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Saccharomyces cerevisiae RAD53 (CHK2) but not *CHK1* is required for double-strand break-initiated SCE and DNA damage-associated SCE after exposure to X rays and chemical agents

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Abstract

Saccharomyces cerevisiae RAD53 (CHK2) and CHK1 control two parallel branches of the *RAD9*-mediated pathway for DNA damage-induced G_2 arrest. Previous studies indicate that *RAD9* is required for X-ray-associated sister chromatid exchange (SCE), suppresses homology-directed translocations, and is involved in pathways for double-strand break repair (DSB) repair that are different than those controlled by *PDS1*. We measured DNA damage-associated SCE in strains containing two tandem fragments of *his3, his3-Δ5′* and *his3-Δ3′::HOcs*, and rates of spontaneous translocations in diploids containing *GAL1::his3-Δ5′* and *trp1::his3-Δ3′::HOcs*. DNA damage-associated SCE was measured after log phase cells were exposed to methyl methanesulfonate (MMS), 4-nitroquinoline 1-oxide (4-NQO), UV, X rays and HO-induced DSBs. We observed that *rad53* mutants were defective in MMS-, 4-NQO, X-ray-associated and HO-induced SCE but not in UV-associated SCE. Similar to *rad9 pds1* double mutants, *rad53 pds1* double mutants exhibited more X-ray sensitivity than the single mutants. *rad53 sml1* diploid mutants exhibited a 10-fold higher rate of spontaneous translocations compared to the *sml1* diploid mutants. *chk1* mutants were not deficient in DNA damage-associated SCE after exposure to DNA damaging agents or after DSBs were generated at *trp1::his3-Δ5′his3-Δ3′::HOcs*. These data indicate that *RAD53*, not *CHK1*, is required for DSB-initiated SCE, and DNA damage-associated SCE after exposure to X-ray-mimetic and UV-mimetic chemicals.

Keywords: *RAD53*; Sister chromatid exchange; *Saccharomyces cerevisiae*; *CHK1*; Double-strand break repair

1. Introduction

Checkpoints play a critical role in maintaining genetic stability by ensuring that DNA repair mechanisms correct DNA damage before replication or segregation of damaged chromatids. In *Saccharomyces cerevisiae* (budding yeast), repair of double-strand breaks (DSBs) occurs by homologous recombination in which the undamaged sister chromatid is the preferred template for gap repair [1]. The G_2 checkpoint mutant, *rad9* [2], is defective in DSB-initiated SCE [3] and non-homologous end joining (NHEJ) [4]. *rad9* diploids exhibit higher rates of homology-directed translocations and higher radiation-associated frequencies of chromosomal rearrangements, compared to wild-type [3]. Both genome instability [3] and NHEJ defects [5] in *rad9* can be partially suppressed by imposing cell cycle delays, implying that G_2 arrest suppresses the segregation of chromosomal fragments and facilitates DSB repair.

MEC1 (ATM/ATR) triggers two parallel *RAD9*-dependent pathways for signaling G_2 arrest (for review, see [6]). After phosphorylation by Mec1, Rad9 associates with DSBs [7] and is required for the phosphorylation of Rad53 and Chk1. The *CHK1*-mediated pathway activates the securin Pds1; the *RAD53* (CHK2)-mediated pathway inhibits the polo-like kinase Cdc5 and maintains Clb2/Cdc28 activity [8]. The *CHK1* and *RAD53* pathways converge by inhibiting cohesin degradation; Chk1 phosphorylates Pds1, thus inhibiting its

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ubiquitination by the Cdc20 anaphase promoting complex (APC), while Rad53 blocks the interaction of Pds1 and Cdc20 [9]. *RAD53* is required for DNA repair throughout the cell cycle [10], while *CHK1* is required for DNA repair only when DNA damage occurs in S phase [8]. Considering that both *pds1* [11] and *rad53* mutants are X-ray sensitive [12,13], it is possible that both *CHK1* and *CHK2* pathways contribute to recombinational repair of DSBs.

However, *PDS1*-mediated X-ray resistance is not *CHK1*-dependent since *chk1* mutants are not X-ray sensitive [14]. Although the *CHK1* pathway proceeds through *PDS1* [15], Pds1 activation in S phase occurs by a mechanism that is *CHK1*-independent but *MEC1*-dependent [16]. *CHK1*, however, has been postulated to mediate homologous recombination in response to replication blocks in both mammalian cells [17] and in yeast [18]. Thus, it is important to determine whether the *RAD9*-dependent pathway that controls SCE is mediated by *RAD53* or *CHK1*.

Mec1-dependent Rad53 phosphorylation occurs when Rad53 associates with Rad9 on DSBs [19]. Rad53 activation is required for DNA damage-induced transcription of DNA repair genes [20]. However, unlike *RAD9*, *RAD53* is essential [21] due to its role in regulating ribonucleotide reductase (*RNR*) [22], and is activated by stalled replication forks [19,23]. The *rad53* null mutant is viable when *SML1*, an inhibitor of *RNR*, is mutated or when *RNR1* is over-expressed [22]. When deoxynucleotide concentration is reduced due to hydroxyurea (HU) exposure, *RAD53* is required to keep stalled replication forks from irreversibly collapsing [24] and slows DNA replication by delaying the initiation of replication from late DNA origins [25]. The checkpoint signaling pathway that is initiated by replication fork stalling is mediated by Mrc1 and Tof1 [26,27]; however, a *RAD9*-mediated response may be initiated if nuclease digestion occurs at stalled forks [6]. Phosphorylated Rad53 triggers multiple changes through its activation of Dun1 and its association with Asf1 (for review, see [28]). *asf1* exhibits higher rates of spontaneous SCE [29], while *dun1* exhibits higher frequencies of UV-associated SCE [30]. Thus, Rad53 could participate in suppressing spontaneous SCE or facilitating DNA damage-associated SCE when both S phase and G_2 checkpoints are triggered.

The *RAD53* requirement for Rad55 phosphorylation [31] and the DNA damage-inducibility of *RAD51* [32,33] further suggest that *rad53* exhibits homologous recombination phenotypes. *rad53* is defective in double-strand gap repair, as demonstrated by experiments in which a linear plasmid is introduced into yeast, and recombination occurs between the linear plasmid and the chromosome [34,35]. *rad53* exhibits higher ratios of crossover events to non-crossover events in gap repair assays, suggesting that Rad53 is tilting the balance towards non-crossovers [35]. The exact molecular explanation for these observations is unknown. Cds1, the *Schizosaccharomyces pompe* Rad53 homologue, interacts with the Mus81 homologue [36], and is required for meiotic crossovers [37]. Evidence that such an interaction occurs in budding yeast is supported by a large scale protein screen [38]. These observations suggest that *RAD53* facilitates DSB-repair in the genome so that crossover events are reduced.

Little is known concerning Rad53 function in regulating recombination between chromosomal sequences. Herein we determined the *RAD53* and *CHK1* requirement for spontaneous and DNA damage-associated SCE. *rad53,* but not *chk1,* mutants exhibited a two-fold higher rate of spontaneous SCE than wild-type. *RAD53,* but not *CHK1*, is required for SCE stimulated by a single HO endonuclease-induced DSB, and for DNA damage-associated SCE after exposure to X rays, 4-NQO and MMS. We thus suggest that *RAD9*-dependent DSB-initiated SCE pathway is mediated by *RAD53*.

2. Materials and methods

2.1. Media, chemicals, and yeast strains

Standard media, including YPD (yeast extract, peptone, dextrose), SC-HIS (synthetic complete lacking histidine), SC-TRP (synthetic complete lacking tryptophan), SC-URA (synthetic complete lacking uracil), and SC-LEU (synthetic media lacking leucine) have been previously described in Burke et al. [39]. YPD kanamycin is YPD supplemented with 200 μg/ml of kanamycin sulfate. YPD(HU) is YPD supplemented with 50 mM HU. Compounds used in this study, including methyl methanesulfonate (MMS) and 4-nitroquinoline 1-oxide (4-NQO), were purchased from Sigma or Aldrich Chemicals. 4-NQO was dissolved in dimethyl sulfoxide (DMSO).

Relevant yeast strains are listed in Table 1. The strains used to measure homology-directed translocations and unequal SCE are derived from a S288c background, and their construction was previously described [3]. These strains contain two tandem truncated fragments of *his3*, *his3-Δ3′* and *his3-Δ5′* as illustrated (Fig. 1). The strains used to measure translocations contain *his3* fragments on chromosomes II and IV, as described in Fasullo et al. [3]. Directed translocations were measured in diploids, which were derived from one haploid containing the *his3* fragments and one haploid lacking the truncated fragments.

We introduced *rad53* (*mec2-1*), an allele that confers a checkpoint defect but not lethality [40], and *rad53* null mutation into strains to measure SCE. We backcrossed *mec2-1* mutant ten times to YB204 (Table 1) and screened for HU sensitive meiotic segregants. Since *RAD53* is essential due to its regulation of ribonucleotide reductase [19], we first deleted *SML1*, a negative inhibitor of *RNR1*. The *sml1*::*KanMX* fragment was amplified by PCR using primers 5′CATATCGTTACTGTTTTGGAACATCGC3′ and 5′ATGAGTAGCAGCACGTTCCTT3′ (Resgen, Inc.) and introduced into the wild-type strains YB203 (*MAT***a**-inc) or YB204 (*MATα*) by selecting for kanamycin resistant transformants. To make a *rad53* null mutant in the *sml1* mutant background, *Bam*HI-digested pWL22 was introduced into