30°C or 3 days at 23°C prior to imaging. SC plates, and SC plates with HU were incubated for 2 days at 30°C or 3 days at 23°C, while SC plates with MMS were incubated for 3 days at 30°C and 4 days at 23°C due to slow growth. For the cycloheximide (CHX) experiments, the yeast were grown to 0.2 OD₆₀₀ at 30°C and then five-fold serially diluted onto YPD + 0.03% dimethylsulfoxide (DMSO), YPD + 0.03% DMSO with 100 ng/mL CHX, and YPD + 0.03% DMSO with 100 ng/mL CHX and 0.012% MMS. The 23°C plates were imaged after 4 days and the 30°C plates were imaged after 3 days.

Recombination analysis

Mitotic recombination assays were performed as described using a direct repeat recombination assay (Godin, et al., 2013). Nine colonies for each strain were grown to saturation at 30°C or 23°C in 2 mL of SC with or without MMS before being diluted to 0.1 OD₆₀₀. 250 uL of this dilution was plated onto SC-LEU plates to measure recombination events. The 0.1 OD₆₀₀ culture was further diluted 1:1000, and then 250 uL were plated onto SC plates to determine viable cells. After two days of growth at 30°C, colonies were counted. Recombination rates and standard deviations were calculated as described (Lea and Coulson, 1949).

RESULTS AND DISCUSSION

Tryptophan biosynthesis is required for resistance to DNA damaging agents at room temperature

To determine if tryptophan biosynthesis genes are involved in resistance to replicative stress, we asked if mutation of *TRP1* would confer similar DNA damage sensitivities. Since the budding yeast tryptophan permease loses functionality at colder temperatures (Abe and Horikoshi, 2000), we examined *trp1-1* cells for MMS and HU sensitivity at 23°C. Whereas, *trp1-1* mutants are slow growing at 23°C upon either MMS or HU exposure, this mutant exhibits no DNA damage sensitivity when grown at 30°C (Figure 1A). Importantly, the DNA damage sensitivity of *trp1-1* cells was specific to tryptophan biosynthesis since *lys2* cells or *ade2-1* mutants exhibit no growth defect relative to WT (Figure 1A). Consistent with *TRP1* mutation being the causative factor, the MMS and HU sensitivity of a *trp1-1* strain is suppressed by a *TRP1* expressing plasmid but not a plasmid expressing *LEU2* (Figure 1B). Since previous work has indicated that tryptophan mutants exhibit different phenotypes depending upon whether YPD or synthetic complete (SC) medium is used (Garrett, 1997), we asked if the *trp1-1* DNA damage defect is dependent upon the medium. Regardless of whether YPD or SC medium is used, *trp1-1* cells exhibit clear growth defects at 23°C upon MMS or HU treatment (Figure 1C). Surprisingly, on SC medium the *trp1-1* mutants exhibit a modest sensitivity to high-dose MMS at 30°C, although the defect at 23°C is more pronounced. These data are consistent with tryptophan mutants exhibiting growth defects at either 28°C or 30°C depending upon the medium or stress induced (Garrett, 1997; Polleys and Bertuch, 2015). Together this data indicates that when tryptophan uptake is limiting, cells become sensitive to replicative stress.

To determine if this is a feature specific to our W303 yeast strain background, we evaluated *trp1* BY cells from the alpha deletion library (Giaever, et al., 2002) and found that the *trp1* mutation also confers sensitivity to HU at 23°C, and this sensitivity is similarly complemented by a *TRP1* but not *LEU2*-expressing plasmid (Figure 2A). We next asked if this defect is specific to *TRP1* deletion or if disruption of any gene in the tryptophan biosynthesis pathway would confer a similar sensitivity to MMS or HU and find that, indeed, they do (Figure 2B). Note that the HU sensitivity in the *trp1* BY cells is modestly more pronounced than in W303, likely reflecting an unknown strain variability (Compare Figure 1 and 2B). Similar to W303, the BY tryptophan mutants also exhibit elevated sensitivity to MMS at 30°C when grown on SC but not YPD medium (Figure 2C and Figure 1C). Interestingly, previous genome-wide studies have implicated individual *TRP* genes to be important for cisplatin, MMS, and HU resistance (Brown, et al., 2006; Svensson, et al., 2011). Together, these results indicate that tryptophan deprivation leads to sensitivity to replicative stress caused by MMS or HU in multiple *Saccharomyces cerevisiae* strain backgrounds.

The sensitivity of *trp1-1* cells to DNA damaging agents is not solely attributed to loss of a single DNA repair pathway

Since we find that *trp1-1* cells are sensitive to replicative stress, we asked whether tryptophan deprivation is due to a defect in a specific DNA repair or damage-signaling

pathway. To do this, we conducted epistasis analysis using a mutant defective for homologous recombination (*rad57*), base excision repair (*mag1*), or translesion synthesis (*rev3*). We five-fold serially diluted the single and double *trp1-1* mutant combinations onto rich medium containing MMS and find that the MMS sensitivity of *trp1-1* double mutant cells is similar or slightly worse growing compared to a *trp1-1* single mutant (Figure 3A). Surprisingly, a *trp1-1* mutant is more MMS sensitive than *rad57*, *mag1* or *rev3* mutant cells at 23°C, whereas at 30°C, the sensitivities conferred by *rad57*, *mag1*, or *rev3* are unaffected by a cell being *TRP1* or *trp1-1* (Figure 3A). Therefore, the growth of *trp1-1* cells on MMS at 23°C is not solely attributed to loss of TLS, BER, or HR and may be multifactorial.

Next we determined if *trp1-1* cells would exhibit altered recombination rates. We measured the recombination rates of *TRP1* and *trp1-1* strains treated with MMS at room temperature and 30°C (Figure 3B), as described previously (Godin, et al., 2013). Consistently, we did not observe a significant difference between the levels of recombination in *trp1-1* and *TRP1* cells, indicating that HR is not impaired in these cells (Figure 3B). Note the low doses of MMS used in this assay reflect the higher sensitivity of yeast cells to MMS in liquid culture compared to on solid medium plates. We next examined whether the MMS sensitivity of *trp1-1* mutants is due to impaired DNA damage-signaling by combining *trp1-1* with deletions of *TOF1*, *RAD9*, and *MRC1*. If *trp1-1* impaired an S phase checkpoint, we would predict that deletion of *TOF1*, *RAD9*, or *MRC1* might give a similar phenotype as the *trp1-1* mutation, and that a double mutant would look like the deletion of the checkpoint gene. Instead, we found that that *trp1-1* cells are more MMS sensitive than any of the checkpoint mutants (Figure 3C).

Since we do not observe a genetic interaction between the *trp1-1* mutant and several DNA repair mutants, we next asked if the tryptophan depletion caused by *trp1-1* induces metabolic changes that could alter DNA repair or damage tolerance. For instance, tryptophan is a precursor for NAD⁺ biosynthesis, and tryptophan depletion may lower cellular levels of NAD⁺ (Bedalov, et al., 2003). Interestingly, sufficient NAD⁺ levels are important for DNA damage repair (Lin and Guarente, 2003). For example, the sirtuin protein family requires sufficient levels of NAD⁺ to effectively remodel chromatin after DNA damage, and disruption of the sirtuins or the required NAD⁺ levels can contribute to misregulated DNA damage repair (Mills, et al., 1999; Tsukamoto, et al., 1997). To test if the DNA damage sensitivity in *trp1-1* cells is due to altered NAD⁺ levels, we deleted *BNA2*, a gene required for biosynthesis of NAD⁺ from tryptophan (Bedalov, et al., 2003). We find that the MMS sensitivity *trp1-1* mutant is independent of *BNA2*, indicating that the DNA damage sensitivity of *trp1-1* cells is not due to impaired NAD⁺ biosynthesis (Figure 4). This likely indicates that sufficient NAD⁺ is generated from the NAD⁺ salvage and import pathways in yeast and that *de novo* biosynthesis of NAD⁺ from tryptophan is not rate limiting in *trp1-1* cells. Similarly deletion of the budding yeast *SIR2*, a sirtuin that utilizes NAD⁺ produced by tryptophan, does not alter the MMS sensitivity of a *trp1-1* cell (Figure 4).

One potential outcome of the tryptophan depletion that occurs in *trp1-1* cells at room temperature is a defect in protein synthesis due to the low abundance of tryptophan. To determine if defective protein synthesis underlies the MMS sensitivity of *trp1-1* cells, we

treated both *TRP1* and *trp1-1* cells with a low-dose of the protein synthesis inhibitor cycloheximide (CHX). If defective protein synthesis causes MMS sensitivity, we would predict that CHX treatment would sensitize both *TRP1* and *trp1-1* cells to MMS equally. Indeed, we find that CHX treatment sensitizes both *TRP1* and *trp1-1* cells to MMS at 23°C (Figure 4B). Since *TRP1* and *trp1-1* cells exhibit the same MMS sensitivity as each other at 23°C (Figure 4B), these results suggest that impaired protein synthesis underlies the MMS sensitivity normally seen in *trp1-1* cells at 23°C.

Together, we show that tryptophan depletion represents a novel method to sensitize eukaryotic cells to DNA damaging agents. This defect may act independently of many of the major DNA damage signaling and repair pathways and of the NAD⁺ dependent regulation of sirtuins, but likely reflects impaired protein synthesis. This novel role for *TRP1* in cells represents a crucial new variable for budding yeast research, as highlighted here and in a recent publication by Polleys and Bertuch which found additional roles for tryptophan biosynthesis in DNA damage repair (Pollys and Bertuch, 2015). A common method of gene disruption is to replace it with the coding sequence of an auxotrophic marker like *TRP1* (Janke, et al., 2004); however, our results indicate that use of *TRP1* auxotrophic marker alone would substantially alter the phenotype of cells grown at colder temperatures upon DNA damage, and for cells on SC medium at 30°C. Furthermore due to the common practice of depleting tryptophan in patients in clinical trials, it is important to determine how tryptophan depletion sensitizes cells to DNA damage and if this mechanism is conserved in higher eukaryotes. Budding yeast are an excellent model system for further evaluation of this damage sensitivity as induction of a tryptophan-limiting state is readily and reproducibly induced by growth at 23°C.

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LITERATURE CITED

- Abe F, Horikoshi K. Tryptophan permease gene TAT2 confers high-pressure growth in *Saccharomyces cerevisiae*. *Mol Cell Biol*. 2000; 20:8093–102. [PubMed: 11027279]
- Bedalov A, Hirao M, Posakony J, Nelson M, Simon JA. NAD⁺-dependent deacetylase Hst1p controls biosynthesis and cellular NAD⁺ levels in *Saccharomyces cerevisiae*. *Mol Cell Biol*. 2003; 23:7044–54. [PubMed: 12972620]
- Brown JA, Sherlock G, Myers CL, Burrows NM, Deng C, Wu HI, McCann KE, Troyanskaya OG, Brown JM. Global analysis of gene function in yeast by quantitative phenotypic profiling. *Mol Syst Biol*. 2006; 2:2006 0001. [PubMed: 16738548]
- Garrett JM. The control of morphogenesis in *Saccharomyces cerevisiae* by Elm1 kinase is responsive to RAS/cAMP pathway activity and tryptophan availability. *Mol Microbiol*. 1997; 26:809–20. [PubMed: 9427410]
- Giaever G, Chu AM, Ni L, Connelly C, Riles L, Veronneau S, Dow S, Lucau-Danila A, Anderson K, Andre B, Arkin AP, Astromoff A, El-Bakkoury M, Bangham R, Benito R, Brachat S, Campanaro S, Curtiss M, Davis K, Deutschbauer A, Entian KD, Flaherty P, Foury F, Garfinkel DJ, Gerstein M, Gotte D, Guldener U, Hegemann JH, Hempel S, Herman Z, Jaramillo DF, Kelly DE, Kelly SL, Kotter P, LaBonte D, Lamb DC, Lan N, Liang H, Liao H, Liu L, Luo C, Lussier M, Mao R, Menard P, Ooi SL, Revuelta JL, Roberts CJ, Rose M, Ross-Macdonald P, Scherens B, Schimmack G, Shafer B, Shoemaker DD, Sookhai-Mahadeo S, Storms RK, Strathern JN, Valle G, Voet M, Volckaert G,

- Wang CY, Ward TR, Wilhelmy J, Winzeler EA, Yang Y, Yen G, Youngman E, Yu K, Bussey H, Boeke JD, Snyder M, Philippsen P, Davis RW, Johnston M. Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature*. 2002; 418:387–91. [PubMed: 12140549]
- Godin S, Wier A, Kabbinavar F, Bratton-Palmer DS, Ghodke H, Van Houten B, Vandemark AP, Bernstein KA. The Shu complex interacts with Rad51 through the Rad51 paralogues Rad55-Rad57 to mediate error-free recombination. *Nucleic Acids Res*. 2013; 41:4525–34. [PubMed: 23460207]
- Janke C, Magiera MM, Rathfelder N, Taxis C, Reber S, Maekawa H, Moreno-Borchart A, Doenges G, Schwob E, Schiebel E, Knop M. A versatile toolbox for PCR-based tagging of yeast genes: new fluorescent proteins, more markers and promoter substitution cassettes. *Yeast*. 2004; 21:947–62. [PubMed: 15334558]
- Lea DE, Coulson CA. The distribution of the numbers of mutants in bacterial populations. *J Genet*. 1949; 49:264–85. [PubMed: 24536673]
- Lin SJ, Guarente L. Nicotinamide adenine dinucleotide, a metabolic regulator of transcription, longevity and disease. *Curr Opin Cell Biol*. 2003; 15:241–6. [PubMed: 12648681]
- Mills KD, Sinclair DA, Guarente L. MEC1-dependent redistribution of the Sir3 silencing protein from telomeres to DNA double-strand breaks. *Cell*. 1999; 97:609–20. [PubMed: 10367890]
- Polleys EJ, Bertuch AA. Tryptophan-Dependent Control of Colony Formation After DNA Damage via Sea3-Regulated TORC1 Signaling in *Saccharomyces cerevisiae*. *G3 (Bethesda)*. 2015; 5:1379–89. [PubMed: 25943524]
- Richard DM, Dawes MA, Mathias CW, Acheson A, Hill-Kapturczak N, Dougherty DM. L-Tryptophan: Basic Metabolic Functions, Behavioral Research and Therapeutic Indications. *Int J Tryptophan Res*. 2009; 2:45–60. [PubMed: 20651948]
- Svensson JP, Pesudo LQ, Fry RC, Adeleye YA, Carmichael P, Samson LD. Genomic phenotyping of the essential and non-essential yeast genome detects novel pathways for alkylation resistance. *BMC Syst Biol*. 2011; 5:157. [PubMed: 21978764]
- Thomas BJ, Rothstein R. Elevated recombination rates in transcriptionally active DNA. *Cell*. 1989; 56:619–30. [PubMed: 2645056]
- Tsukamoto Y, Kato J, Ikeda H. Silencing factors participate in DNA repair and recombination in *Saccharomyces cerevisiae*. *Nature*. 1997; 388:900–3. [PubMed: 9278054]
- Young SN. Acute tryptophan depletion in humans: a review of theoretical, practical and ethical aspects. *J Psychiatry Neurosci*. 2013; 38:294–305. [PubMed: 23428157]
- Zhao X, Muller EG, Rothstein R. A suppressor of two essential checkpoint genes identifies a novel protein that negatively affects dNTP pools. *Mol Cell*. 1998; 2:329–40. [PubMed: 9774971]

FIGURE 1.

trp1-1 W303 cells are sensitive to MMS and HU. A. The indicated strains were five-fold serially diluted onto YPD medium or YPD containing 0.02% MMS or 100mM HU and incubated for 2 days at 30°C or four days at 23°C. B. *TRP1* and *trp1-1* cells or *trp1-1* cells containing the indicated plasmid were five-fold serially diluted onto YPD medium or YPD containing 0.02% MMS or 100mM HU and incubated for two days at 30°C or four days at 23°C. C. The indicated strains were five-fold serially diluted onto SC medium or SC containing 0.02% MMS or 100mM HU and incubated for 2 days at 30°C or four days at 23°C.

FIGURE 2.

The defect caused by *trp1-1* is observed in multiple yeast strain backgrounds and is observed with deletion of any tryptophan biosynthesis gene in the BY strain background. A. *TRP1* and *trp1* cells containing the indicated plasmid are five-fold serially diluted onto YPD medium or YPD containing 100mM HU and incubated for two days at 30°C or four days at 23°C. B. *TRP+*, *trp1*, *trp2*, *trp3*, *trp4*, or *trp5* cells are five fold serially diluted and plated onto YPD, YPD with 0.02% MMS, or YPD with 100mM HU as in A. C. *TRP+*, *trp1*, *trp2*, *trp3*, *trp4*, or *trp5* cells are five fold serially diluted and plated onto SC, SC with 0.02% MMS, SC with 100mM HU as in B. Note that the tryptophan mutants, while

always more sensitive than *TRP⁺* cells to medium containing MMS or HU, exhibited variable growth between trials.

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FIGURE 3.

trp1-1 cells MMS sensitivity is independent of the major DNA repair pathways in W303 yeast. A. WT, *rev3*, *mag1*, and *rad57* cells that are *TRP1* or *trp1-1* were grown overnight at 23°C and five-fold serially diluted onto YPD medium or YPD with 0.006% MMS and incubated for two days at 30°C or four days at 23°C. B. *TRP1* and *trp1-1* cells were grown at 30°C or 23°C in SC or SC containing 0.0005% MMS to measure recombination rates as described for the direct repeat recombination assay (Godin, et al., 2013). C. WT, *tof1*, *rad9*, and *mrc1* cells that are *TRP1* or *trp1-1* were grown overnight at 23°C and diluted as in A.

FIGURE 4.

The MMS sensitivity of a *trp1-1* cell is not due to defects in NAD⁺ metabolism in W303 yeast but is due to slowed protein synthesis. A. WT, *bna2*^Δ, and *sir2*^Δ cells that are *TRP1* or *trp1-1* are grown overnight at 23°C and five-fold serially diluted onto YPD medium or YPD with 0.012% MMS and incubated for two days at 30°C or four days at 23°C. B. WT (*TRP1*) and *trp1-1* cells were grown overnight at 30°C and five-fold serially diluted onto rich YPD medium + 0.03% DMSO or YPD medium + 0.03% DMSO containing 100 ng/mL cycloheximide (CHX) either with or without 0.012% MMS. The plates were incubated at 23°C for four days or 30°C for three days prior to imaging.

Table 1

Strains used in this study

Name	Description
KBY138-1C	<i>MAT^a ADE2 TRP1 LYS2 leu2-3,112 his3-11,15 ura3-1</i>
KBY138-4A	<i>MAT^a ADE2 TRP1 lys2 leu2-3,112 his3-11,15 ura3-1</i>
KBY138-3D	<i>MAT^a ADE2 trp1-1 LYS2 leu2-3,112 his3-11,15 ura3-1</i>
KBY138-2B	<i>MAT^a ade2-1 TRP1 LYS2 leu2-3,112 his3-11,15 ura3-1</i>
W9100-2D	<i>MAT^α TRP1 lys2 ADE2 leu2-3,112 his3-11,15 ura3-1</i>
W9100-12C	<i>MAT^α trp1-1 LYS2 ADE2 leu2-3,112 his3-11,15 ura3-1</i>
BY4742 [*]	<i>MAT^α his3 1 leu2 0 ura3 0 lys2 0 RAD5</i>
KBY661 [*]	<i>MAT^α his3 1 leu2 0 ura3 0 lys2 0 RAD5 trp1::KAN</i>
KBY662 [*]	<i>MAT^α his3 1 leu2 0 ura3 0 lys2 0 RAD5 trp2::KAN</i>
KBY663 [*]	<i>MAT^α his3 1 leu2 0 ura3 0 lys2 0 RAD5 trp3::KAN</i>
KBY664 [*]	<i>MAT^α his3 1 leu2 0 ura3 0 lys2 0 RAD5 trp4::KAN</i>
KBY665 [*]	<i>MAT^α his3 1 leu2 0 ura3 0 lys2 0 RAD5 trp5::KAN</i>
KBY657-44B	<i>MAT^a LYS2 trp1-1 rad57::LEU2 ADE2 his3-11,15 ura3-1</i>
KBY657-44A	<i>MAT^a lys2 TRP1 rad57::LEU2 ADE2 his3-11,15 ura3-1</i>
KBY667-23A	<i>MAT^α rev3::HISMx6 bar1::LEU2 TRP1 LYS2 ADE2 his3-11,15 ura3-1</i>
KBY667-42B	<i>MAT^α rev3::HISMx6 bar1::LEU2 trp1-1 LYS2 ADE2 his3-11,15 ura3-1</i>
KBY353-28B	<i>MAT^a TRP1 lys2 mag1::NatNT1 ADE2 leu2- 3,112 his3-11,15 ura3-1</i>
KBY353-30D	<i>MAT^a trp1-1 LYS2 mag1::NatNT1 ADE2 leu2- 3,112 his3-11,15 ura3-1</i>
KBY225-5B	<i>MAT^a leu2 EcoRI::URA3-HO::leu2 BstII LYS2 trp1-1 ADE2 leu2-3,112 his3-11,15 ura3-1</i>
KBY995-28B	<i>MAT^α TRP1 leu2 EcoRI::URA3-HO::leu2 BstII LYS2 ADE2 leu2-3,112 his3-11,15 ura3-1</i>
KBY724-1D	<i>MAT^a TRP1 LYS2 tof1::KanMX4 ADE2 leu2-3,112 his3-11,15 ura3-1</i>
KBY724-4C	<i>MAT^a trp1-1 LYS2 tof1::KanMX4 ADE2 leu2- 3,112 his3-11,15 ura3-1</i>
KBY725-3D	<i>MAT^a rad9::HIS3 TRP1 LYS2 ADE2 leu2-3,112 his3-11,15 ura3-1</i>
KBY725-4A	<i>MAT^a rad9::HIS3 trp1-1 LYS2 ADE2 leu2-3,112 his3-11,15 ura3-1</i>
KBY726-2D	<i>MAT^α trp1-1 lys2 mrc1::KanMX4 ADE2 leu2- 3,112 his3-11,15 ura3-1</i>
KBY726-5A	<i>MAT^a TRP1 LYS2 mrc1::KANMX ADE2 leu2- 3,112 his3-11,15 ura3-1</i>
KBY853-10C	<i>MAT^α bna2::His3 TRP1 lys2 ADE2 leu2-3,112 his3-11,15 ura3-1</i>
KBY853-2A	<i>MAT^α bna2::HIS3 trp1-1 lys2 ADE2 leu2-3,112 his3-11,15 ura3-1</i>
KBY852-6A	<i>MAT^α TRP1 LYS2 sir2::NatNT2 ADE2 leu2-3,112 his3-11,15 ura3-1</i>
KBY852-10A	<i>MAT^a trp1-1 LYS2 sir2::NatNT2 ADE2 leu2-3,112 his3-11,15 ura3-1</i>

^{*} Denotes a BY alpha deletion library strain or the corresponding control (Giaever, et al., 2002).