Supplemental text and Supplemental Table legends for:

Mitochondrial genome variation affects multiple respiration and non-respiration phenotypes in *Saccharomyces cerevisiae*

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**Supplemental text:** Analysis of the oligomycin phenotype-*WHI2* genotype association, identification of additional *WHI2* genotype-dependent phenotypes, and assessment of epistasis.

**Introduction:**

In a species as well-studied as *S. cerevisiae*, previously described phenotypes, such as oligomycin sensitivity/resistance, and annotated functions, such as genes encoding components of the oligomycin sensitive ATP synthase, offer a direct way to assess nuclear genotype associations. One phenotypic/functional annotation-based hypothesis is that respiration inhibitor phenotype-nuclear genotype associations will identify one or more of the nuclear genes that encode most of the components of mitochondrially-localized complexes, such as the mitochondrial ribosome (inhibited by chloramphenicol, erythromycin, and spiramycin), cytochrome bc1 (inhibited by antimycin A and myxothiazol), and ATP synthase (inhibited by oligomycin). However, none of the respiration phenotype-nuclear genotype associations identified nuclear genes functionally annotated as encoding components of the corresponding mitochondrially-localized complexes (Table S8). Thus, we chose to experimentally test the annotation-independent oligomycin phenotype-*whi2* genotype association.

**Methods:**

**Deletion of *WHI2*:** The *whi2*∆ amplicons were generated by PCR amplification (using OneTaq polymerase (NEB)) with oligos SV125 and SV126 (Table S12) using either pUG-amdSYM (Solis-Escalante *et al.* 2013) or pAG25 (Goldstein and McCusker 1999) as templates, which generated 2.4 kb *whi2*∆::amdSYM and 1.3kb *whi2*∆::NATMX4 amplicons, respectively. The *whi2*∆::amdSYM and *whi2*∆::NATMX4 amplicons were introduced into haploid *WHI2* strains (Table S1) using the standard PEG/LiAc procedure (Gietz and Schiestl 2007); transformants were selected on synthetic dextrose minimal media containing 0.6g/L acetamide as the nitrogen source (Solis-Escalante *et al.* 2013) or on YPD supplemented with 100mg/L nourseothricin (Goldstein and McCusker 1999), respectively. The *whi2*∆ genotypes of transformants were verified by PCR using oligos SV127 and SV128 (Table S12). The pUG-amdSYM plasmid was obtained from, and should be requested from, EUROSCARF. The pAG25 plasmid has been deposited with, and should be requested from, Addgene <http://www.addgene.org/John_McCusker/>.

**Construction of pAG36::WHI2:** Using genomic DNA from an S288c background strain as template, *WHI2* (from 450 bp upstream of the start codon and 200 bp downstream of the stop codon) was amplified (using OneTaq polymerase (NEB)) with oligos SV141 and SV142 (Table S12). The resulting 2.1 kb *WHI2*-containing amplicon was purified and digested with *Sal*I-HF and *Pac*I (NEB). The *Sal*I-*Pac*I digested *WHI2*-containing amplicon was purified and ligated to *Sal*I-*Pac*I digested and purified pAG36 (Goldstein and McCusker 1999) using T4 DNA ligase (NEB). All enzymatic reactions were carried out as per the manufacturer’s recommendations. The ligation mix was introduced into *E.coli* DH5α (Thermo, sub-cloning efficiency) and transformants were screened for *WHI2*-containing pAG36 by restriction digestion and agarose gel electrophoresis. The pAG36 and pAG36::*WHI2* plasmids have been deposited with, and should be requested from, Addgene <http://www.addgene.org/John_McCusker/>.

**Results and Discussion**

**General stress response regulator *WHI2* genotype affects oligomycin and other inhibitor phenotypes:** Although the Saccharomyces Genome Database showed no previous descriptions of *whi2* null effects on sensitivity to oligomycin or to any of the other inhibitors analyzed in this study, grouping seven strains with loss-of-function *whi2* polymorphisms identified an association with oligomycin sensitivity (Table S8). Analysis of full-length *WHI2* ORF sequences identified 33 DNA and 20 protein sequences (Table S13). As a first test of the hypothesis that *WHI2* genotype contributes to the oligomycin phenotype, we introduced *whi2*∆ into haploid derivatives of the YJM627, YJM1443, YJM195, YJM145 (isogenic with YJM789 (Wei *et al.* 2007)), and S288c genetic backgrounds, all of which have full-length, presumably functional *WHI2* ORFs. We determined the oligomycin phenotypes of the isogenic *WHI2* vs. *whi2*∆ strain pairs and found in all cases that the *whi2*∆ strains were more oligomycin sensitive (Fig. S7, Table S14). We also determined additional inhibitor phenotypes (dextrose carbon source: ± ketoconazole, ± cycloheximide; ethanol carbon source: ± spiramycin, ± myxothiazol) of the isogenic *WHI2* vs. *whi2*∆ haploid strain pairs. We found that *whi2*∆, while having no effect on growth on control media, had background-specific effects on cycloheximide, spiramycin, and myxothiazol phenotypes (Fig. S7, Table S14). To confirm the *WHI2*-dependence of phenotypes, we separately introduced pAG36 (vector control) (Goldstein and McCusker 1999) and pAG36::*WHI2* (containing S288c-derived *WHI2*) into the haploid *whi2*∆ derivatives of the YJM627 (OliS) and YJM195 (OliS, MyxS) backgrounds; pAG36::*WHI2* complemented the inhibitor sensitive phenotypes in both genetic backgrounds (Fig. S7).

 As a second test of the hypothesis that *WHI2* genotype contributes to oligomycin and other inhibitor phenotypes, we separately introduced pAG36 (Goldstein and McCusker 1999) and pAG36::*WHI2* into 20 OliS *ATP6*S (ORF SNPs: 753, 763, 766 bp) diploid strains with a variety of *WHI2* genotypes (*whi2* loss-of-function polymorphisms (n=7); *WHI2* full-length ORFs with different amino acid sequences (n=13)) and determined six inhibitor phenotypes. We found that pAG36::*WHI2* had no effect on growth on the YPE control medium of 19 strains. However, in YJM1199, one of six strains with the most common *WHI2* amino acid sequence, pAG36::*WHI2* improved growth on control media (Fig. S8), which precluded determining plasmid-borne *WHI2* effects on its inhibitor phenotypes. In 10 of the 19 strains, pAG36::*WHI2* had no effect on any of the tested inhibitor phenotypes. However, in the 9 remaining strains, pAG36::*WHI2* increased some inhibitor resistance phenotypes (Table S15, Fig. S8, Fig. S9).

 Consistent with the oligomycin phenotype-*whi2* genotypeassociation, analysis of *WHI2* vs. *whi2*∆ strain pairs showed that *WHI2* contributes to oligomycin resistance. In addition, *WHI2* also contributed to other respiration inhibitor resistance phenotypes, as well as one non-respiration inhibitor resistance phenotype, with epistasis that likely contributes to the lack of association with these other inhibitor phenotypes. Similarly, analysis of vector- vs. CEN plasmid-borne, S288c-derived *WHI2*-containing strains also showed that *WHI2* contributes to oligomycin and/or other resistance phenotypes, again with epistasis. In some cases, contributions of CEN plasmid-borne, S288c-derived *WHI2* are likely due to increased Whi2 levels. For example, for two of the five strains that have the same *WHI2* amino acid sequence as S288c, YJM193 and YJM428, the effect of CEN plasmid-borne, S288c-derived *WHI2* were consistent with increased Whi2 levels. In other cases, epistatic contributions of CEN plasmid-borne, S288c-derived *WHI2* may arise from the sequences or expression levels of genes that encode Whi2 binding partners, such as *PSR1*, *MSN2*, and *AKR1* (Kaida *et al.* 2002; Hwang *et al.* 2016), and/or from differences in *WHI2* genotype; that is, CEN plasmid-borne, S288c-derived *WHI2* vs. chromosomal *WHI2*.

**Hypotheses for Whi2 effects on respiration inhibitor phenotypes:** *WHI2* encodes a general stress response activator that contributes to stress response (Kaida *et al.* 2002) and to mitophagy (Mendl *et al.* 2011), as well as to nutritional sensing and mitochondrial morphology (Leadsham *et al.* 2009) but Whi2 does not localize to the mitochondrion but rather to the cell periphery (Yofe *et al.* 2016). Negative genetic interactions have been described between *whi2*∆ and two components of the retrograde signaling pathway, *rtg1*∆ and *rtg2*∆ (Aguilar *et al.* 2010; Costanzo *et al.* 2010). Interestingly, *whi2* loss-of-function mutations have been repeatedly isolated in experimental evolution studies and identified as suppressors of deletion mutations in multiple genes (Cheng *et al.* 2008; Mendl *et al.* 2011; Lang *et al.* 2013; Teng *et al.* 2013; Kryazhimskiy *et al.* 2014; Szamecz *et al.* 2014; van Leeuwen *et al.* 2016; Comyn *et al.* 2017; Gorter *et al.* 2018).

 One hypothesis for *WHI2* genotype-dependent effects on respiration inhibitor phenotypes is that mitochondrial genome copy number is reduced in *whi2* strains, which might increase sensitivity to respiration inhibitors. Although there was no significant association between *whi2* loss-of-function polymorphisms and mitochondrial genome copy number, the sample size was small (n=7) and mitochondrial genome copy number was determined in the presence of glucose and at one cell density. A second hypothesis for these results is that functional Whi2, and presumably the Whi2-activated general stress response, may aid adaptation to some inhibitor-induced stresses with both inhibitor- and genetic background-specificity. Conversely, based on the frequency of *whi2* loss-of-function polymorphisms, as well as advantageous *whi2* loss-of-function mutations (Cheng *et al.* 2008; Mendl *et al.* 2011; Lang *et al.* 2013; Teng *et al.* 2013; Kryazhimskiy *et al.* 2014; Szamecz *et al.* 2014; van Leeuwen *et al.* 2016; Comyn *et al.* 2017; Gorter *et al.* 2018), functional Whi2, and possibly the Whi2-activated general stress response, may be disadvantageous with environment- and/or genetic background-specificity.

**Supplementary Table legends**

**Table S1: *S. cerevisiae* strains**

The 100-genomes strains (Strope *et al.* 2015) and additional isogenic haploid strains used to construct *whi2*∆ strains and iso-nuclear F1 diploids are listed. YJM145 is the prototrophic *HO* (2N) parent of and is isogenic with YJM789 (Wei *et al.* 2007). YJM627 (*HO*, 2N) is a single spore clone of and is isogenic with Y55 (*HO*). YJM1552 (2N) is isogenic with S288c. YJM1870 (*ho*∆/*ho*∆) is isogenic with sequenced YJM1250 (*HO*). YJM1846 is isogenic with sequenced YJM1388 (*ho*), which was diploidized to generate YJM1846. YJM1847 is isogenic with sequenced YJM1419 (*ho*), which was diploidized to generate YJM1847. YJM1869 (*HO*/*HO* *HIS3*/*HIS3*) is isogenic with sequenced YJM1433 (*HO*/*HO* *his3*/*his3*). YJM1628 (*HO*/*HO* *COX15*/*COX15*) is isogenic with YJM1615 (*HO*/*HO* *cox15*-ochre/*cox15*-ochre) and YJM421 (*HO*/*HO* *SUP7*-ochre/*sup7* *cox15*-ochre/*cox15*-ochre) (Ito-Harashima *et al.* 2002).

**Table S2: Respiration and non-respiration inhibitors**

The inhibitors used in this study, along with the concentrations and media used, target(s), and mitochondrial genes in which resistance mutations have been previously identified, are listed. Spot dilution and/or high throughput inhibitor phenotypes on non-fermentable carbon source(s): Nourseothricin, Paromomycin, Hygromycin B, G418, Chloramphenicol, Erythromycin, Spiramycin, Oligomycin, Antimycin A, Myxothiazol, Ethidium bromide, Methotrexate, 5-fluoro-uracil, 5-fluoro-cytosine. Spot dilution inhibitor phenotypes on dextrose carbon source: Cycloheximide, Ketoconazole, Cupric sulfate (copper); high throughput inhibitor phenotypes were previously determined (Strope *et al.* 2015). ND = not determined or unknown; NA = not applicable.

**Table S3: *S. cerevisiae* inhibitor phenotypes**

High throughput respiration inhibitor phenotypes are ratios of + inhibitor/- inhibitor colony sizes, as previously described (Strope *et al.* 2015). Respiration inhibitor concentrations and media are listed in Table S2. Media + inhibitor (incubation time): SE+Chloramphenicol (48h); SE+Erythromycin (96h); SE+Spiramycin (96h); YPE+Paromomycin (48h); YPE+Hygromycin (48h); YPE+G418 (48h); YPE+nourseothricin (48h); SE+AntimycinA (48h); SE+Myxothiazol (96h); SE+Oligomycin (96h); SE+Ethidium Bromide (96h); YPE+ Ethidium Bromide (48h); SE+Methotrexate (96h); SE+5-fluorocytosine (96h); SE+5-fluorouracil (96h).

**Table S4: *S. cerevisiae* mitochondrial polymorphisms used for association**

The subset of mitochondrial genome polymorphisms (n=180; bi-allelic and minor allele frequency ≥ 5%) used for testing mitochondrial genotype associations with strain origins, populations, and phenotypes. The full set of *S. cerevisiae* mitochondrial polymorphisms are listed in Table S6 and Table S7.

**Table S5: *S. cerevisiae* mitochondrial genome sizes and copy numbers**

Mitochondrial genome copy numbers for the 93 strains were determined as described in Methods. ND = Not Determined.

**Table S6: *S. cerevisiae* mitochondrial polymorphisms**

For the RNA-encoding *15S*, *21S*, *RPM1*, and tRNA genes, the locations of SNPs (e.g. T->A), insertions (# ins), and deletions (# del) are denoted. For the protein-encoding genes, the locations of synonymous SNPs (e.g. T->A), non-synonymous SNPs (e.g. G->T(N)), insertions (# ins), and deletions (# del) are denoted. For *RF1*, the sequence in the Saccharomyces Genome Database is the reference. For *ENS2*, the sequence in (Nakagawa *et al.* 1991) is the reference. For all other genes, the S288c mitochondrial gene (Foury *et al.* 1998) is the reference. For Ens2 cut sites (i.e. recognition sequences), the sequences identified by Nakagawa, et al. (Nakagawa *et al.* 1992) are given as are the sequences in the 96 strains (n=# of the 96 strains with a specific Ens2 recognition sequence) and the Sc ATP6 group in which each is found; polymorphisms relative to S288c (our reference) are underlined.

 For *ATP6*, the six *S. cerevisiae* *ATP6* groups (Sc Group1 – 5 and 1399) are listed; see also Fig. 1, Fig. 3, Fig. S1, Fig. S2; in addition, strains are grouped by Ens2 recognition sequence (713-738 bp) (Nakagawa *et al.* 1992), oligomycin phenotype-associated SNPs (753, 763, 766 bp), the presence/absence of *ENS2* sequences, and *ENS2* group (A, B, 1399). Also shown are the distances between the 3’ end of the 780 bp *ATP6* ORF and the 5’ end of the *ENS2* ORF; *ENS2* ORF sizes; *ENS2* groups (A, B, 1399), and oligomycin phenotypes. (+/- Oli ratio): High throughput oligomycin phenotypes are ratios of + oligomycin/- oligomycin colony sizes (SE ± 0.5 mg oligomycin/L at 96 h), as we previously described (Strope *et al.* 2015). For Ens2 recognition sequences, the two Ens2 recognition sequences (713-738 bp of *ATP6* ORF) identified by (Nakagawa *et al.* 1992) are shown, as well as the corresponding sequences (polymorphisms underlined) identified in *ATP6* in this study, relative to S288c (our reference), as well as the numbers of strains (n=#) and the ATP6 groups with each sequence. For *ENS2*, the three *S. cerevisiae* *ENS2* groups (Sc Group A, B, and 1399) are listed.

**Table S7: *S. cerevisiae* mitochondrial introns**

Introns in the *21S*, *COX1*, and *COB* genes are shown.

**Table S8: *S. cerevisiae* respiration inhibitor phenotype associations with nuclear and mitochondrial polymorphisms**

Respiration inhibitor abbreviations: Antimycin A (Ant), Erythromycin (Ery), Ethidium Bromide (EtBr), Oligomycin (Oli), Spiramycin (Spi), Hygromycin B (Hyg), chloramphenicol (CAM), Myxothiazol (Myx). Respiration inhibitor phenotypes are listed in Table S3. The mitochondrial polymorphisms used to assess inhibitor associations are listed in Table S4.

**Table S9:** **Mann-Whitney U-tests of *ENS2*-oligomycin phenotype associations.**

For each condition (SE ± 0.5 mg oligomycin at 72 hrs; SE ± 0.5 mg oligomycin at 96 hrs; YPE ± 1 mg oligomycin at 24 hrs; YPE ± 1 mg oligomycin at 48 hrs), the association of oligomycin phenotype (ratios of + oligomycin/- oligomycin colony sizes) was assessed with *ens2* sequence presence vs. *ens2*0 (i.e. absence of *ens2* sequences); full-length, potentially functional *ENS2* ORFs vs. *ens2* with ORF shortening polymorphisms plus *ens2*0; and full-length, potentially functional *ENS2* ORFs vs. *ens2* with ORF shortening polymorphisms. Only the *ens2* sequence presence vs. *ens2*0 association with oligomycin phenotype was significant. The other two oligomycin phenotype associations that considered *ENS2* function were not significant, consistent with Ens2 function(s) not contributing to oligomycin phenotype.

**Table S10:** **Mitochondrial genotype-dependent spot dilution phenotypes in eight iso-nuclear F1 pairs from 12 genetic backgrounds.**

Background: genetic backgrounds of haploid ρ+ and ρ0 strains crossed to generate iso-nuclear F1 pairs. Oli: the high throughput ± oligomycin phenotype ratio for each of the 12 genetic backgrounds (Table S8). *ATP6* genotype: *ATP6* SNPs (ORF: 753, 763, 766), which are in complete linkage disequilibrium and associate with oligomycin resistance (R) vs. sensitivity (S) for each genetic background (Table S6, Table S8). Iso-nuclear F1 ρ genotype-dependent phenotypes (ethanol carbon source): Oli (oligomycin), Myx (myxothiazol), Spi (spiramycin), Mtx (methotrexate), Ant (antimycin A), Ery (erythromycin), Chl (chloramphenicol), EtBr (ethidium bromide), YPE (growth temperature). Iso-nuclear F1 ρ genotype-dependent phenotypes (dextrose carbon source): Cyh (cycloheximide), Ket (ketoconazole), Cu (copper), YPD (growth temperature). T: Transgressive (i.e. opposite of expected from parental phenotypes) ρ genotype-dependent phenotypes in iso-nuclear F1.

**Table S11: Iso-nuclear F1 pairs from the 14×14 matrix with major effect mitochondrial genotype-dependent phenotypes.**

Iso-nuclear F1: crosses of 14 different genetic backgrounds. *ATP6*: ORF SNPs (753, 763, 766) that are in complete linkage disequilibrium and associate with oligomycin resistance (R; n=7 genetic backgrounds) vs. sensitivity (S; n=7 genetic backgrounds) (Table S6). Mitochondrial genotype-dependent phenotypes in iso-nuclear F1 pairs (ethanol carbon source): Oli (oligomycin), Myx (myxothiazol). ρ genotype-dependent phenotypes in iso-nuclear F1 pairs (dextrose carbon source): Cyh (cycloheximide), Ket (ketoconazole). T: Transgressive iso-nuclear F1 ρ genotype-dependent phenotypes. Iso-nuclear F1 pairs not listed had equivalent phenotypes, including some *ATP6*R vs. *ATP6*S iso-nuclear F1 pairs with equivalent oligomycin phenotypes, consistent with epistasis.

**Table 12: *WHI2* primers**

SV125 and SV126: Primers used to construct *whi2*∆; genomic *WHI2* homology (upper case); MX cassette homology (lower case).

SV127 and SV128: Primers used to verify *whi2*∆.

SV141 and SV142: Primers used to clone S288c *WHI2*; *Sal*I and *Pac*I sites used in cloning (lower case).

**Table S13: *S. cerevisiae* *WHI2* polymorphisms**

Non-synonymous (N) and synonymous (S) SNP locations are shown. Shown in red are the locations of loss-of-function polymorphisms: ATG (start) 🡪 ACG; sense to nonsense/premature STOP codon; and out-of-frame insertions.

**Table S14: *WHI2* *vs*. *whi2*∆ inhibitor phenotypes in five genetic backgrounds**

Inhibitor phenotypes of isogenic strain pairs: (=) *WHI2* = *whi2*∆; (>) *WHI2* > *whi2*∆; (ND) = Not Determined. Inhibitors (dextrose as carbon source): Ket (ketoconazole); Cyh (cycloheximide). Inhibitors (ethanol as carbon source): Spi (spiramycin); Myx (myxothiazol); Oli (oligomycin).

**Table S15: Inhibitor phenotypes of isogenic pAG36::WHI2- vs. pAG36-containing strain pairs**

Phenotypes were determined on YPE + nourseothricin to select for plasmid maintenance. Inhibitor phenotypes of isogenic pAG36::WHI2- vs. pAG36-containing strain pairs: (=) pAG36::WHI2 = pAG36; (>) pAG36::WHI2 > pAG36; (NA) = Not Applicable; the effect of pAG36::WHI2 on inhibitor phenotypes could not be determined because of its effect on growth on YPE control medium. YPE: inhibitor-free control medium. Inhibitors (ethanol as carbon source): EtBr (Ethidium Bromide); Ery (erythromycin); Spi (spiramycin); Antimycin A (Ant); Myx (myxothiazol); Oli (oligomycin). YJM244, 248, 1242, 1244, 1342, 1355, and 1479 have different inactivating *whi2* polymorphisms. YJM189, 1199, 1326, 1332, 1336, and YJM1628 (isogenic with YJM1615) have the same, and most common, *WHI2* coding sequence. YJM193, 271, 428, 689, and 1083 have the same *WHI2* coding sequence as S288c.

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