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DNA Repair 4 (2005) 59–69
DNA REPAIR
www.elsevier.com/locate/dnarepair

The *Saccharomyces cerevisiae PDS1* and *RAD9* checkpoint genes control different DNA double-strand break repair pathways

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Available online 1 October 2004

Abstract

In response to DNA damage, the *Saccharomyces cerevisiae* securin Pds1 blocks anaphase promotion by inhibiting *ESP1*-dependent degradation of cohesins. *PDS1* is positioned downstream of the *MEC1*- and *RAD9*-mediated DNA damage-induced signal transduction pathways. Because cohesins participate in postreplicative repair and the *pds1* mutant is radiation sensitive, we identified DNA repair pathways that are *PDS1*-dependent. We compared the radiation sensitivities and recombination phenotypes of *pds1*, *rad9*, *rad51* single and double mutants, and found that whereas *pds1 rad9* double mutants were synergistically more radiation sensitive than single mutants, *pds1 rad51* mutants were not. To determine the role of *PDS1* in recombinational repair pathways, we measured spontaneous and DNA damage-associated sister chromatid exchanges (SCEs) after exposure to X rays, UV and methyl methanesulfonate (MMS) and after the initiation of an HO endonuclease-generated double-strand break (DSB). The rates of spontaneous SCE and frequencies of DNA damage-associated SCE were similar in wild type and *pds1* strains, but the latter exhibited reduced viability after exposure to DNA damaging agents. To determine whether *pds1* mutants were defective in other pathways for DSB repair, we measured both single-strand annealing (SSA) and non-homologous end joining (NHEJ) in *pds1* mutants. We found that the *pds1* mutant was defective in SSA but efficient at ligating cohesive ends present on a linear plasmid. We therefore suggest that checkpoint genes control different pathways for DSB repair, and *PDS1* and *RAD9* have different roles in recombinational repair.

Keywords: *Saccharomyces cerevisiae*; *PDS1*; Double-strand break repair; Cell cycle checkpoint; *RAD* genes

1. Introduction

The faithful transmission of genetic information relies on DNA repair and replication mechanisms and the mechanical separation of sister chromatids to opposite poles. Cell cycle checkpoints are surveillance mechanisms that ensure the accurate completion of each stage of the cell cycle [1]. The G_2 checkpoint pathway is triggered by either DNA damage, spindle damage, or by improper attachment of microtubules to the kinetechore. The checkpoint pathway is divided into protein sensors that detect damage, transducers that transmit the DNA damage signal, and effectors that block cell cycle progression (for review, see [2]). The DNA damage signal, postulated to be single-stranded DNA, is detected by a complex that includes Rad9 and Rad17 proteins (for review, see [2]).

In budding yeast, checkpoint-dependent G_2 arrest is achieved by inhibiting the transition from metaphase to anaphase using two parallel *MEC1* (ATM/ATR)-dependent pathways [3]. The *CHK1*-activated pathway prevents securin (Pds1) ubiquitination and subsequent degradation by the anaphase promoting complex (APC/C^{Cdc20}) [4], thus preventing Esp1-dependent degradation of the cohesins Scc1/Mcd1 [5] and sister chromatid separation. The *RAD53* (CHK2)-activated pathway results in the inhibition of the polo kinase Cdc5, which prevents the degradation of the G_2 cyclins Clb2 and Clb5, and thus inhibits mitotic exit [3]. Rad53 inhibits Cdc20–Pds1 interaction, and Pds1 inhibits APC^{Cdh1} degradation of Clb2, thus indicating cross talk between these pathways [4]. However, *rad53* or *pds1* single mutants only exhibit a partial defect in DNA damage-induced G_2 arrest; whereas *rad53 pds1* double mutants [6] and *rad9* mutants [7], are completely deficient in DNA damage-induced G_2 arrest.

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doi:10.1016/j.dnarep.2004.08.007

G_2 arrest provides time for gap repair between sister chromatids, which are the preferred substrates for the recombinational repair of double-strand breaks (DSBs) [8,9]. Compared to wild type, *rad9* mutants exhibit lower frequencies of sister chromatid exchange (SCE) after exposure to either X rays or after HO endonuclease-induced DSBs are targeted to specific chromosomal sites [10]. *rad9* mutants also exhibit higher rates of spontaneous, mitotic chromosomal rearrangements and higher frequencies of DNA damage-associated translocations [10]. Thus, a higher frequency of promiscuous recombinational repair may occur if the timing of recombination is altered.

How each of the parallel branches of the G_2 checkpoint pathway contributes to recombinational repair of DSBs is unclear. Because cohesins are required for postreplicative DSB repair [11], recombinational repair may be enhanced by *pds*/inhibition of cohesin degradation. pds1 mutants share phenotypes with other checkpoint mutants, including hypersensitivity to ionizing radiation, elevated frequencies of chromosome loss [12], and higher levels of translocations and gross chromosomal rearrangements in haploid strains [13]. These studies thus suggest that *PDS1* is important in preventing promiscuous repair of DSBs.

Unlike other checkpoint mutants that are hypersensitive to DNA damaging agents, *pds1* mutants are also hypersensitive to nocodazole, a drug that depolymerizes microtubules [14]. Mad1/Mad2/Mad3/Bub1 pathway, which monitors kinetechore attachment to the microtubules, inhibits anaphase by preventing Pds1 degradation [15]. Thus, *pds1* mutants may exhibit complex phenotypes due to the role of *PDS1* in both DNA damage and spindle checkpoints.

We investigated whether *PDS1*-dependent checkpoints are important in DNA damage-associated SCE, and whether *PDS1*-mediated radiation repair participates in the same *RAD9*-recombinational repair pathway. Our studies revealed that *pds1 rad9* but not *pds1 rad51* double mutants exhibit synergistic sensitivity to X rays, compared to the single mutants. In contrast to *rad9* mutants, *pds1* mutants are not deficient in sister chromatid gap repair, but are deficient in DSB repair that is mediated by single-strand annealing (SSA). To determine whether recombination phenotypes of *pds1* were similar to *rad51* or *rad9* mutants, we also measured and frequencies of spontaneous and X ray associated translocations. These studies reveal that *PDS1* and *RAD9* control different DSB repair pathways.

2. Materials and methods

2.1. Media and yeast strains

Standard media for the culture of yeast, SC (synthetic complete, dextrose), SC-HIS (SC lacking histidine), SC-LEU (SC lacking leucine), SC-TRP (SC lacking tryptophan), SC-URA (SC lacking uracil), YP (yeast extract, peptone), and YPD (YP, dextrose), are described by Sherman et al. [16]. YPL medium contains YP with 2% lactate (pH 5.8); YPGlu medium contains YP medium with 2% ultra-pure glucose; YPGal medium contains YP medium with 2% ultra-pure galactose (Sigma, St. Louis, MO).

Relevant yeast strains are listed in Table 1 and are derived from a S288c background. Strains used to measure sister chromatid exchange (SCE) contain two *his3* fragments, positioned in tandem at *trp1*, as previously described [10]. The strains used to measure translocations contain *his3* fragments on chromosomes II and IV, as described in Fasullo et al. [10]. The *pds1::LEU2* disruption in the designated strains was made by a one-step gene replacement [17], using previously described transformation protocols [10] except that plates were incubated at room temperature. A *Bam*H1 restriction fragment from plasmid pAY55 [14], containing *pds1::LEU2*, was introduced into YB204, YB109, and YA148 by selecting for Leu^+ transformants. Leu^+ transformants were screened for temperature sensitivity, and the *pds1::LEU2* disruption was confirmed by Southern blots. The *pds1* diploid strain used to measure directed translocations was constructed by mating the haploid strain YB304, which contains recombination substrates, with the *pds1* (YB300) strain congenic to YA148.

Double mutants were derived from diploid crosses of the appropriate single mutants and obtaining a meiotic segregant with the desired phenotype. The *pds1rad9* (YB302), *pds1 rad51* (YB303) and *pds1 rad1* (YB304) double mutants are meiotic segregants derived from diploid crosses of *pds1* (YB301) and *rad9* (YB147), *pds1* (YB301) and *rad51* (YB177) and *pds1* (YB301) and *rad1* (YB221) strains, respectively (see Table 1). The *rad51 rad9* double mutant is derived from a diploid cross of *rad51* (YB177) and *rad9* (YB147) haploids.

Plasmids expressing HO endonuclease or *Eco*RI nuclease were introduced into yeast strains by selecting for Trp^+ or Ura^+ transformants, respectively. pGHOT-*GAL3* has been previously described [10]. p*GAL3* was constructed by *Bam*H1 digestion of pGHOT-*GAL3* and religation of the *Bam*H1 restriction fragment lacking *GAL10::HO*.

2.2. Measuring X ray and UV sensitivity

Both wild type and mutant strains were inoculated into 2 ml of YPD and incubated at 23 °C. Cells were grown to stationary phase. Appropriate serial dilutions were plated onto YPD medium and exposed to X rays or UV. All plates were incubated at room temperature. The percent survival was calculated by dividing the number of CFU obtained after radiation exposure by the number of CFU before exposure and multiplying by 100%. Statistical significance of the differences between radiation sensitivities was determined by the two-tailed *t*-test [18].