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COMMENTARY

Evolutionary Divergence in DNA Damage Responses among Fungi

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ABSTRACT Cell cycle checkpoints and DNA repair pathways contribute to maintaining genome integrity and are thought to be evolutionarily ancient and broadly conserved. For example, in the yeast *Saccharomyces cerevisiae* and humans, DNA damage induces activation of a checkpoint effector kinase, Rad53p (human homolog Chk2), to promote cell cycle arrest and transcription of DNA repair genes. However, recent studies have revealed variation in the DNA damage response networks of some fungi. For example, Shor et al. (mBio 11:e03044-20, 2020, https://doi.org/10.1128/mBio.03044-20) demonstrate that in comparison to *S. cerevisiae*, the fungal pathogen *Candida glabrata* has reduced activation of Rad53p in response to DNA damage. Consequently, some downstream targets that contribute to *S. cerevisiae* genome maintenance, such as DNA polymerases, are transcriptionally downregulated in *C. glabrata*. Downregulation of genome maintenance genes likely contributes to higher rates of mitotic failure and cell death in *C. glabrata*. This and other recent findings highlight evolutionary diversity in eukaryotic DNA damage responses.

KEYWORDS cell cycle, DNA damage, DNA repair, budding yeasts, Saccharomycotina

Organisms are challenged by a constant barrage of exogenous and endogenous DNA-damaging agents (1). To cope with the threat of potentially deleterious mutations, an intricate network of DNA damage response processes, including cell cycle checkpoints and DNA repair pathways, help detect and repair DNA lesions (2). Due to their fundamental importance to life, most cell cycle and DNA repair processes are thought to be ancient in origin and broadly conserved (3).

One broadly conserved process is phosphorylation-based activation of the checkpoint effector kinase Rad53p (human homolog Chk2) in the presence of DNA damage or replication fork stalling (4–7). Phosphorylation of Rad53p by upstream sensor kinases and autophosphorylation amplifies the DNA damage signal, leading to cell cycle arrest, transcription of DNA repair genes, replication fork stabilization, and the activation of other processes that contribute to genome stability (8, 9). In the model yeast *Saccharomyces cerevisiae*, *RAD53* mutants are more sensitive than the wild type to DNA damage and fail to slow cell cycle progression (10); in humans, mutations in *CHK2* are associated with increased breast cancer risk (11).

Despite broad evolutionary conservation in the DNA damage response, recent studies have revealed variation in numerous cell cycle and DNA repair processes among fungi (12–18). For example, the bipolar yeast lineage *Hanseniaspora* has experienced substantial losses among cell cycle and DNA repair genes, which are associated with a punctuated burst of sequence evolution and an increased mutational burden (12). Notwithstanding these losses, *Hanseniaspora* yeasts have successfully diversified and are frequently isolated from the grape and wine must environment (19, 20). Genome instability and hypermutation can also stem from loss of function in a single gene. For example, in a lineage of *Cryptococcus deuterogattii*

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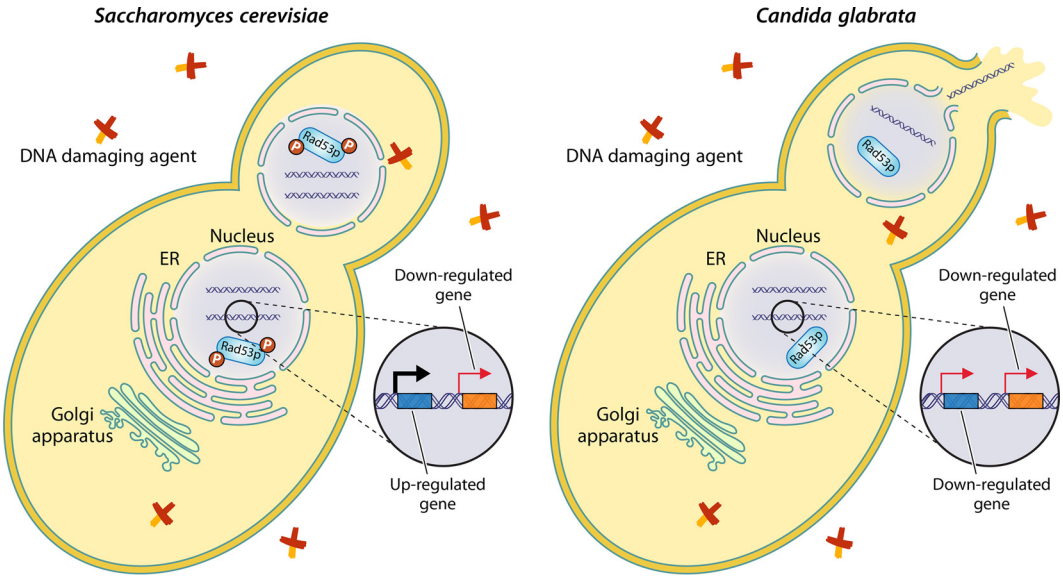

FIG 1 Divergence in the DNA damage response in a model yeast and a major yeast pathogen. (Left) In the model yeast *Saccharomyces cerevisiae*, the presence of DNA-damaging agents, like methyl methanesulfonate, activates the DNA damage response to help ensure genome integrity. A key step in the DNA damage response is phosphorylation-based activation of Rad53p, which activates multiple downstream processes, including upregulated expression of DNA repair genes, thereby providing the cell with an opportunity to repair DNA damage. (Right) In contrast, the noncanonical DNA damage response in the major yeast pathogen *Candida glabrata* is marked by reduced Rad53p phosphorylation and is associated with divergent expression of DNA repair genes, which may be responsible for higher rates of mitotic failure and cell death. ER, endoplasmic reticulum.

yeasts, a nonsense mutation in a DNA mismatch repair gene is associated with an increased mutation rate and rapid evolution of antifungal drug resistance (13, 21).

Beyond fungi, presence and absence patterns among genes responsible for efficacious chromosome segregation also vary across eukaryotes (22). Unraveling how the underlying biological networks compensate for gene losses in living organisms is a challenging task. To overcome this challenge, experimental evolution can be used to provide insight into possible mechanisms that compensate for network perturbation. For example, experimental evolution studies of *S. cerevisiae* mutants lacking genes responsible for proper chromatid cohesion have revealed distinct evolutionary routes that overcome disrupted chromosome metabolism pathways (23, 24). These findings, together with those in the previous paragraph, support a view that pathways once thought to be resistant to evolutionary change can diverge.

However, the extent and functional outcome of variation among cell cycle and DNA repair processes remain poorly understood. Furthering our understanding of the diversity and function of the DNA damage response network, Shor et al. (25) characterize a noncanonical DNA damage response in *Candida glabrata*, a genetically diverse major fungal pathogen comprised of at least seven major lineages (26–28). Of note, in comparison to the more-well-known *Candida* yeast *Candida albicans*, which is roughly as divergent from *S. cerevisiae* as humans are from sponges, *C. glabrata* and *S. cerevisiae* are more closely related and their divergence is on par with that of humans and zebrafish (29). Shor et al. demonstrate that DNA damage induced less Rad53p phosphorylation in *C. glabrata* than in *S. cerevisiae* (Fig. 1), which is likely due to sequence divergence at key phosphorylation sites. In contrast, DNA damage is known to induce robust Rad53p phosphorylation in more distantly related species, including *C. albicans* (5, 30, 31). These findings suggest that differences likely exist between the DNA damage responses of *C. glabrata* and *S. cerevisiae*.

To this end, the researchers examined the DNA damage-induced transcriptome and activities of Rad53p targets in *C. glabrata* and *S. cerevisiae* and revealed a divergent

architecture, expression, and transcriptional rewiring of the DNA damage response network in *C. glabrata* (Fig. 1). For example, genes present in the genome of *C. glabrata* but absent in the genome of *S. cerevisiae* were frequently upregulated in response to DNA damage. Additionally, genes that encode known targets of Rad53p in *S. cerevisiae*, such as *RNR3*, which encodes a ribonucleotide reductase subunit, and *HUG1*, which encodes a ribonucleotide reductase inhibitor, are absent from the genome of *C. glabrata*. Among orthologous genes that contribute to genome integrity, numerous genes were found to be upregulated in *S. cerevisiae* but downregulated in *C. glabrata*, including the proliferating cell nuclear antigen (or PCNA, which is encoded by *POL30*), a subunit of the prereplicative complex (*CDC6*), several subunits of the minichromosome maintenance replicative helicase, and a subunit of DNA polymerase δ (*POL31*). Among genome integrity genes that were lost in *Hanseniaspora* yeasts (12), divergent expression profiles were also pronounced, revealing that some genome integrity genes differentially contribute to the DNA damage response networks in diverse yeasts. Despite substantial divergence in the architectures and expression of the DNA damage response networks, some targets of Rad53p that are present in the genomes of both yeasts had similar expression profiles, indicating that their expression was likely mediated through a Rad53p-independent mechanism, a signature of transcriptional rewiring (32).

Downregulation of multiple genes that are responsible for genome integrity suggests that the checkpoint signaling module is less robust in *C. glabrata* than in *S. cerevisiae*. Supporting this hypothesis, the researchers reveal that *C. glabrata* had an attenuated cell cycle checkpoint response in the presence of DNA damage, which may contribute to higher rates of mitotic failure and cell lethality than in *S. cerevisiae* (Fig. 1). Although hypermutation contributes to pathogenicity-related traits, such as multidrug resistance in diverse fungi, including *C. glabrata* (13, 21, 33, 34), the contribution of the noncanonical DNA damage response in *C. glabrata* to pathogenicity and pathogenicity-related phenotypes is unknown but holds promise as an exciting area for future research.

This study, taken together with other recent findings (12, 13, 17, 18, 21, 23, 24), expands our knowledge of the variation among fungal DNA damage response networks. These observations raise the question of what events lead to DNA damage response network perturbation and, in some cases, subsequent stabilization in different lineages. More broadly, these studies contribute to a timely discussion of variation in the architecture, wiring, and function of the eukaryotic DNA damage response.

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