The non-stereotypical DNA damage response of budding yeast *Candida glabrata*

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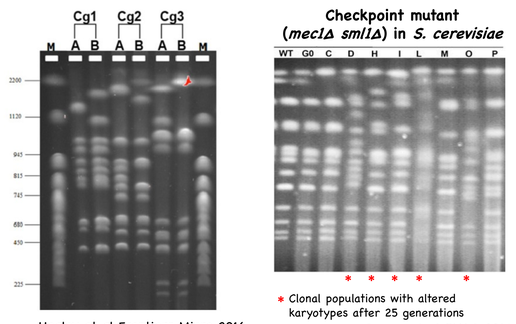
Abstract

Budding yeast *Candida glabrata* is an opportunistic pathogen closely related to *Saccharomyces cerevisiae.* Unlike *S. cerevisiae*, however, *C. glabrata* is a leading cause of life-threatening invasive fungal infections, particularly in North America and Europe. *C. glabrata* rapidly develops resistance to antifungal drugs and exhibits high genomic heterogeneity among clinical isolates in terms of both nucleotide polymorphisms and chromosomal rearrangements, suggesting that this organism can rapidly generate and tolerate high levels of genetic change. The mechanisms underlying this genetic flexibility are still unclear. In the closely related *S. cerevisiae*, DNA damage checkpoint pathways are critical for maintaining a stable genome. Whether analogous checkpoints are active in *C. glabrata* and function to preserve genome integrity in response to DNA damage in this organism is not known. We began investigating the DNA damage checkpoint in *C. glabrata* by analyzing DNA damage-induced phosphorylation of the highly conserved effector kinase Rad53. Western blotting and mass spectrometry analysis showed that, although DNA damage induces the expected robust phosphorylation of histone H2A (aka "DNA damage histone" or γH2A.X) in *C. glabrata*, it does not induce CgRad53 phosphorylation. Consistent with this finding, CgRad53 lacks the Ser/Thr clusters that are most heavily phosphorylated in ScRad53 upon DNA damage. We also analyzed DNA damage-induced transcriptomic changes in *C. glabrata* and identified several important genes and gene categories differentially regulated by DNA damage in the two species. Consistent with altered DNA damage checkpoint function, *C. glabrata* was more susceptible to higher doses of DNA damage. Finally, we analyzed the *C. glabrata* cell division cycle in the presence of DNA damage and found that many cells continue to divide under these conditions and that these divisions give rise to cells with aberrant DNA content. Together, results from these studies indicate that DNA damage-induced checkpoint activation is attenuated in *C. glabrata*, suggesting a possible molecular mechanism for rapidly generating genetic change, including antifungal drug-resistant mutations, in this organism.

Fig. 1 Chromosomal variations among clinical *C. glabrata* strains resemble DNA damage/S-phase checkpoint mutants in *S. cerevisiae*



Healey et al Frontiers Micro 2016

* Clonal populations with altered karyotypes after 25 generations
Serero et al PNAS 2014

Fig. 2. CgRad53 does not show the stereotypical mobility shift due to extensive phosphorylation upon DNA damage

C. glabrata

No mobility shift was detected with either Myc-tagged endogenous CgRad53 or using rabbit polyclonal Abs raised against CgRad53 (Genscript). (These Abs could not detect endogenous CgRad53 but could detect a slightly [4X] overexpressed version)

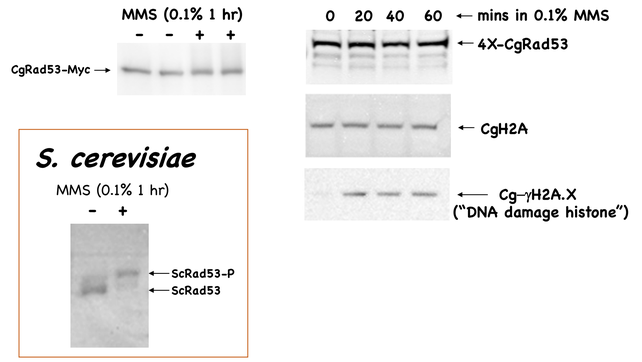
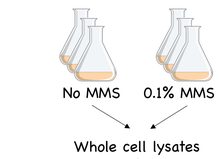
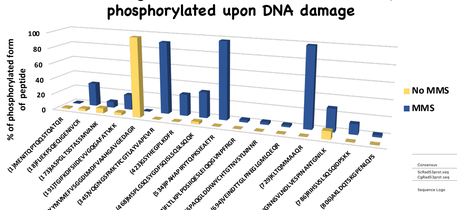


Fig. 3 Mass spectrometry of CgRad53 confirms lack of DNA-damage-induced phosphorylation

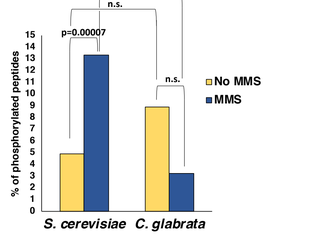


	# of recovered peptides	# (%)of phospho-peptides	% of Rad53p coverage
S. cerevisiae no MMS	346	17 (4.9)	75
S. cerevisiae MMS	451	60 (13.3)	82
C. glabrata no MMS	45	4 (8.9)	44
C. glabrata MMS	63	2 (3.2)	49

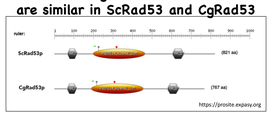
Consistent with previous studies, MS analysis identified several regions in ScRad53 that were heavily phosphorylated upon DNA damage



The abundance of recovered Rad53 phosphopeptides was significantly increased by MMS treatment in *S. cerevisiae* but not in *C. glabrata*



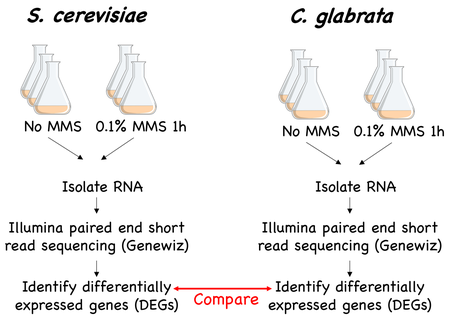
Overall domain organization and % of Ser/Thr are similar in ScRad53 and CgRad53



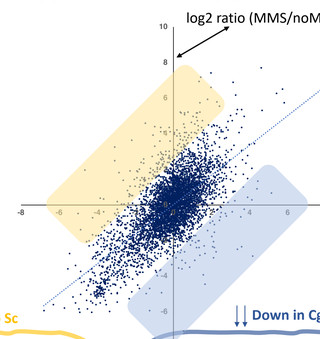
	ScRad53p	CgRad53p
serines	73/821 (8.89%)	62/767 (8.1%)
threonines	42/821 (5.12%)	38/767 (5.0%)

BUT: a number of ScRad53 Ser/Thr-containing regions phosphorylated upon DNA damage (——) are missing (( )) in CgRad53

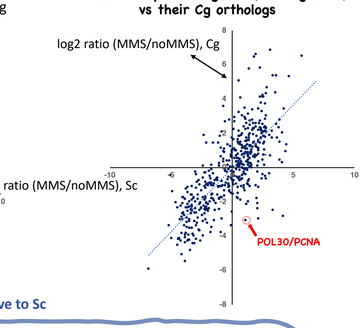
Fig. 4. Transcriptional DNA damage response in *C. glabrata*: similarities & differences with *S. cerevisiae*



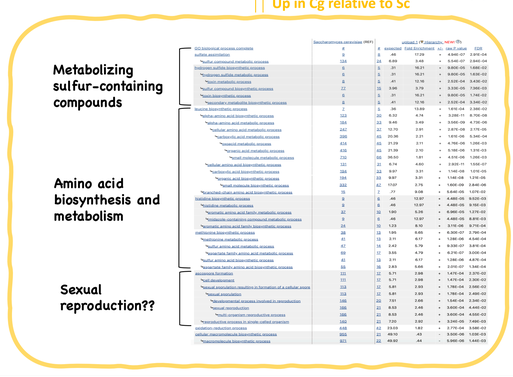
Cg genes vs their Sc orthologs (~70% of all genes)



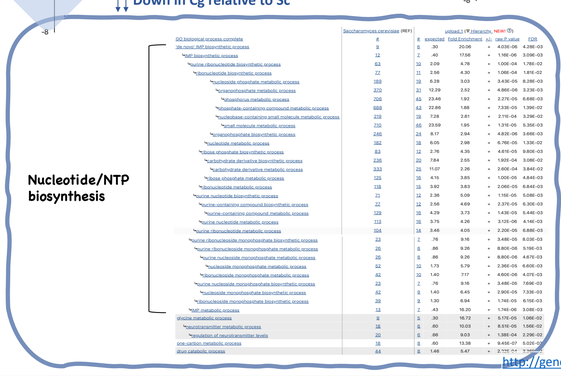
Sc Rad53-dependent genes (Jaehnig 2013) vs their Cg orthologs



Up in Cg relative to Sc



Down in Cg relative to Sc



http://geneontology.org/

Fig. 5. *C. glabrata* and *S. cerevisiae* show differential sensitivity to DNA damage (MMS) but not to S-phase arrest (HU)

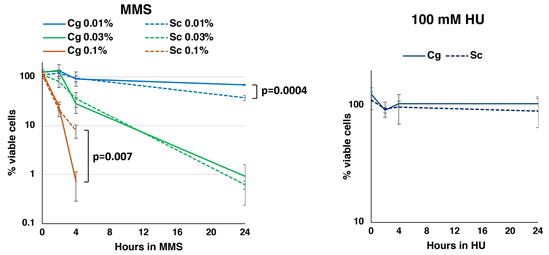
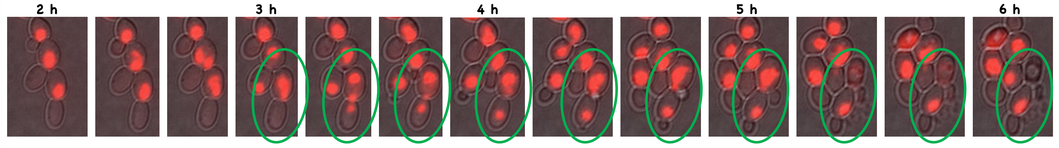


Fig. 6. *C. glabrata* cells undergo aberrant cell divisions in the presence of DNA damage

Time-lapse of *C. glabrata* cells carrying NLS-RFP (**red nuclei**) in a YPD agar patch containing 0.03% MMS, 20 min intervals



Cell division was completed before the nuclear division, resulting in unequal distribution of genetic material between mother and daughter cells. Both mother and daughter proceeded to bud, but the mother "exploded" 1.5 hours later.

Experiments, interrupted:

- Classical checkpoint analysis by flow cytometry
- Measuring dNTP levels after DNA damage (anyone want to help??)
- Measuring PCNA protein abundance after DNA damage
- Analyzing Rad53 phosphorylation in response to other types of DNA damage (e.g. oxidative stress)