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Letter to the Editor

Gregory C. Howard, Galen A. Collins[#], and William P. Tansey^{*}

Department of Cell and Developmental Biology, Vanderbilt University School of Medicine,
Nashville, TN, USA

Dear Editor

We recently reported a chemical-genetic strategy for inhibiting proteasome function in *Saccharomyces cerevisiae* (Collins *et al.*, 2010). In this approach, genetic lesions in the tryptic (*pup1-T30A*)- and caspase (*pre3-T20A*)-like sites of the proteasome are combined with chemical inhibitors of the chymotryptic site (e.g. MG132) to effect potent and comprehensive inhibition of proteasomal proteolysis. As part of this strategy, yeast are also deleted for the gene encoding the drug efflux pump *Pdr5*, a standard manipulation that increases intracellular drug levels. We have now learned that the combination of *pup1*, *pre3* and *pdr5* mutations results in loss of mitochondrial DNA, an unexplained phenomenon that may limit the utility of this approach in circumstances where mitochondrial genome integrity is important. We report here a revised version of the chemical-genetic strategy that is equally as effective but avoids this complication.

We first became aware of this phenomenon when we performed gene expression profiling and observed that, compared to *PUP1PRE3pdr5* (GAC201) cells, *pup1pre3pdr5* (GAC202) cells express >10-fold lower levels (near background) of 13 mitochondrially-encoded genes (Q0045, Q0050, Q0065, Q0070, Q0075, Q0055, Q0080, Q0085, Q0105, Q0110, Q0130, Q0160 and Q0275), even in the absence of MG132. We subsequently learned that this difference is due to an absence of mitochondrial DNA in GAC202 (but not GAC201) cells, which occurred commensurate with our deletion of *PDR5* from the MHY1178 parental strain (Arendt and Hochstrasser, 1997). We re-derived GAC202 and observed the same phenomenon; we also observed loss of mitochondrial DNA when we deleted *PDR5* in *pup1pre3* cells of a different genetic background. Subsequent experiments showed that loss of mitochondrial DNA is the result of a specific combination of the $\delta pdr5$ and *pup1-T-30A* alleles.

We do not understand the basis of this phenomenon. We do note, however, that loss of the proteasome assembly factor Ump1 leads to mitochondrial DNA damage via accumulation of reactive oxygen species (Malc *et al.*, 2009), suggesting that mitochondrial DNA may be under stress when full proteasome function is impaired. Perhaps accumulation of toxic

metabolites or other compounds occurs in $\delta pdr5$ cells, adding an additional stress that further destabilizes the mitochondrial genome.

To avoid having to delete *PDR5*, we took advantage of the fact that MG132 uptake in yeast can be increased by growing cells in media containing L-proline as the sole nitrogen source and by addition of low levels of sodium dodecyl sulphate (SDS; we use 0.004%) at the same time as MG132 (Liu *et al.*, 2007). Congenic strains WCG4a (MATa *leu2-3, 112 ura3 his3-11,15 CanS GAL2*) and YUS5 (WCG4a except *pup1-T30A pre3-T20A*), carrying integrated copies of the mutant *pup1* and *pre3* alleles, were obtained from Dieter Wolf of the University of Stuttgart (Heinemeyer *et al.*, 1997) and confirmed for presence of mitochondrial DNA. We then treated these cells with 50 μ M MG132, as described (Liu *et al.*, 2007), and monitored both yeast cell growth (Figure 1A) and induction of the Gcn4 target gene, *ARG1* (Figure 1B). As we observed in our original study (Collins *et al.*, 2010), the *PUP1PRE3* WCG4a cells responded only modestly to MG132 under these conditions, whereas the *pup1pre3* YUS5 strain showed reduced proliferation and failed to induce *ARG1* transcription in the presence of MG132.

We conclude that the chemical–genetic strategy for inhibition of the proteasome is a useful way to probe proteasome function in *S. cerevisiae*, and that complicating issues of mitochondrial DNA loss can be avoided by combining the YUS5 strain with transient treatment with L-proline/SDS/MG132. We suggest that researchers wishing to use the chemical–genetic strategy use these strains and approach in the future.

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Figure 1.

Revised chemical-genetic strategy to inhibit proteasomal proteolysis. (A) Congenic strains WCG4a (*PUP1PRE3*) and YUS5 (*pup1pre3*) were grown in complete synthetic medium (CSM) containing 0.1% proline, SDS added to 0.004%, and treated with either DMSO or 50 μ M MG132. Growth at 30°C was measured as an increase in the absorbance (A_{600 nm}) of the culture. (B) Strains in (A) were grown in CSM containing 0.1% proline, SDS added to 0.004%, and treated with either DMSO or 50 μ M MG132 for 30 min. Gcn4-target genes were then induced for 30 min by treatment with 3-amino-1,2,4-triazole (ON) or uninduced with a vehicle (ethanol) control (OFF), RNA harvested and *ARG1* transcript levels monitored relative to an *ACT1* control by reverse-transcription quantitative PCR ($n = 3$), SEM