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Loss of heterozygosity in yeast can occur by ultraviolet irradiation during the S phase of the cell cycle

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Abstract

A *CAN1/can1Δ* heterozygous allele that determines loss of heterozygosity (LOH) was used to study recombination in *Saccharomyces cerevisiae* cells exposed to ultraviolet (UV) light at different points in the cell cycle. With this allele, recombination events can be detected as canavanine-resistant mutations after exposure of cells to UV radiation, since a significant fraction of LOH events appear to arise from recombination between homologous chromosomes. The radiation caused a higher level of LOH in cells that were in the S phase of the cell cycle relative to either cells at other points in the cell cycle or unsynchronized cells. In contrast, the inactivation of nucleotide excision repair abolished the cell cycle-specific induction by UV of LOH. We hypothesize that DNA lesions, if not repaired, were converted into double-strand breaks during stalled replication and these breaks could be repaired through recombination using a non-sister chromatid and probably also the sister chromatid. We argue that LOH may be an outcome used by yeast cells to recover from stalled replication at a lesion.

Keywords: Loss of heterozygosity; Canavanine-resistance; Ultraviolet light; Cell cycle; *Saccharomyces cerevisiae*

1. Introduction

Ultraviolet (UV) irradiation generates mostly pyrimidine dimers and other base modifications [1] that are normally removed by nucleotide excision repair (NER) [2]. However, unexcised lesions can interfere with the passage of DNA polymerase during replication [2,3]. DNA recombination is one of the predominant pathways used to rescue a replication fork stalled on the template [4–9]. In this case, UV-induced DNA lesions can cause the fork to arrest during S phase. The replication arrest thus formed can be rescued by recombination pathways by using either the undamaged sister chromatid or a non-sister chromatid, resulting in sister chromatid exchange or LOH, respectively.

LOH represents an important step in neoplastic transformation, which is responsible for the loss of a functional copy of a heterozygous tumor suppressor gene. Cell proliferation is a risk factor in the long process of cancer development [10,11]. Molecular genetic analysis of human cancers has shown that tumor cells exhibit multiple forms of genetic instability including chromosomal deletion, mitotic non-disjunction, and recombina-

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tion between homologous chromosomes [12], resulting in LOH; these phenomena all require cell division for their occurrence and fixation. Although the mechanism responsible for a particular LOH may be difficult to determine, homologous recombination can contribute to 88% of the LOH events in *Saccharomyces cerevisiae* [13], and 75% in human retinoblastoma locus [12]. Despite that several pathways are known to be involved in the generation of LOH, little is understood about the molecular events that lead to LOH, including those involving mitotic recombination.

Chromosomal lesions are extremely potent inducers of homologous recombination, raising the issue of whether they are causative lesions for LOH by allelic recombination. The repair of DNA lesions by homologous recombination involves the invasion of the 3′ end of the strand into an intact homologous chromosome, which serves as a template for repair by gene conversion or break-induced replication (BIR) [14,15].

In this study, we investigated the effect of UV radiation on the LOH of yeast cells throughout the cell cycle. We show that exposure of S phase cells to UV radiation results in an increase of LOH. The result may suggest that double-strand breaks are generated in S phase, but not G1 or G2/M phase, becoming an inducer of LOH. In contrast, irradiation of NER-deficient strains at any stage of the cell cycle causes equal induction of LOH. The result indicates that most UV-induced DNA lesions remain until the next S phase where double-strand breaks occur in the NER-deficient strains, hence irradiation leads to LOH equally at any stage of the cell cycle.

2. Materials and methods

2.1. Yeast strain, media and reagents

The diploid strain YAS3001 (*MATa/MATα CAN1/can1Δ::LEU2 ade2-1/ade2-1 lys2-1/lys2-1 ura3-52/ura3-52 leu2-3Δ112/leu2-3Δ112 HIS3/his3-Δ200 TRP1/trp1-901 ILV2/ilv2 V-11::LYS2 V-562::ADE2*) was constructed by mating YAS101 (*MATα can1Δ::LEU2 ade2-1 lys2-1 ura3-52 leu2-3,112 his3-Δ200 trp1-901*) with YAS106 (*MAT**a** ade2-1 lys2-1 ilv2 ura3-52 leu2-3,112 V-11::LYS2 V-565::ADE2*) [15]. *V-11::LYS2* and *V-562::ADE2* signifying the LYS2 fragment and ADE2 fragment, respectively, are inserted at the 11-kb and the 565-kb positions of chromosome V where the wild-type CAN1 gene resides at the 32-kb position. YAS109 and YAS110, rad2 derivatives of YAS101 and YAS106, respectively, carried a *rad2::KANMX* allele generated by PCR amplification of the *KAN* gene from pFA6a-kanMX [16]. The sequences of primers used are available upon request. YAS3002, a *rad2* derivative of YAS3001, was constructed by mating YAS109 with YAS110. All reagents and media were as described by Daigaku et al. [15].

2.2. Characterization of canavanine-resistant (Can^R) mutations in synchronized cells

Yeast cells were grown in YPD medium to the exponential phase and were arrested at the S phase by adding hydroxyurea (HU) to a final concentration of 200 mM and culturing at 30 °C for 3 h. Cultures were then monitored microscopically for unbudded S-arrested cells. When 90% or more of cells were in the S phase, cells were collected, washed twice in sterile distilled water, resuspended in the YPD medium, and incubated at 30 °C for 100 min. Every 25 min, cell samples were withdrawn, washed and resuspended in sterile distilled water, and irradiated with UV using a germicidal UV lamp. After an additional 2 h of incubation at 30 °C, cells were collected and the Can^R mutants were enumerated as described previously [13]. The chromosome structures associated with the formation of Can^R mutations were analyzed using V-11::*LYS2* and V-565::*ADE2* alleles as described previously [15].

2.3. Flow cytometric analyses

The S-arrested cells were regrown and cell samples were withdrawn every 25 min as described above. Cell samples were fixed with 70% ethanol overnight, washed twice with 50 mM sodium citrate, and resuspended in the 50 mM sodium citrate containing 0.25 mg/ml of RNase A. Following incubation at 37 °C for 1 h, these cells were treated again with 0.25 mg/ml of proteinase K at 37 °C for 1 h. After a brief sonication, 0.4 ml of propidium iodide (16 μg/ml) was added to the samples. A Becton Dickinson flow cytometer (FACS Calibar) was used to analyze the samples. For each time point, 20,000 cells were assayed.

3. Results and discussion

To investigate the contribution of allelic recombination to loss of heterozygosity (LOH) events, the diploid yeast strain YAS3001 was used. This strain carries *CAN1* and *can1Δ::LEU2* at the corresponding position of each homologous chromosome V, that is the *CAN1/can1*-heterozygote. As the *CAN1* gene product is responsible for sensitivity to canavanine, mutation of the wild-type *CAN1* gene results in resistance to canavanine (Can^R). Because mitotic recombination, which includes gene conversion, allelic crossover and break-induced replication (BIR) [14], is one of the DNA damage tolerance pathways, damage-induced allelic recombination could result in LOH. Actually, 90 J/m^2 of UV-radiation to YAS3001 increased the frequency of Can^R mutant cells by 35-fold (Fig. 1). Similarly, 9 J/m^2 of UV-radiation to an NER-deficient (*rad2Δ*) *CAN1/can1-*