Supplementary Material (ESI) for Molecular BioSystems
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A metabolomic and multivariate statistical process to assess the effects of genotoxins in Saccharomyces cerevisiae

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Detailed description of compounds:

Methyl Methane Sulfonate (MMS – Aldrich 129925): MMS is a direct DNA alkylating agent (Lee et al., 1992). It is classified as a clastogen and causes alkylation which can lead to strand breaks (Lundin et al., 2005). It will activate the Base Excision Repair pathway and causes inhibition of DNA synthesis. MMS causes methylation of 7-deoxyguanine as well as 3-deoxyadenine. Initial experiments used an exposure level of 0.2 % (v/v) in liquid rich YPA-D media. The sensitivity of the yeast to this chemical was also assessed using a number of doses in rich YPA-D media with the lowest being 0.005 % (v/v) (Dose series 0.2 %, 0.15 %, 0.1 %, 0.05 %, 0.02 %, 0.01 %, 0.005 %). The final experiment in Footprinting media MMS was used at 0.0025 % based on the concentration used to assess the Green Screen methods (Cahill et al., 2004).

Ethyl Methane Sulfonate (EMS – Sigma M0880): EMS is an alkylating agent similar to MMS; it is a direct ethylating agent and is classified as both a mutagen and a carcinogen. EMS leads to an activation of Base Excision Repair pathways along with Non-Homologous End Joining. In the Footprinting media this was used at a concentration of 0.005 %.

Dimethyl sulfoxide (DMSO – Sigma D8418): This is the main vehicle for all the agents used and was assessed to determine its effect on the yeast. It is known that at higher concentrations DMSO can affect the integrity of the cell wall. In the initial experiments DMSO was tested at 0.25 % (v/v) in Rich YPA-D media. In the final experiments in Footprinting media DMSO was tested at two different concentrations, one at 0.2 % (v/v) and the second at a higher concentration of 1 % (v/v).

4-nitroquinoline 1-oxide (4NQO – Sigma N8141): This was solubulised in DMSO as a stock solution of 10 mg/ ml. 4NQO was used in the initial experiments at 10 μg/ ml and then in the final experiments in the Footprinting media it was used at 1 μg/ml according to the concentration used in the Green Screen methods.

Phleomycin (Phl – Sigma P9564): Phleomycin is structurally related to the glycopeptide antibiotic Bleomycin (Gavin et al., 1998) which causes a broad spectrum response. It similarly blocks S-phase entry into the cell cycle by intercalating into the DNA which is then degraded. It can also cause perturbances to membrane stability (Bennett, 1999) and DNA damage, requiring RAD6 repair in yeast and homologous recombination pathways. Phl was prepared as a stock solution of 10 mg/ ml and used at 10 μg/ ml in the initial experiments in Rich YPA-D media; this is consistent with the Green Screen methods that used 12 μg/ ml. In the final experiment in the Footprinting media Phl was added at 1 μg/ ml.

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N-nitroso-N-methylurea (MNU – Sigma N1517): MNU is an alkylating agent and is classified as a carcinogen (Gorin et al., 1976). MNU stock solution of 10 mg/ ml was made up in 1:1 water: DMSO. It was not used in the initial experiments but was used at 10 μg/ ml in the Footprinting media.

N-nitroso-N-ethylurea (ENU – Sigma N3385): ENU is a DNA alkylating agent similar to MNU. It was prepared as a 25 mg/ ml solution in 1:1 water: DMSO. It was not used in the initial experiment but was used at 25 μg/ ml in the Footprinting media.

Nocodazole (NOC – Sigma N1404): This antimicrobial is not a genotoxin but disrupts microtubules as it binds to β-tubulin and prevents the formation of an essential disulphide bond. The consequence of this is a reduction or inhibition in microtubule movement which results in cell cycle arrest at G2/M phase. NOC was made as a 15 mg/ ml solution in water and added to the initial experiments in Rich YPA-D media at 15 μg/ ml.

Actinomycin D (ACT – Sigma A4262): ACT is an antibiotic that inhibits cell proliferation by binding to but not directly damaging DNA. It blocks the RNA polymerase and also DNA polymerase, which results in apoptosis of cells (Pincus and Rekosh, 1984). A stock solution was made at 60 mg/ ml and added to the Footprinting media at 60 μg/ ml.

Mitomycin C (MIT – Aldrich 69824): MIT inhibits DNA synthesis and is used as an antibacterial for both gram positive and gram negative bacteria (Galloway et al., 1998). MIT was prepared as a 100 mg/ ml stock and added to the Footprinting media at 100 μg/ ml.

Ampicillin (AMP – Sigma A9516): β-Lactam antibiotic that affects the peptidoglycan cross linkages in bacterial membranes. It is not a genotoxin and is not known to cause DNA damage in yeast. A stock solution of 0.8 g/ ml was prepared in water and AMP was added to the Footprinting media at 8 mg/ ml.

Methotrexate (MET – Sigma M9929): Methotrexate targets dihydrofolate reductase and as folate is required for purine synthesis inhibits DNA synthesis (Giaever et al., 2004). A 1 mg/ ml stock solution was prepared and MET was added to the footprinting media at 1μg/ ml (Miyajima et al., 1984).

5-Fluorocytosine (5-FU – Sigma F7129): 5-FU causes inhibition of the thymidylate synthase and leads to a misincorporation of fluoronucleotides into RNA, which affects RNA processing and incorporation of these into DNA (Giaever et al., 2004). From a 10 mg/ ml stock of 5-FU 10 μg/ ml was added to Fingerprinting media (Seiple et al., 2006).

Ethanol (EtOH– HPLC Grade): Ethanol is not a mutagen. It was added to the Fingerprinting media at 5 % (v/v) (Hu et al., 2007; You et al., 2003).

Carbendazim (CAR – Aldrich 45368): This is a benzimidazole derivative and has properties similar to nocodazole. A 1 % stock was prepared in DMSO and added to the Finger printing media to a final concentration 25 μg/ ml (Poddar et al., 2005; Soustelle et al., 2004).

Thiabendazole (THIA – Sigma T8904): This anti-fungal is a used as a common treatment to athlete's foot. It affects tubulin and causes an arrest in the spindle apparatus (Bylebyl et al., 2003).

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Cytosine β-D arabinofuranoside (araC – Sigma C1768): araC is converted into cytosine arabinoside triphosphosphate which then affects DNA incorporation, DNA and RNA polymerases and causes cell cycle arrest in S-phase (McIntosh and Haynes, 1984). A 10 mM stock was prepared and then added to a final concentration of 10 μM in the Footprinting media.

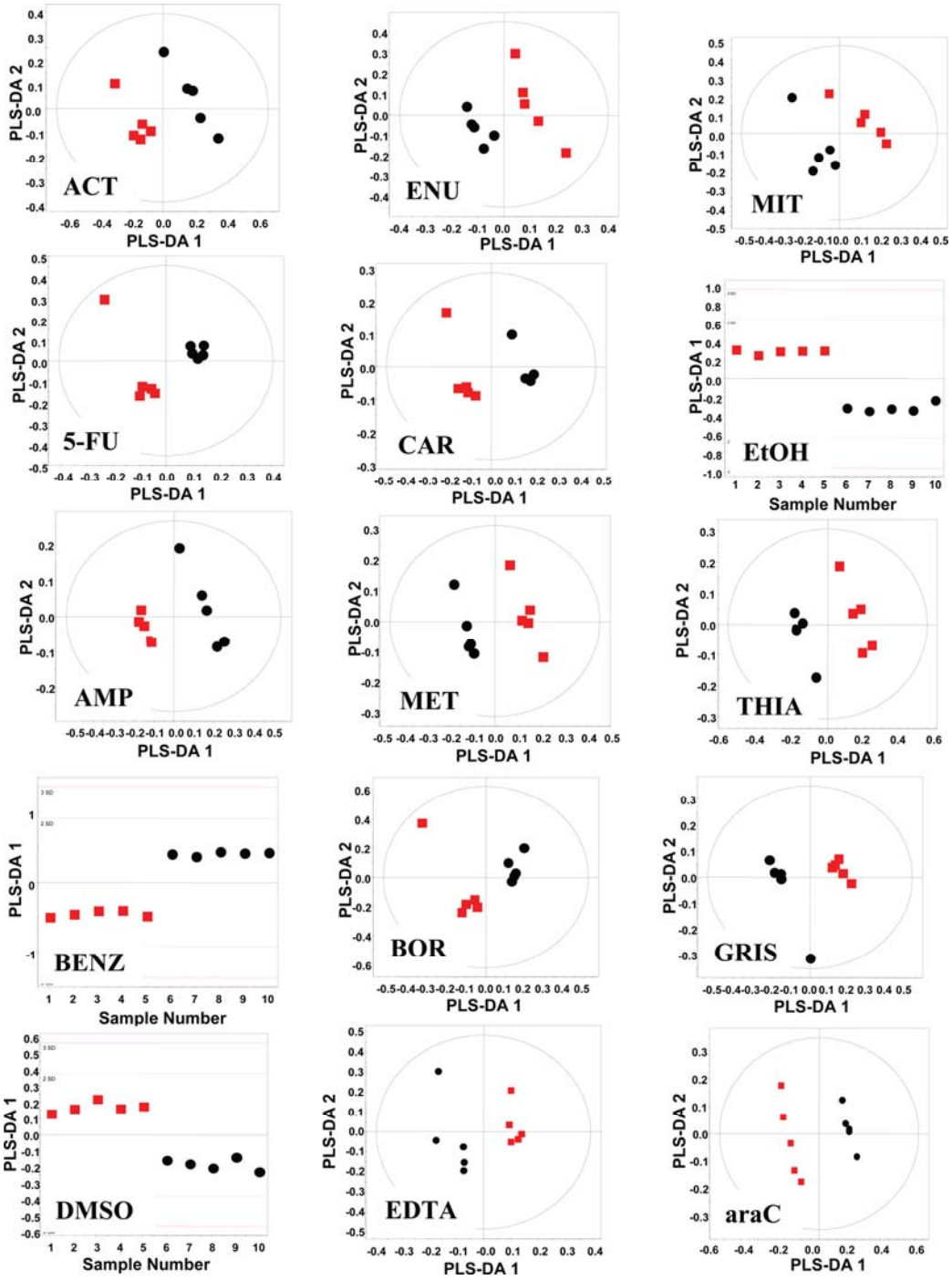
Griseofulvin (GRIS – Sigma G4753): GRIS is an anti-fungal that binds to β-tubulin and affects the spindle apparatus causing spindle disruption and ultimately growth inhibition by cell cycle arrest (Savoie et al., 2003).

Boric acid (BOR – Aldrich 31146): A Non-Genotoxin, added to the Footprinting media at a final concentration of 10mM.

Ethylene diaminetetra-acetic acid (EDTA – Sigma E5134): This is a chelating agent and not a mutagen. It was added at 0.3 mM final concentration.

Benzyl acetate (BENZ – Aldrich 50475): **Benzyl acetate is not a mutagen. It was added to the Footprinting media at a final concentration of 0.05 %.**

Supplementary Figure 1: Analyses of NMR spectra of yeast extracts by PLS-DA from yeast samples exposed to **ACT** ($R^2X = 0.582$, $R^2Y = 0.977$, $Q^2 = 0.850$), **ENU** ($R^2X = 441$, $R^2Y = 0.905$, $Q^2 = 0.776$), **MIT** ($R^2X = 0.561$, $R^2Y = 0.998$, $Q^2 = 0.581$), **5-FU** ($R^2X = 0.445$, $R^2Y = 0.972$, $Q^2 = 0.800$), **CAR** ($R^2X = 0.391$, $R^2Y = 0.982$, $Q^2 = 0.777$), **EtOH** ($R^2X = 0.629$, $R^2Y = 0.988$, $Q^2 = 0.980$), **AMP** ($R^2X = 0.369$, $R^2Y = 0.994$, $Q^2 = 0.741$), **MET** ($R^2X = 0.373$, $R^2Y = 0.991$, $Q^2 = 0.777$), **THIA** ($R^2X = 0.458$, $R^2Y = 0.983$, $Q^2 = 0.798$), **BENZ** ($R^2X = 0.736$, $R^2Y = 0.994$, $Q^2 = 0.988$), **BOR** ($R^2X = 0.606$, $R^2Y = 0.932$, $Q^2 = 0.687$, **GRIS** ($R^2X = 0.432$, $R^2Y = 0.988$, $Q^2 = 0.675$), **araC** ($R^2X = 0.435$, $R^2Y = 0.997$, $Q^2 = 0.992$), **EDTA** ($R^2X = 0.299$, $R^2Y = 0.950$, $Q^2 = 0.245$) and **DMSO-2** (at 1 %) ($R^2X = 0.326$, $R^2Y = 0.977$, $Q^2 = 0.826$). Control yeast cultures are shown in black, yeast exposed to chemical are shown in red.



Supplementary Table 1: Summary of metabolite changes following PLS-DA analysis of NMR data from extracts of yeast cells exposed to Actinomycin D (ACT), N-nitroso-N-ethylurea (ENU), Mitomycin C (MIT), 5-fluorocyosine (5-FU), Carbendazim (CAR), Ethanol (EtOH), Ampicillin (AMP), Methotrexate (MET), Thiabendazole (THIA), Benzyl acetate (BENZ), Boric acid (BOR), Griseofulvin (GRIS), demiethyl sulfoxide (DMSO), ethylene diaminetetra- acetic acid (EDTA), cytosine β-D arabinofuranoside (araC), methyl methane sulfonate (MMS), ethyl methane sulfonate (EMS), 4-nitroquinoline 1-oxide (4NQO), Phleomycin (Phl), N-nitroso-N-methylurea (MNU), N-nitroso-N-ethylurea (ENU) and DMSO.

Chemical	Metabolite Change — Increase / Decrease
ACT	Acetate (1.88), Arginine/ Lysine (1.72, 3.24, 3.20, 3.00), Succinate (2.40) Glutamine (2.12, 2.16, 2.44), Glutamate (2.36, 2.32), N-acetyl groups (2.00, 2.04), Isoleucine/ Leucine/ Valine (0.92, 0.96) Histidine (7.72)
ENU	Lysine (3.00, 3.56), Arginine (3.24, 3.56) Alanine (1.48), Glutamine (2.12, 2.44), Isoleucine/ Leucine/ Valine (0.96, 1.04), Lactate (1.32)
MIT	Lysine (3.76, 3.04), Succinate (2.40), Glucose (3.64, 3.88), Aspartate (2.84) Glutamine (2.44, 2.12, 2.08), Glutamate (2.36), N-acetyl groups (2.00), Histidine (7.72),
5-FU	Glutamine (2.16), Lysine (1.72, 3.04) Arginine (1.76, 3.20, 3.24), Isoleucine/ Leucine/ Valine (1.00) Lactate (1.32), Alanine (1.44), N-acetyl groups (2.00, 2.04),
CAR	Glucose (3.84), Glutamate (2.36), Lysine (3.04, 1.72, .176) Glutamine (2.12, 2.44), Isoleucine/ Leucine/ Valine (0.92, 0.96), Lactate (1.32), Histidine (7.72)
EtOH	Glutamine (2, 08, 2.12, 2.44), Glutamate (2.36, 2.32), N-acetyl groups (2.00, 2.04), Alanine (1.44), Lactate (1.32), Isoleucine/ Leucine/ Valine (1.00) Acetate (1.88), Lysine (3.00, 1.72), Arginine (1.68, 3.20, 3.24), Histidine (7.72)
AMP	Alanine (1.48), Arginine (3.20, 1.72, 3.76), Succinate (2.40), Glucose (3.88, 3.64) Isoleucine/ Leucine/ Valine (0.92, 0.96), Glutamate/ Glutamine (2.44, 2.32, 2.12), Lactate (1.32), N-acetyl groups (2.00, 2.04) Threonine (4.20, 4.24)
MET	Glutamate (2.36), Lysine/ Arginine (3.04, 3.24, 1.72, 3.76), Succinate (2.40) Isoleucine/ Leucine/ Valine (0.92, 0.96, 1.00), Lactate (1.32), Histidine (7.72)
THIA	Alanine (1.44, 1.48), Lysine/ Arginine (1.72, 3.00, 3.24) Glutamine (2.12, 2.44), Isoleucine/ Leucine/ Valine (0.92, 0.96), Lactate (1.32), Glutamate (2.32), N-acetyl groups (2.00)
BENZ	Alanine (1.44), Arginine (3.20, 3.24, 3.76), Glutamate (2.32, 2.36), Threonine (4.20, 4.24), Lactate (1.32), N-acetyl groups (2.04) Acetate (1.88), Lysine (3.00, 1.72), Glutamine (2.44, 2.12), Isoleucine/ Leucine/ Valine (0.92, 0.96), Succinate (2.40)
BOR	Glutamate (2.32, 2.36), Succinate (2.40), Arginine (3.24), Isoleucine/ Valine/ Leucine (1.00) Acetate (1.88), Alanine (1.44), Glucose (3.80)
GRIS	Glutamine (2.12), Lysine/ Arginine (3.72),Glutamate (2.32), Lactate (1.32), Succinate (2.40), Threonine (4.24), Isoleucine/ Leucine/ Valine (1.00) Acetate (1.88), Alanine (1.44), N-acetyl groups (2.00), Phenylalanine (3.96)
DMSO-2	Lysine (3.00, 3.04, 1.72), Alanine (1.44, 1.48) Acetate (1.88), Glutamine (2.12, 2.44), Lactate (1.32), Glucose (3.88, 3.80) Isoleucine/ Leucine/ Valine (0.96, 1.00)
EDTA	Arginine/ Lysine (1.84, 3.24, 3.08) Acetate (1.88), Glutamine (2.12, 2.16)
araC	Arginine (1.68, 3.68), Alanine (1.44), Lysine (1.72, 3.00, 3.04) Acetate (1.88), Isoleucine/ Leucine/ Valine (0.96, 1.00), Lactate (1.32), Glutamine (2.12, 2.44), Glucose (3.80, 3.88, 3.84)