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Yap1: A DNA damage responder in *Saccharomyces cerevisiae*

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ABSTRACT

Activation of signaling pathways in response to genotoxic stress is crucial for cells to properly repair DNA damage. In response to DNA damage, intracellular levels of reactive oxygen species increase. One important function of such a response could be to initiate signal transduction processes. We have employed the model eukaryote *Saccharomyces cerevisiae* to delineate DNA damage sensing mechanisms. We report a novel, unanticipated role for the transcription factor Yap1 as a DNA damage responder, providing direct evidence that reactive oxygen species are an important component of the DNA damage signaling process. Our findings reveal an epistatic link between Yap1 and the DNA base excision repair pathway. Corruption of the Yap1-mediated DNA damage response influences cell survival and genomic stability in response to exposure to genotoxic agents.

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1. Introduction

Cells are continually exposed to both endogenous and exogenous sources of reactive oxygen species (ROS). High levels of ROS are detrimental to cells leading to oxidative stress and to impaired physiological function through damage to DNA, proteins, and lipids (Apel and Hirt, 2004; Cadenas and Sies, 1985; Scandalios, 2005) In addition, chronic exposure to ROS has been associated with several human pathologies including cancer (Dreher and Junod, 1996), neurodegenerative disorders (Droge, 2002), and cardiovascular disease (Alexander, 1995), as well as with the process of aging (Harman, 1981).

DNA damage signaling confers on eukaryotic cells an efficient and rapid response to genotoxic insults. Mechanistic studies of DNA damage signaling pathways involving the detailed dissection of the interactions between dozens of previously identified components has proven to be beneficial in both clinical applications as well as for our basic understanding of signal transduction mechanisms. However, the vast majority of past and current investigations have focused on DNA damage signaling processes caused by either DNA single or double strand breaks (SSBs and DSBs, respectively) (Ciccia and Elledge, 2010). Although a significant fraction of cellular DNA damage is, indeed, contributed by SSBs and DSBs, oxidative damage is a major source of the deleterious modifications of DNA and, in certain cases thought to be a precursor of SSBs and DSBs (Sage and Harrison, 2011). In humans, recent studies have shown that defects in base excision repair and/or aberrant repair of oxidative DNA damage could contribute to tumorigenesis (Kryston et al., 2011; Nemec et al., 2010).

Elucidation of oxidative DNA damage signaling is not a straightforward task since oxidative modifications of DNA are efficiently repaired by base excision repair enzymes with overlapping specificities (Doetsch et al., 2001). However, previous studies from our group have demonstrated that DNA damage itself causes increases in cellular ROS (Evert et al., 2004; Salmon et al., 2004; Rowe et al., 2008). We hypothesized that DNA damage-induced moderate increases in ROS levels could be beneficial for cells if such levels function as a molecular switch of the redox status of transcriptional activators of DNA repair and/or ROS-scavenging genes. Here we report that signaling of non-oxidative, alkylation agent-induced DNA damage could be relayed by the transcription factor Yap1 providing insight into the mechanisms DNA base damage response networks.

In order to maintain genomic stability, cells have evolved a number of DNA damage management pathways that include direct reversal, base excision repair (BER), nucleotide excision repair (NER), mismatch repair, translesion synthesis, and recombination (Friedberg et al., 2006). As BER and NER are key systems for the

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removal of numerous deleterious lesions from the genome (Friedberg et al., 2006), the influence of ROS signaling on the function of these pathways may be crucial for an appropriate cellular response to genotoxic stress. BER is primarily responsible for the repair of small, non-bulky lesions and abasic sites, such as those caused by oxidizing and alkylating agents (Lindahl et al., 1997). NER is thought to be the major pathway for the repair of DNA helix distorting lesions such as ultraviolet light (UV-C)-induced cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts (6–4 PP) as well as a variety of bulky adducts (Lindahl et al., 1997). In addition to its major role in repair of bulky DNA lesions, NER can also contribute to the repair of oxidative DNA damage (Evert et al., 2004; Friedberg et al., 2006).

Yeast is an informative model system for dissection of eukaryotic DNA repair pathways as there is a high degree of conservation with complex organisms. In yeast, BER and NER are the major repair pathways for many types of DNA lesions, and the specific range of DNA damages that are primarily handled by each are generally distinct. By examining cells deficient in one or both of these repair pathways it is possible to delineate the relationship between DNA damaging agent cytotoxicity and ROS levels and determine whether modulation of ROS levels constitutes a general response to DNA damage or is limited to specific classes of DNA lesions.

We have recently documented that increased levels of DNA damage, regardless of type or degree of cytotoxicity, cause elevations in intracellular ROS (Rowe et al., 2008). These studies also revealed that cells deficient both in BER and NER harbor greatly elevated (~800-fold) levels of oxidative DNA damage, and substantially increased ROS levels (Salmon et al., 2004). Furthermore, intracellular ROS levels in both WT and repair-deficient strains (BER$^-$, NER$^-$, and BER$^-$/NER$^-$ strains) are elevated in a dose-dependent manner following exposure to the DNA alkylating agent methyl methanesulfonate (MMS) or UV-C (Rowe et al., 2008). These studies indicate that DNA damage per se can cause an increase in intracellular ROS regardless of the type of DNA lesion or the primary pathway involved in its repair, suggesting that the ROS produced may function in DNA damage-induced signaling processes (Rowe et al., 2008).

The transcription factor Yap1 is activated by oxidative stress (Coleman et al., 1999; Delaunay et al., 2000, 2002; Moye-Rowley et al., 1989; Rodrigues-Pousada et al., 2004). Under normal cellular growth conditions Yap1 is localized in the cytoplasm due to constitutive nuclear export by the classical nuclear export receptor, Crm1 (Delaunay et al., 2002; Yan et al., 1998). However, in response to oxidative stress, intermolecular disulfide bonds form in Yap1 blocking the binding of Crm1. In absence of Crm1 Yap1 cannot be exported to the cytoplasm and accumulates in nucleus where it activates transcription of over 70 genes (Gulshan et al., 2005; Okazaki et al., 2007). The best characterized group of transcriptional targets of Yap1 are the genes involved in ROS scavenging, such as superoxide dismutase (*SOD1*), catalase (*CTT1*), and thioredoxin (*TRX2*) (Temple et al., 2005). Several studies indicate that genes involved in DNA repair, replication, and cell cycle check point control can also be activated by Yap1, including *NTG1*, *POL1*, *MAG1*, *MEC1*, and *POL3* (Monteiro et al., 2008). We have recently reported that as the endogenous levels of ROS increase following exposure to MMS, Yap1 accumulates within the nucleus (Rowe et al., 2008). Collectively, these findings suggest that Yap1 may be directly involved in the DNA damage response via ROS signaling.

The goal of the present study was to determine the involvement of Yap1 in the DNA damage response. We examined several biological endpoints in a set of isogenic repair-proficient (WT, *yap1Δ*) and repair-deficient (BER$^-$, BER$^-$ *yap1Δ*, NER$^-$, and NER$^-$ *yap1Δ*) strains with or without functional Yap1. We determined the sub-cellular localization of Yap1 in cells exposed to different DNA damaging agents and found that Yap1 accumulates within the nucleus in response to MMS and H_2O_2, but not UV-C exposure. Analysis of the role of Yap1 in modulating cytotoxicity, intracellular ROS levels, and levels of chromosomal aberrations caused by these agents allowed us to conclude that ROS generated in response to DNA damage mediates signaling processes resulting in Yap1 activation. Such activation appears to be invoked in response to certain types of DNA damage, specifically, DNA damage repaired by BER.

2. Material and methods

2.1. Strains, media, and growth conditions

All the strains utilized in this study (with exception of DSC0025, DSC0035 and DSC0036) are haploid isogenic derivatives of heterozygous diploid hDNP42 (Supplemental Table 1). Two sets of isogenic haploid strains were obtained by sporulation and dissection of independent isolates of the parental diploid hDNP42. Each isogenic set contains a repair proficient strains (wild type – WT and WT *yap1Δ*) and repair deficient strains (BER$^-$, NER$^-$, BER$^-$ *yap1Δ*, and/or NER$^-$ *yap1Δ*). hDNP42 diploid strain was constructed by the replacement of one copy of *YAP1* gene in heterozygous hDNP19 diploid with a PCR fragment containing the *natNT2* gene flanked by *YAP1* upstream and downstream sequences, conferring resistance to nourseothricin and deletion of the entire *YAP1* open reading frame. Plasmid pYM17 (Euroscarf) was used as a template for amplification of *natNT2* gene (Degtyareva et al., 2008). Primer sequences are available upon request. Isogenic strains DSC0025 (WT), DSC0035 (BER$^-$) and DSC0036 (NER$^-$) had been described previously (Swanson et al., 1999) and were utilized in Yap1 localization studies and measurement of $O_2^{\bullet-}$ levels. The hDNP42-derived strains were utilized in the cytotoxicity, mutation rate, and chromosomal aberration (karyotype analysis) studies. All yeast strains were grown in YPD media (1% yeast extract, 2% peptone, 2% dextrose). All YPD media were supplemented with 0.5% adenine sulfate. For selection of strains containing the Yap1-GFP plasmid, the strains were grown on synthetic complete media lacking uracil (0.5% ammonium sulfate, 0.17% yeast nitrogen base without amino acids, 2% dextrose). Synthetic complete media lacking arginine and supplemented with 60 mg/mL L-canavanine was used for mutation rates measurement. Solid media contained 2% agar (Guthrie and Fink, 1991).

Strains referred to in the following text as BER$^-$ strains contain disruptions of three genes *NTG1*, *NTG2*, and *APN1* (due to the redundancy in functional activities of BER enzymes lack of one or two BER genes is very efficiently compensated by other BER proteins (Swanson et al., 1999). NER$^-$ strains contain a disruption of the *RAD1* gene. Strains that also have a disruption in *YAP1* gene are referred to as *yap1Δ*, BER$^-$ *yap1Δ*, and NER$^-$ *yap1Δ* strains.

2.2. Cell growth and viability

Liquid YPD media was inoculated with yeast cells and grown at 30 °C for ~24 h to saturation (>7×10^7 cells/mL). Fifty milliliter of liquid YPD was inoculated with an appropriate amount of cells, such that the culture would reach a density of 2×10^7 cells/mL after 12 h of growth at 30 °C. To determine cell viability, cultures were plated on YPD after exposure to MMS or UV-C and incubated for 48 h at 30 °C. Cultures were diluted to a density that would yield approximately 100–200 colonies per plate.

2.3. Yap1 cellular localization studies

For studies examining the sub-cellular localization of Yap1, strains DSC0025, DSC0035and DSC0036 (WT, BER$^-$, and NER$^-$ strains, respectively) were transformed with a centromeric plasmid pLR1 (Rowe et al., 2008) encoding Yap1-GFP fusion protein. Cells transformed with the plasmid were grown to mid-log phase (~2×10^7 cells/mL) as described above in YPD at 30 °C overnight, counted, and washed twice with H_2O. The density of the cells was adjusted to 2×10^7 cells/mL in H_2O. Cells were stained with 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen) to visualize DNA in nuclei and mitochondria. Cells were incubated with 1 μL DAPI (100 nM) per 1 mL of cells for 5 min, washed once with H_2O, and then re-suspended in the original volume in H_2O. Cells were then exposed to MMS (0.5 or 25 mM), H_2O_2 (0.5 or 25 mM), or UV-C (2 or 25 J/m^2). Cells were incubated in MMS or H_2O_2 throughout the time course. Cells were exposed to UV-C at the start of the time course and then placed in the dark for the duration of the experiment. Cells were subjected to fluorescence confocal microscopy (Zeiss LSM510 META) and images were analyzed using Carl Zeiss LSM Image Browser software.

2.4. Measurement of $O_2^{\bullet-}$ levels

$O_2^{\bullet-}$ levels were detected using the fluorescent probe dihydroethidium (DHEt) (Benov et al., 1998; Carter et al., 1994). Cells were grown to mid-log phase (~2×10^7 cells/mL) in YPD at 30 °C overnight. Cells were counted in haemacytometer, washed twice in H_2O and then adjusted to 2×10^7 cells/mL in H_2O. Cells were then exposed to various doses of either MMS or UV-C as described in the text.

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