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# DNA damage checkpoint and repair: From the budding yeast *Saccharomyces cerevisiae* to the pathogenic fungus *Candida albicans*



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#### ABSTRACT

Cells are constantly challenged by internal or external genotoxic assaults, which may induce a high frequency of DNA lesions, leading to genome instability. Accumulation of damaged DNA is severe or even lethal to cells and can result in abnormal proliferation that can cause cancer in multicellular organisms, aging or cell death. Eukaryotic cells have evolved a comprehensive defence system termed the DNA damage response (DDR) to monitor and remove lesions in their DNA. The DDR has been extensively studied in the budding yeast *Saccharomyces cerevisiae*. Emerging evidence indicates that DDR genes in the pathogenic fungus *Candida albicans* show functional consistency with their orthologs in *S. cerevisiae*, but may act through distinct mechanisms. In particular, the DDR in *C. albicans* appears critical for resisting DNA damage stress induced by reactive oxygen species (ROS) produced from immune cells, and this plays a vital role in pathogenicity. Therefore, DDR genes could be considered as potential targets for clinical therapies. This review summarizes the identified DNA damage checkpoint and repair genes in *C. albicans*. © 2021 The Authors. Published by Elsevier B.V. on behalf of Research Network of Computational and

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#### Contents

1.	Introduction	5344						
2.	DNA damage response	5344						
3.	DNA damage checkpoints in C. albicans	5345						
4.	DNA damage checkpoint related protein phosphatases in <i>C. albicans</i>							
	4.1. Protein phosphatase 4 (PP4) complex	5346						
	4.2. Type 2C protein phosphatase (PP2C)	5347						
	4.3. Protein phosphatase 1 (PP1)	5347						
5.	DNA damage repair pathways in C. albicans	5347						
	5.1. Homologous recombination (HR)	5347						
	5.2. Non-homologous end joining (NHEJ)	5348						
	5.3. Base excision repair (BER)	5348						
	5.4. Nucleotide excision repair (NER)	5348						
	5.5. Postreplication repair (PRR)	5348						
	5.6. Mismatch repair (MMR)	5349						
6.	DNA damage response allows pathogenesis in <i>C. albicans</i>	5349						
	6.1. DNA damage response contributes to morphogenesis	5349						
	6.2. DNA damage response contributes to biofilm formation	5349						
	6.3. Positive roles of DNA damage response in pathogenicity	5349						
	6.4. Negative roles of DNA damage response in pathogenicity	5349						
7.	DNA damage response in other fungal pathogens	5349						

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8.	Discussion	6350
	Declaration of Competing Interest	6351
	Acknowledgments	6351
	References	6351

# 1. Introduction

Humans are constantly exposed to fungi capable of causing a variety of diseases. Numerous medically important fungi, especially *Candida* species, including *Candida* albicans, *Candida* tropicalis, *C.* dubliniensis and the highly pathogenic fungus *Candida* auris, have been identified to cause mild infections, severe cutaneous infections, or life-threatening systemic infections [1]. Among them, *C.* albicans is the most frequently isolated agent of candida-sis, accounting for 64% of *Candida* infections [2,3]. *C.* albicans is skilled at adapting to various environments since it can switch between either yeast or hyphae forms as well as white, grey or opaque forms, each exhibiting distinct pathogenicities [1,4]. In addition to morphology switching, other virulence factors, such as adhesins and invasive enzymes, also contribute to the pathogenicity of *C.* albicans [1].

The invasion and spreading of *C. albicans* cells within the human body are challenged by the immune system. In particular, macrophages and neutrophils can recognize and phagocytose invading pathogens and release reactive oxygen species (ROS) or other active substances to attack the invaders' proteins or DNA. This protective mechanism represents a key part of the first line of defence against such pathogens as *C. albicans* [5–7]. However, eukaryotic cells have evolved a set of responses, termed the DNA damage response (DDR), to fight against DNA damage stresses and maintain the stability of the genome. This predicts that the DDR system in *Candida* cells will be critical for the pathogen's resistance to the attack from the host immune system and its survival and proliferation inside the host.

#### 2. DNA damage response

The integrity of the genome is critical for the transfer of genetic information between generations [8]. During growth and development, cells may be challenged by both extrinsic factors, such as radiation, chemicals, chemotherapeutic drugs, and intrinsic factors, such as replication errors, reactive oxygen species [9]. As a result, these stresses introduce modified bases, mismatches, intra- or inter-strand crosslinks, and single- or double-strand breaks (DSBs) to the genome [10,11]. The accumulation of DNA lesions may result in direct cell death, apoptosis or can trigger the aberrant proliferation that characterizes cancer in mammals. To defend against such DNA-damaging events, cells have evolved a set of conserved mechanisms, termed the DNA damage response (DDR), to sense and repair damaged DNA, and thus ensure the fidelity of genetic information transfer [12]. In general, this DDR includes cell cycle checkpoints, chromatin remodeling, DNA repair and DNA-damage tolerance pathways (Fig. 1).



Fig. 1. Schematic diagram of DNA damage response. External and internal stresses induce DNA lesions. The damaged DNA may activate the DNA damage response signaling pathway, where DNA damage checkpoints play a central role in arresting the cell cycle and mediating the DNA repair process. The unsuccessful repairing of DNA lesions may cause apoptosis, cell death or cancer. Homologous recombination (HR); Non-homologous end joining (NHEJ); Base excision repair (BER); Nucleotide excision repair (NER); Postreplication repair (PRR); Mismatch repair (MMR).

Checkpoints function in coordinating cell cycle progression and DNA repair and thus play central roles in DDR. The checkpoints have been extensively studied in the budding yeast Saccharomyces cerevisiae due to its relatively simple genetic framework compared to mammals [13–16]. Several sensor proteins function to receive the signal of damaged DNA and transfer the damage signal to effector kinase. Among the identified sensor proteins, the checkpoint kinase ScRad53 is a widely studied G2/M checkpoint in S. cerevisiae. In the presence of DNA damage, ScRad53 can be activated through a set of regulatory phosphorylations by the upstream damage sensor kinases ScMec1 or ScTel1 [17–19]. Adaptor proteins ScRad9 or ScMrc1 are indispensable in mediating the recruitment of ScRad53 to DNA lesions [17-19]. Once the checkpoint is activated, it mediates a series of cellular processes, including cell cycle arrest, inhibition of origin firing, protection and restart of stalled replication forks, as well as DNA damage repair [13]. Generally, different types of DNA lesions rely on specific repair pathways. After DNA damage is corrected, the checkpoint kinase needs to be deactivated to permit resumption of the normal cell cycle.

The genetic framework of *C. albicans* is close to that of *S. cerevisiae*, and thus comparative genomics between these two fungi can help to illuminate many points. Such points include the evolutionary processes that led to the pathogenicity of *C. albicans* in contrast to the nonpathogenic yeast *S. cerevisiae*. This review summarizes current observations on DNA damage checkpoint and repair genes in *C. albicans*, mainly structured around the findings in *S. cerevisiae*.

#### 3. DNA damage checkpoints in C. albicans

Activation of DNA damage checkpoint is the core event of DDR, which further regulates downstream cell cycle arrests, histone modifications, transcriptional changes and post-translational modifications of proteins to facilitate the repair process. In *S. cerevisiae*, this activation involves two highly conserved apical protein kinases ScMec1 (ATM) and ScTel1 (ATR), initiating evolutionarily conserved signal transduction cascades. Generally, ScMec1 functions to sense a wide range of DNA lesions that induces the generation of ssDNA (single-stranded DNA), whereas ScTel1 is mainly activated by DSBs [20].

For checkpoint signal initiation, ScMec1 associates with ScDdc2, which recognizes and binds RPA-coated ssDNA in DNA lesions [21]. Moreover, activation of ScMec1 relies on other factors, including the 9-1-1 complex (ScDcd1-ScMec3-ScRad17), the replication factor ScDpb11 and the DNA helicase ScDna2 [22]. Subsequently, activated ScMec1, with the participation of adaptors ScRad9 or ScMrc1, phosphorylates and activates a series of effector kinases, including ScRad53, ScChk1 and downstream ScDun1 [13]. In addition, ScMec1 (or ScTel1) also phosphorylates ScSae2, which interacts with ScRad53 and ScDun1, contributing to DNA repair and genome maintenance [23]. Generally, ScMec1 is considered as the principal sensor kinase since mec1 mutants confer comprehensive genotoxic defects, including sensitivity to hydroxyurea (HU) and methyl methane sulfonate (MMS), persistent replication fork stalling followed by irreversible fork collapse, DSB formation and cell death [14,24,25]. In particular, ScMec1 was found to coordinate checkpoint signaling and HR-mediated DNA repair through regulating the ScSgs1-ScTop3-ScRmi1 (STR) complex [26]. Intriguingly, unlike ScMec1 in S. cerevisiae, this checkpoint kinase is not essential in C. albicans. These differences in gene essentiality also happen to other genes, which may arise during evolutionary progress. Since C. albicans cells inhabit mammals, they have more chances, compared to the budding yeast, to be stressed by the host immune system and evolve substituted DDR signaling pathways. However, CaMec1 in C. albicans plays a similarly critical role in

maintaining genome stability. The *Camec1* deletion mutants show strong sensitivity to various genotoxic stresses [27]. Deleting *CaMEC1* increases cell size, suggesting cell division defects that may support, at least partially, a checkpoint-related function [28]. In addition, CaMec1 was found to be involved in phosphorylation of CaRfa2, an ssDNA-binding protein, which provides evidence for the role in checkpoint activation [29].

ScTel1 acts partially redundant with ScMec1 in mediating cell cycle checkpoints to initiate DNA repair, particularly DSB repair in S. cerevisiae [19,30,31]. ScTel1 may participate, together with the MRX (ScMre11-ScRad50-ScXrs2) complex, in ScMec1dependent DSB-induced checkpoint activation by increasing the efficiency of ssDNA accumulation at the ends of DSBs [32]. The ScTel1-ScMre11 complex triggers the activation of ScRad53 and its interaction with ScRad9 in mitotic cells [33]. Moreover, ScTel1 may also activate the checkpoint response to DSBs independently of ScMec1 [32]. In C. albicans, the function of CaTel1 remains uncharacterized, but sequence analysis suggests it contains a similar N terminus TAN domain to that found in ScTel1, implying DNA damage repair-related functions. However, unlike ScTel1, sequence analysis also shows that there is no PI3Kc domain at the C terminus of CaTel1 in C. albicans, suggesting a potential functional diversity of Tel1 in these two yeast species.

ScRad53, the main checkpoint effector, is classified as an essential element in DDR in S. cerevisiae [34]. In C. albicans, CaRad53 was first identified by the Wang Lab in 2007 [35]. CaRad53 can be phosphorylated by the treatment of either HU or MMS [35,36]. Similar to the pattern of ScRad53, the phosphorylated CaRad53 in C. albicans shows a slower gel migration, supplying an effective tool to study its phosphorylation status [35,36]. Nevertheless, according to current data, CaRAD53 is not essential in C. albicans, either the BWP17, SN148 or CEC3194 backgrounds [35,37,38]. Deleting CaRAD53 does, however, cause severe sensitivity to various genotoxic stresses, including MMS and HU [35]. Loss of function of CaRad53, either through deletion or point mutations, causes defects in G2/M arrest, suggesting functional similarity to ScRad53 [35]. Raphaël Loll-Krippleber et al. further reported that CaRad53 maintains heterozygosity in *C. albicans*, and its deletion results in frequent aneuploidies, due to break-induced replication/mitotic cross-over or chromosome loss [38]. Site-directed point mutation on CaRad53 reveals that its kinase activity and N-terminal phosphorylation sites are crucial for its function in the resistance to genotoxic stress [38]. Therefore, CaRad53 shows critical roles in regulating the cell cycle and maintaining genome integrity in C. albicans.

Checkpoint kinase ScChk1 plays an additional role to ScRad53 in coordinating a DNA damage signal to cell cycle arrest [39]. ScChk1 shows ScMec1-dependent phosphorylation in response to DNA damage [14]. Several studies reveal that ScChk1 functions to arrest the cell cycle by regulating the stability of ScPds1 [40,41]. However, there is no ortholog of *ScCHK1* in either *C. albicans*, *C. dubliniensis* or *C. parapsilosis*. Although there is a protein in *C. albicans* named CaChk1, it is not an ortholog of ScChk1, showing only 2.8% sequence identity with ScChk1, and has a completely different function from ScChk1. The possibility could be that *C. albicans* cells lost the ortholog of *ScCHK1* during the evolutionary progress, and other checkpoint kinases take the function of ScChk1 in response to DNA damage stress.

Adaptors CaMrc1 and CaRad9 are required for activating CaRad53 in *C. albicans*, but may function in response to different genotoxic stresses [35]. Deleting either *CaRAD9* or *CaMRC1* generates sensitivity to MMS and HU, but *Carad9* mutant cells were more sensitive to MMS, whereas *Camrc1* mutant cells were more sensitive to HU [42]. Consistently, deleting *CaRAD9* blocks the filamentous growth induced by MMS or UV light specifically, but not that induced by HU or aphidicolin [35]. In contrast, *CaMRC1* dele-

#### Table1

Г	vpical	DNA	damage	check	point	and	repair	genes	in S.	cerevisiae	and C	. albicans.
								0				

Functional Group	S. cerevisiae	C. albicans	Name Description	Function Description
<b>Checkpoint</b> Sensors	activation DDC2/ LCD1	LCD1	<u>L</u> ethal, <u>C</u> heckpoint- defective	Interacts physically with Mec1 and contributes to its activation; uncharacterized in <i>C. albicans</i> .
	DDC1	DDC1	<u>D</u> NA <u>D</u> amage Checkpoint	9–1-1 complex for Mec1 activation; uncharacterized in <i>C. albicans.</i>
	RAD17	RAD17	<u>RAD</u> iation sensitive	9–1-1 complex for Mec1 activation; uncharacterized in <i>C. albicans.</i>
	MEC3	MEC3	<u>M</u> itosis <u>E</u> ntry Checkpoint	9–1-1 complex for Mec1 activation; uncharacterized in <i>C. albicans.</i>
	RAD24	RAD24	RADiation senstive	Loads Rad17-Mec3-Ddc1 onto DNA; uncharacterized in C. albicans.
	DPB11	DPB11	<u>D</u> NA <u>P</u> olymerase <u>B</u> (II)	DNA replication initiation protein; prevents accumulation of chromatin bridges by stimulating the Mec1 kinase and suppressing homologous recombination; uncharacterized in <i>C. albicans.</i>
	DNA2	DNA2	<u>DNA</u> synthesis defective	Tripartite DNA replication factor; involved in DNA repair/processing of meiotic DSBs; uncharacterized in C. albicans.
	MRE11	MRE11	<u>M</u> eiotic <u>RE</u> combination	Nuclease subunit of the MRX complex with Rad50 and Xrs2; complex functions in repair of DSBs and in telomere stability.
	RAD50	RAD50	<u>RAD</u> iation sensitive	Subunit of MRX complex with Mre11 and Xrs2; complex is involved in processing DSBs.
	XRS2	NA	<u>X-R</u> ay <u>S</u> ensitive	FHA domain-containing component of the Mre11 complex; no ortholog in C. albicans.

tion impairs DNA synthesis and causes cell elongation even in the absence of external genotoxic agents, suggesting it has different roles from CaRad9 in DDR [35]. Moreover, phosphorylation of CaRad53 was significantly impaired in response to MMS in strains deleted for *CaRAD9*, while phosphorylation of CaRad53 was nearly blocked in response to HU treatment in cells with deletions of *CaMRC1* [42]. In particular, CaRad9 and CaMrc1 mediate the phosphorylation of CaRad53 through different residues. Specifically, CaRad9 mediates the phosphorylation of CaRad53 in response to MMS at T7, S9, T24 of the TQ domain and S350 residues [42]. In contrast, CaMrc1 mediates the phosphorylation of Rad53 mainly at different residues in response to HU [42]. These observations support that adaptors CaRad9 and CaMrc1 mediate the phosphorylation of the checkpoint kinase CaRad53 in response to DNA damage or DNA replication stress via different signaling pathways.

ScDun1 is a downstream kinase of ScRad53 and regulates a wide range of cellular responses to DNA damage, including dNTP concentrations, by inhibiting the ScCrt1 repressor of RNR genes [22]. In *C. albicans*, CaDun1 also plays crucial roles in response to genotoxic stresses, suggesting a potential signaling pathway in the DDR [35]. However, *CaDUN1* in *C. albicans* is not significantly overexpressed with the treatment of HU, whereas *ScDUN1* is strongly upregulated in *S. cerevisiae*, showing a difference in response to genotoxic stress [43]. Thus, the specific function and working model of CaDun1 in *C. albicans* remain to be uncovered.

In general, candidate orthologs of checkpoint-related genes in budding yeast have been identified in *C. albicans*. The essentiality of the checkpoint elements is quite different from their orthologs in *S. cerevisiae*, yet they show similar functionality (Table 1). Furthermore, the signal transduction pattern of checkpoint elements in *C. albicans* remains to be clarified, which may be uncovered by protein interaction assays, genetic interaction assays or RNAseq assays in the future.

# 4. DNA damage checkpoint related protein phosphatases in *C. albicans*

The timely deactivation of checkpoint kinases, especially ScRad53, is critical for the essential resumption of cell cycle progression during recovery from or adaption to DNA damage. Ser/ Thr phosphatases are assumed to play crucial roles during this progress since many checkpoint kinases are Ser/Thr kinases [38]. Up to now, phosphatase ScPph3, ScPtc2, ScPtc3 as well as ScGlc7, have been identified as necessary for the dephosphorylation of checkpoint kinase ScRad53 in *S. cerevisiae* [44].

#### 4.1. Protein phosphatase 4 (PP4) complex

The protein phosphatase 4 (PP4) complex consists of the catalvtic subunit ScPph3 and the regulatory subunits ScPsv2 and ScPsy4 in S. cerevisiae and is indispensable for the dephosphorylation of Rad53 and yH2A. Similar to the mechanism by which ScPph3 dephosphorylates checkpoint kinase ScRad53, CaPph3 and its regulator CaPsy2 are essential for the dephosphorylation of CaRad53 in C. albicans [42]. The single gene deletion or the double gene deletion of CaPPH3 and CaPSY2 both cause strong genotoxic sensitivities and persistent phosphorylation status of CaRad53 during the recovery from genotoxic stress [36]. A protein interaction assay revealed that CaPsy2 associates with both CaPph3 and CaRad53 in C. albicans, but the specific mechanism is quite different from the pattern in S. cerevisiae; ScPys2 interacts with ScPph3 in S. cerevisiae, while CaPsy2 shows no direct interaction with CaPph3 according to current data in *C. albicans* [36,45]. Instead, we found CaTip41 may act as an adaptor protein for CaPph3 in C. albicans, since it interacts with both CaPph3 and CaPsy2, and contributes to deactivating CaRad53 [46]. In addition, ScPsy2 interacts with the kinase domain (KD) of ScRad53; but CaPsy2 interacts with the FHA2 domain (C terminus) of CaRad53 via its N terminus sequence in C. albicans. In consistent, blocking the interaction between CaPsy2 and CaRad53 through a point mutation of the Y33 residue at the N-terminus of CaPsy2, inhibits the dephosphorylation of CaRad53 and causes strong sensitivity to genotoxic stress in C. albicans (Fig. 2) [36]. This finding suggests a model where the direct interaction between CaPsy2 and CaRad53 is crucial for the dephosphorylation of CaRad53 in C. albicans.

Furthermore, the ScPph3-ScPsy2-ScPsy4 complex takes part in regulating the phosphorylation status of  $\gamma$ H2AX in *S. cerevisiae*, whose phosphorylation at the carboxy-terminal SQE motif to cre-



**Fig. 2. Phosphatases involved in the dephosphorylation of checkpoint kinase Rad53.** (A) In *S. cerevisiae*, ScPsy2 and ScPtc2 are required for the dephosphorylation of ScRad53; ScPsy2 interacts with the kinase domain (KD) and ScPtc2 interacts with the FHA (N terminus) domain. In *C. albicans*, Psy2 interacts with the FHA (C terminus) domain, but Ptc2 shows no clear interaction with Rad53. (B) Pph3 and Psy2 form a complex and play a dominant role in the dephosphorylation of Rad53 in *C. albicans*. Psy4 and Tip41 act as adaptors for Pph3 and Psy2. In particular, Tip41 plays an important role in the dephosphorylation of Rad53 during the recovery from DNA damage stress, while Psy4 seems dispensable for the dephosphorylation of Rad53. Unlike the pattern in *S. cerevisiae*, the Pph3-Psy2-Psy4 complex is not involved in the dephosphorylation of Rad53 both in *S. cerevisiae* and *C. albicans*, but the direct interaction between Rad53 and Glc7 is unclear. Moreover, Glc7 is involved in the dephosphorylation of *Y* H2A in *S. cerevisiae*, but the role in *C. albicans* to be established. The red question mark means uncovered interaction according to current data. (For interpretation of the references to colour in this figure legend, the reader is referred to the we bersion of this article.)

ate  $\gamma$ H2AX-containing nucleosomes is the earliest mark of DSBs [47]. In contrast, CaPph3 and CaPsy2 do not show direct roles in dephosphorylating  $\gamma$ H2AX, even though CaPph3, CaPsy2 and CaPsy4 can form a complex in *C. albicans* [48]. Therefore, the regulating functions on  $\gamma$ H2AX by Pph3-Psy2-Psy4 complex appear different in these two yeast species.

# 4.2. Type 2C protein phosphatase (PP2C)

Functional differences in DDR are also observed in Ptc2, a type 2C protein phosphatase. In *S. cerevisiae*, ScPtc2 interacts with the FHA1 domain (N terminus) of ScRad53 and contributes to ScRad53 dephosphorylation together with ScPtc3 (Fig. 2-A) [49]. In *C. albicans*, the identified CaPtc2 shows strong sequence identity with both ScPtc2 and ScPtc3. No specific ortholog of ScPtc3 was found in this pathogen, suggesting a potential dual function of CaPtc2 in *C. albicans* that was distributed to the two orthologs in *S. cerevisiae* after the whole-genome duplications (WGD) [50]. Deletion of *CaPtC2* causes moderate sensitivity to genotoxic stresses [51]. In addition, CaPtc2 offers no direct interaction with CaRad53 and no significant roles in the dephosphorylation of CaRad53 [36,51,52]. Therefore, CaPtc2 may not play a dominant role in the dephosphorylation of checkpoint kinase CaRad53 in *C. albicans*.

#### 4.3. Protein phosphatase 1 (PP1)

In *S. cerevisiae*. ScGlc7, a protein phosphatase of the PP1 family, is necessary for the dephosphorylation of ScRad53 during the recovery from replication fork stalling caused by HU stress [53]. In addition, ScGlc7 is involved in the dephosphorylation of  $\gamma$ H2AX at S129 residue and is required for the recovery from replication fork stalling caused by dNTP depletion [53]. In *C. albicans*, CaSds22 mediates CaRad53 dephosphorylation probably through inhibitory binding to CaGlc7 [54]. However, no direct interaction between Glc7 and Rad53 was reported in either of these two yeast species, suggesting an indirect role of Glc7 in regulating Rad53. We also can not preclude the transient interaction between Glc7 and Rad53; therefore, combined protein interaction assays may be adopted to further check the interaction between Glc7 and Rad53.

Compared to Rad53, dephosphorylation of other checkpoint kinases remains less studied in both *S. cerevisiae* and *C. albicans*. In general, protein phosphatases function differentially during the response to the genotoxic stress in these two yeast species. Here, we propose a working model of checkpoint-related phosphatases in *C. albicans*, according to the signaling pathway in *S. cerevisiae* (Fig. 2-B).

#### 5. DNA damage repair pathways in C. albicans

# 5.1. Homologous recombination (HR)

In the presence of DNA damage, specific repair pathways are utilized to remove different DNA lesions. External stress like MMS may induce DNA alkylation, yielding single-strand breaks (SSBs) and also DSBs, resulting in lethality. Fixing DSBs relies on homologous recombination (HR) and also non-homologous end joining (NHEJ). HR in *S. cerevisiae* depends on phosphorylation controlled by ScMec1 [13]. Established HR repair proteins are ScRad51, ScRad52, ScRad54, ScRad55, ScRad57, ScRad59, ScSlx4, ScRad50, ScMre11, ScMms4, ScMus81, ScSgs1, ScDna2, ScMms22 and ScMms1[55].

ScRad51 plays a central role in this process, and its binding to ssDNA is a key step in HR [55]. In addition, ScRad52 acts to attenuate resection of the ends of DSB and stimulate strand exchange by facilitating ScRad51 binding to single-stranded DNA [56,57]. In C. albicans, CaRAD51 appears to be involved in HR since its deletion causes increased sensitivity to compounds that cause DSBs [58]. Furthermore, deletion of CaRAD52 results in strong sensitivity to genotoxic stress and increased loss of heterozygosity in C. albicans [59,60]. In S. cerevisiae, ScRad52 plays a more prominent role than ScRad51 in HR, where ScRad59 functions cooperatively with ScRad52 in inverted-repeat recombination by a strand-annealing mechanism in a ScRad51-independent pathway [61]. In C. albicans, CaRAD52 is epistatic to CaRAD51 for growth rate, colony morphology, viability and filamentation, suggesting the more prominent role of CaRAD52 than CaRAD51 in the repair of DSBs in C. albicans [58,62]. Additionally, ScRad51 functions cooperatively with ScRad54, a member of the SWI/SNF family of DNA translocases, to mediate HR, where ScRad54 acts as a motor protein to translocate along dsDNA and performs several important functions in HR [63]. In *C. albicans*, deletion of *CaRAD54* creates hypersensitivity to MMS and menadione, and causes an aberrant cell and nuclear morphology [64], but a potential cooperation with Rad51 in HR remains to be established.

In *S. cerevisiae*, ScRad50, ScMre11 and ScXrs2 take part in the early steps of DSB repair, which is required for both HR and NHEJ. In *C. albicans*, both *CaRAD50* and *CaMRE11* genes were identified, and their deletions cause dramatic sensitivity to UV-induced DNA damage stress and oxidative stress. As well, deleting *CaRAD50* and *CaMRE11* both increase genome instability [65]. But strikingly, *CaXRS2* gene is missing from the genome of *C. albicans*, suggesting different DDR pathways in these two yeast species. These observations indicate that the DNA damage repair related functions of CaRad50 and CaMre11 are also active in *C. albicans*.

ScSLX4 encodes an endonuclease and acts as a scaffold with ScSlx1 in genome maintenance, supplying a role in HR [66-68]. ScSlx4 works together with PP4 phosphatase to down-regulate ScRad53 activity to allow HR machinery to repair DNA lesions [69]. In C. albicans, the Caslx4 mutant exhibits increased sensitivity to the DNA damaging agent MMS but not to the DNA replication inhibitor HU, thus showing a DNA damage related role [70]. Similarly, CaSLX4 expression is activated in a checkpoint kinase CaRad53 dependent manner during MMS-induced DNA damage, implying a checkpoint-related regulating mechanism in C. albicans [70]. In addition, ScMms22-ScMms1 complex is involved in HR and mainly functions as S-phase specific recombination-promoting factor [71]. In C. albicans, CaMMS22 is essential for the HR induced by camptothecin, while CaMMS1 is missing from the genome according to current data [72]. Moreover, the ScRad55-ScRad57 complex shows unique nonredundant functions in recombination, and mutations in any one of these components can lead to recombination defects. In C. albicans, CaRAD57 is critical for responding to MMS, HU, or ionizing radiation (IR) damage but is only essential for camptothecin-incuded damage repair in the absence of *CaMMS22* [72]. However, *CaRAD55* is also missing from the genome of C. albicans. according to current data. These differences in MMS1 and *RAD55* may supply another evidence for the idea that DDR in *C*. albicans does not completely follow the pattern in S. cerevisiae. A list of the potential HR-involved genes in both S. cerevisiae and C. albicans is found in Table 1.

#### 5.2. Non-homologous end joining (NHEJ)

NHEJ provides a relatively simple bypass strategy for repairing DSBs, allowing the free ends to be ligated without the participation of homology. In C. albicans, CaLIG4 is an orthologue to both yeast DNL4/LIG4 and human ligase IV, which are involved in NHEJ to correct DSBs [73]. *CaLIG4* can complement the defect of *Sclig4* in NHEJ, suggesting an NHEJ related role of CaLig4 in C. albicans [74]. However, CaLIG4 is not essential for DNA replication or for repairing DNA damage induced by ionizing radiation or UV light in C. albicans, suggesting that these lesions may be repaired primarily by HR [73,75]. Moreover, in S. cerevisiae, ScRtt109 functions in the NHEJ pathway through the interaction with ScVps75 [76]; and in C. albicans, deletion of CaRTT109 generates strong sensitivity to DNA damage stress and elevated transcription of DDR genes [77], but the specifics of the role in NHEJ remains to be established. In addition, ScYku80, a subunit of the telomeric Ku complex, and ScDoa1, a Trp-Asp (WD)-repeat protein involved in ubiquitinmediated protein degradation, seem to play roles in NHEJ, since their orthologs in S. cerevisiae promote NHEJ process [78]. Nevertheless, their detailed roles in NHEJ are unknown in C. albicans up to now.

#### 5.3. Base excision repair (BER)

The base excision repair (BER) pathway repairs the damage that creates minor disturbances in the DNA helix [37]. This process is initiated by a series of glycosylases, including ScUng1, ScMag1, ScOgg1, ScNtg1, ScOgg2 and ScNtg2 in S. cerevisiae (Table 1). They recognize damaged or abnormal bases and cleave the glycosylic bond linking the base to the sugar-phosphate backbone [79]. Moreover, apurinic/apyrimidinic (AP) endonucleases, ScApn2, ScApn1, and other additional factors, ScRad27, ScPol30, ScPol4, ScPol3, ScPol2, ScCdc9, seem necessary for the BER pathway (Table 1) [80]. In C. albicans, the BER genes CaNTG1, CaAPN1 and CaOGG1 have been characterized, and deletion mutants of these genes show no change in susceptibility to DNA-damaging agents, yet ScAPN1 shows essential roles in response to MMS, UV light and gamma rays [81]. The possibility could be that BER and other DNA repair pathways have overlapping functions in correcting DNA lesions in C. albicans.

# 5.4. Nucleotide excision repair (NER)

The nucleotide excision repair (NER) pathway represents a key repair system to remove distorted DNA regions induced by UV light. In S. cerevisiae, the damage binding factors ScRad14, RPA, the ScRad4-ScRad23 and the ScRad7-ScRad16 complex bind to UV damaged DNA; DNA helicases ScRad3 and ScRad25/ScSsl2 create a bubble structure; two endonucleases, the ScRad1-ScRad10 complex and ScRad2 may incise the damaged DNA strand on the 5'- and 3'-side of the lesion [82]. In C. albicans, CaRad23 and CaRad4 have been identified to have redundant roles in UV induced DDR, with CaRAD4 epistatic to CaRAD23 [83]. CaRAD3 in C. albicans seems essential, and when expressed in yeast has a helicase activity on a duplex DNA substrate and complements the defects in both NER pathway and transcription [84]. Furthermore, C. albicans versions of NER genes CaRAD2 and CaRAD10 were identified, but the level of UV sensitivity of the C. albicans Carad2 and *Carad10* mutants was higher than that of the *Scrad2* and *Scrad10* mutants [81]. This difference could be caused by different levels of exposure to the external environment, where S. cerevisiae cells have more chance to be stressed by UV light, compared to C. albicans cells.

#### 5.5. Postreplication repair (PRR)

The DNA postreplication repair (PRR) pathway fills DNA damage-induced single-stranded gaps without removing the replication-blocking lesions [85]. In S. cerevisiae, ScRad6 forms a heterodimer with ScRad18, which is central to translesion synthesis [86–88]. PRR can be further divided into two pathways: translesion DNA synthesis mode, that involves ScRev3, ScRev7, ScRev1 and error-free mode, which includes ScUbc13, ScMms2, ScRad5, Polô-PCNA (Proliferating Cell Nuclear Antigen) [85]. In C. albicans, the identified CaRAD6 gene can complement the defect of an Scrad6 null mutation in UV sensitivity [37,89]. In addition, CaRad6 depletion causes UV sensitivity in C. albicans, showing a conservative DNA damage-related role [37,89]. We have also characterized the phenotype of deleting CaRAD18, a putative transcription factor with a zinc finger DNA-binding motif, which causes increased sensitivity to the genotoxic stresses induced by MMS and HU [37]. Moreover, ScRad6-ScRad18 complex regulates monoubiquitination at the Lysine (K) 164 residue of PCNA, which can be recruited to DNA lesions in S. cerevisiae [90]; in contrast, the detailed mechanism by which CaRad6-CaRad18 regulates PPR remains unclear in C. albicans.

#### 5.6. Mismatch repair (MMR)

The DNA mismatch repair (MMR) pathway functions to correct mismatches generated during DNA replication and recombination. In *S. cerevisiae*, the ScMsh2–ScMsh6 complex is responsible for the repair of mispaired bases, while the ScMsh2–ScMsh3 complex is primarily responsible for the repair of larger insertion/deletion mispairs [91,92]. Other proteins, including ScMlh1–ScPms1, ScMlh1–ScMlh3, DNA polymerase  $\delta$ , RPA, PCNA, RFC, ScExo1, ScRad27 and the DNA polymerase  $\delta$  and  $\varepsilon$  associated exonucleases, are implicated in this process [91]. In *C. albicans*, MMR genes have not been extensively characterized; only *CaMSH2* and *CaPMS1* mutants have been constructed, but they cause no apparent genotoxic phenotypes [65].

#### 6. DNA damage response allows pathogenesis in C. albicans

#### 6.1. DNA damage response contributes to morphogenesis

Morphology of *C. albicans* cells is the key pathogenic character and can be affected by damage response. In cases of DNA damage, the checkpoints may be activated to arrest the cell cycle and further induce polarization/extension of *Candida* cells, generating a form of filamentous-like growth. For instance, genotoxic stresses like HU and MMS induce filamentous growth of *Candida* cells, accompanied by the activation of checkpoint CaRad53 [36,38]. In addition, the DDR deficiency in *C. albicans* caused by deleting the DNA damage repair genes *CaRAD52*, *CaMMS22* and *CaMMS21*, results in a change in cell morphology, creating an increased ratio of elongated and connected cells [59,93]. However, this checkpoint-induced filamentous growth is distinct from classical hyphal forms, and the pathogenicity of this kind of filamentous form remains to be established.

#### 6.2. DNA damage response contributes to biofilm formation

Biofilm formation is also a critical virulence factor for *C. albicans* cells and is closely associated with morphogenesis. Since many DDR genes influence morphogenesis, they may show critical roles in biofilm formation. For instance, *CaDDR48* is a stress-associated gene involved in DDR, and its transcription is upregulated in biofilm formation. In consistent, deletion of *CaDDR48* decreases biofilm formation in *C. albicans* [94]. Moreover, deletion of NER genes, *CaRAD4* or *CaRAD23*, significantly increases the biofilm formation ability in *C. albicans* [83]. Since deleting *CaRAD4* or *CaRAD23* does not influence morphology or ture hyphae formation, the increased biofilm formation may attribute to altered transcription of several cell wall genes. Therefore, the potential regulation on cell wall structure or biofilm formation by DDR deserves further studying.

#### 6.3. Positive roles of DNA damage response in pathogenicity

DDR genes may contribute to pathogenicity, which is consistent with their roles in defending against the DNA damage stress generated by host immune cells. For instance, deleting *CaRTT109* increases sensitivity to  $H_2O_2$  and other DNA damage stressing agents in *C. albicans* [77]. Moreover, *CaRTT109* deletion results in significantly less pathogenicity in the murine model and increased susceptibility to killing by macrophages that can be suppressed by the NADPH oxidase (NOX) inhibitor diphenylene-iodonium chloride (DPI) [77]. This is a piece of direct evidence that the DNA damage response in the fungal pathogen is necessary for fighting against oxidative DNA damage stress. Similarly, deletion of *CaRAD52* confers strong filamentous growth, but the filamentous growth does not increase the pathogenicity, with the mutant showing decreased virulence in a murine model [95]. In addition, *CaLIG4*, a typical NHEJ gene in *C. albicans*, also plays a positive role in regulating pathogenicity [73]. Additionally, *CaRFX2* is a DNA damage responsive gene that regulates the expression of *CaRAD6* and *CaDDR48* in *C. albicans*, and the *CaRFX2* deletion significantly reduces virulence in a murine model [96]. Those DDR genes, like *CaRTT109*, *CaRAD52*, *CaLIG4*, show indispensable roles in pathogenicity and cell morphogenesis in *C. albicans*.

Some other DDR genes, with no significant roles in morphogenesis, show clear roles in pathogenicity. Recently, we reported that NER protein CaRad23 is needed for pathogenicity in a murine model but plays no significant role in morphogenesis [83]. The mechanism may be the regulation by CaRad23 on virulencerelated genes. But whether the regulation of pathogenicity mediated by CaRad23 is related to NER or other DDR pathways remains to be established.

## 6.4. Negative roles of DNA damage response in pathogenicity

The DDR-related genes can also show negative roles in pathogenicity. Depleting CaPph3, one of the phosphatases involved in deactivating CaRad53, increases fungal pathogenicity in the murine model [36]. A similar consequence also happens to the deletion of or even point mutation in *CaPSY2* [36]. One possibility could be the increased true hyphae formation caused by deleting CaPPH3. Interestingly, the double gene deletion of CaPPH3 and CaPTC2 shows decreased pathogenicity, although the CaPTC2 single gene deletion shows no significant role in pathogenicity [52]. In budding yeast. ScPph3 acts redundantly with ScPtc2 and ScPtc3 in DSB repair [97]. Kim et al. showed that the ScPPH3 ScPTC2 ScPTC3 triple gene depletion sensitizes cells to several genotoxic drugs such as MMS, HU and cisplatin [97]. Similarly, in C. albicans, double deletion of both CaPPH3 and CaPTC2 results in dramatic genotoxic sensitivity, which may reflect the cells' poor survival ability in the host. Therefore, multiple functions of DDR genes in pathogenicity may be observed according to the different genetic backgrounds in C. albicans.

In addition, *CaTOP2*, which encodes DNA topoisomerase II, also plays a negative role in pathogenicity [98]. The underlying mechanism could be the high capacity to produce true hyphae and the increased phospholipase as well as proteinase activities in the mutant [98]. By contrast, deletion of *CaTOP1*, encoding DNA topoisomerase I, results in reduced pathogenicity [99]. This difference in pathogenicity may suggest that CaTop1 and CaTop2 regulate virulence independent of the activity of topoisomerase.

In general, whether the negative roles of DDR genes in regulating the pathogenicity is a direct or independent role from DDR signaling pathways remains unclear. Since the deletion of PP4 genes and *CaTOP2* promotes hyphae growth, it is possible that DDR genes function in pathogenicity, at least partially, via affecting morphogenesis.

Taken together, many DNA damage response genes are indispensable for pathogenicity (Fig. 3). Some DDR genes, like *CaRTT109*, are needed for alleviating cellular DNA damage stress; some, like *CaPPH3*, are linked to morphogenesis and the remaining genes have, up to now, unclear involvement. The pathogenicity in the host is a comprehensive effect that depends on the direct and indirect effects of DDR genes. Therefore, the specific mechanisms by which DDR genes control pathogenicity need to be uncovered through the model cell and animal assays.

# 7. DNA damage response in other fungal pathogens

DNA damage response in many other medical fungi also plays significant roles in regulating morphology, resistance to host cells



**Fig. 3. DNA damage response genes that are responsible for the pathogenicity of** *C. albicans* **cells.** Typical genes involved in different DDR pathways are selected. *RAD52, LIG4, RTT109, RFX2, RAD23* and *TOP2* play positive roles in pathogenicity. *TOP1, PPH3, PSY2* and *PSY4* play negative roles in pathogenicity. *SDS22* overexpression attenuates pathogenicity.

and pathogenicity. For instance, deletion of DNA damage repair genes CgRTT107, CgSGS1 or chromatin organization genes CgRSC3, CgRTT109 in C. glabrata, results in decreased virulence in the murine model of disseminated candidiasis [100]. In addition, CgRAD53, the ortholog of ScRAD53 and CaRAD53, has been identified in C. glabrata. Constitutive expression of CgRAD53 supports the growth of C. glabrata cells in the presence of MMS, HU and cobalt chloride. CgRAD53 is also involved in biofilm formation and thus shows a potential correlation with virulence in *C. glabrata* [101]. Furthermore, it is well known that ScRad53 and CaRad53 are phosphorylated in response to genotoxic stresses, but strikingly in C. glabrata, exposure to DNA damage stresses does not induce CgRad53 phosphorylation, suggesting alterable signaling DDR pathways. Additionally, several key protectors of genome stability are upregulated in response to DNA damage stress in S. cerevisiae but downregulated in C. glabrata [102]. Therefore, the DNA damage response pathway could be different in these eukaryotic cells, which may contribute to rapidly generating genetic change, drug resistance and pathogenicity of C. glabrata . Finally, the C. auris cells usually do not produce hyphae, but the induction of DNA damage can cause filamentation growth, supplying a potential correlation to its pathogenicity [103].

A recent study in *Cryptococcus neoformans* reveals that perturbation of both *CnRAD53* and *CnCHK1*, two main checkpoints, attenuates the virulence, probably by promoting phagosome maturation within the macrophage, reducing melanin production and increasing susceptibility to oxidative stresses [104]. This observation directly supports the idea that DNA damage checkpoint kinases are linked to pathogenesis. It also supplies the underlying mechanism by which checkpoints regulate pathogenicity during host infection. More recently, BER genes *CnAPN1* and *CnAPN2*, were reported to modulate the DDR, melanin production, tolerance to drugs and also virulence of *C. neoformans* [105]. In addition, loss of *CnPMS1*, a mismatch repair gene in *C. neoformans*, increases mutation rates and reduces virulence [106]. These observations suggest that direct DNA damage response genes regulate the pathogenicity of *C. neoformans*.

In *Aspergillus fumigates*, several studies demonstrate that DDR or related pathways contribute to virulence and drug resistance. For instance, AfRtt109, a canonical histone acetyltransferase, functions in response to genotoxic agents but also regulates development as well as virulence [107]. Moreover, another study suggests that genetic instability caused by deleting AfAtmA and AfAtrA (ATM and ATR homologs in *A. fumigates*) can confer an adaptive advantage, mainly in the intensity of voriconazole resistance acquisition [108].

In the phytopathogenic fungus *Ustilago maydis*, during induction of the virulence program, the cell cycle is arrested on the plant surface and it is not resumed until the fungus enters the plant [109]. During the formation of the infective hypha in this phytopathogenic fungus, two DDR kinases, UnAtr1 and UmChk1, are required to induce a G2 cell cycle arrest, which in turn is essential to facilitate the virulence program [109,110]. These observations suggest that DDR in *U. maydis* is associated with pathogenicity through regulating the cell cycle.

In general, current studies in these pathogenic fungi support the idea that DDR is closely related to pathogenicity, mainly through coordinating cell cycle progression. These findings may also help to understand the underlying mechanism by which DDR regulates pathogenicity in *C. albicans.* 

# 8. Discussion

The phagocytosis of pathogens by host immune cells may stimulate the release of active molecules, including ROS or nitric oxide (NO) to damage the proteins, lipids and DNA as part of the pathogen destruction program. After the invading pathogens are engulfed by host immune cells, ROS stressing can induce various DNA lesions, including modified bases, apurinic/apyrimidinic (AP) sites, SSBs or DSBs inside the pathogen cells [111]. Several studies identify the crosstalk between the DDR and oxidative stress response. In humans, oxidative stresses, including ROS induce ATM activation, link to metabolic regulation and cancer progression [111,112]. In addition, checkpoint activation through ATR can also be triggered by oxidative stress, but independent of ATM [111]. In S. cerevisiae, ScMec1 functions in sensing, responding to and affecting the redox state of the cell [111]. And in *C. albicans*, the potential function of CaMec1 in oxidative stress is unknown. Instead, the checkpoint kinase CaRad53 is found to be activated in response to oxidative stress in a CaTrx1 dependent manner [5]. These observations suggest the close relationship between DDR and oxidative stress. In addition, DNA damage repair genes, such as CaAPN1, CaSRS2, CaPOL30, CaRAD57, CaRDH54 and several oxidative stress response genes in C. albicans cells are induced during the incubation with macrophages [113]. More immediately, several DDR genes, like CaRTT109, are essential for the pathogenicity in dependent of its role in fighting against ROS [77]. Taken together, the DDR in response to oxidative stress seems crucial for the resistance to host immune cells. On the other hand, DDR genes are involved in damaging host cells. A large-scale analysis of interactions between C. albicans cells with macrophages reveals that several DNA damage response genes, such as CaRAD3 and *CaRAD26* are required to induce macrophage pyroptosis [114]. In general, the DDR genes seem indispensable for the pathogenicity of C. albicans cells, either for colonization in or escaping from host immune cells. But the specific mechanism for different DDR genes in pathogenicity remains to be determined.

Up to now, numerous DDR genes have been identified in C. albicans. The majority of them act following the rules of their orthologs in S. cerevisiae, but the specific mechanism can be inconsistent, especially for the checkpoint protein phosphatases. This difference may arise during the evolutionary process, probably due to the different external living environments. In contrast to the DDR-related function, some other points of DDR genes may need to be further explored in the future. In particular, the detailed genetic interaction relationships between different DDR genes, which are critical for understanding the DDR signaling pathway, remain less examined in C. albicans. The main reason could be that C. albicans cells are diploid: to get visual phenotypes, two copies of a single gene should be deleted with two sequential rounds of transformation, leading to low genetic manipulation efficiency. Recently, CRISPR/Cas9-mediated gene editing has been developed to enable facile knockout in Candida species [115,116]. As a consequence, the large-scale genetic interaction assay may be accomplished in the future, which may help understand the specific regulating mechanism of DDR in C. albicans. Furthermore, the potential involvement of DDR genes in pathogenicity remains poorly understood. The difficulty could be that it is time- and fund-consuming to perform classical virulence assay through murine models. Nowadays, several alternative model organisms like Galleria mellonella or Caenorhabditis elegans [117,118], which show significant advantages, can be used to test pathogenicity. Finaly, some computational systems, like the C. albicans coexpression network (CalCEN) seem useful for identifying virulence factors, which could be used for studying the importance of DDR genes [119].

Taken together, DDR signaling pathways and genes in *C. albicans* show significant roles in fighting against DNA damage stress, including the internal ROS stress induced by the host immune system. Therefore, they may supply the potential value for clinical therapy.

# **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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