DNA Repair 24 (2014) 98–106

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Fission yeast Drp1 is an essential protein required for recovery from DNA damage and chromosome segregation

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ARTICLE INFO

Article history:
Received 28 May 2014
Received in revised form 8 August 2014
Accepted 16 September 2014
Available online 28 September 2014

Keywords:
S. pombe
Rint1
Drp1
rad50
DNA repair
Chromosomes

ABSTRACT

DNA double strand breaks (DSBs) are the most critical types of DNA damage that can leads to chromosomal aberrations, genomic instability and cancer. Several genetic disorders such as Xeroderma pigmentosum are linked with defects in DNA repair. Human Rint1, a TIP1 domain containing protein is involved in membrane trafficking but its role in DNA damage response is elusive. In this study we characterized the role of Drp1 (damage responsive protein 1), a Rint1 family protein during DNA damage response in fission yeast. We identified that Drp1 is an essential protein and indispensable for survival and growth. Using *in vitro* random mutagenesis approach we isolated a temperature sensitive mutant allele of *drp1* gene (*drp1-654*) that exhibits sensitivity to DNA damaging agents, in particular to alkylation damage and UV associated DNA damage. The *drp1-654* mutant cells are also sensitive to double strand break inducing agent bleomycin. Genetic interaction studies identified that Rad50 and Drp1 act in the same pathway during DNA damage response and the physical interaction of Drp1 with Rad50 was unaffected in *drp1-654* mutant at permissive as well as non permissive temperature. Furthermore Drp1 was found to be required for the recovery from MMS induced DNA damage. We also demonstrated that the Drp1 protein localized to nucleus and was required to maintain the chromosome stability.

1. Introduction

The fission yeast *Schizosaccharomyces pombe* is a useful model system for studies of cell cycle events, like DNA replication, repair, mitosis and cytokinesis. All these events must be performed with high accuracy to ensure proper genomic integrity [1]. Genomic instability can arise due to the defects in DNA replication, repair or chromosome segregation [2]. If these defects are not repaired, damaged DNA may be replicated and segregated into daughter cells that can lead to chromosome instability [3] which is a major reason for tumour development [4]. DNA double strand breaks (DSBs) can also leads to chromosomal aberrations, disruption of genome integrity and cancer. Several genetic disorders such as Xeroderma pigmentosum are linked with defects in DNA repair [5]. The mechanisms of DNA repair have been investigated in the lower eukaryotes, especially in *Saccharomyces cerevisiae* and *S. pombe* and have been shown to be highly conserved among eukaryotes [5]. DSBs are generated not only by exogenous sources such as ionizing irradiation but also by endogenous factors such as free radicals generated during cellular metabolism [6]. DSBs can also arise due to the replication fork collapse. However, programmed DSBs are created at recombination sites during meiosis [7] they can also activate DNA damage checkpoints leading to cell cycle arrest and induction of appropriate repair machinery [8].

DSBs in eukaryotes are repaired by two major DNA repair pathways: homologous recombination (HR) and nonhomologous end joining (NHEJ) [9–11]. In *S. cerevisiae* recombination repair genes belongs to the Rad52 epistasis group [9] that include RAD50, MRE11, XRS2, RAD51, RAD52, RAD54, RAD55, and RAD57 [12]. Mutants of these genes are sensitive to ionizing radiation and other DNA damaging agents [13–15]. The *S. pombe rad50* gene was isolated on the basis of its sequence similarity to its homologues from *S. cerevisiae*, *Caenorhabditis elegans*, and humans [15], The Rad50 deletion mutant is sensitive to ionizing radiation and produce inviable spores during meiosis [16]. Rad50 belongs to the structural maintenance of chromosome (SMC) family of proteins. This protein family has its role in chromosome condensation and segregation, transcriptional repression and recombination [17]. The Rad50 protein contains an N-terminal Walker A and C-terminal Walker B NTP binding domains, linked by two extensive coiled-coil regions [18,19]. Rad50 acts as the binding sites for Mre11 at the N- and C-terminal ends of the coiled-coil region [20,21].

The Mre11 protein is the catalytic subunit of the Mre11/Rad50/Nbs1 complex that dimerizes through its phosphodiesterase

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http://dx.doi.org/10.1016/j.dnarep.2014.09.006
1568-7864/

domains [21]. It has 3′–5′ dsDNA exonuclease and ssDNA endonuclease activities [22]. The Nbs1/Xrs2 protein is the least conserved protein of the MRN(X) complex and interacts with different proteins during DNA repair [23,24]. The Mre11-Rad50-Xrs2 protein complex (MRX complex) from *S. cerevisiae* has been reported to be involved in the homologous recombination and non homologous end joining [25,26].

Fission yeast Drp1 is an essential protein [27] that belongs to RINT1/TIP-1 family. Human Rint1, a Drp1 homologous protein was first identified as a Rad50-interacting protein that participates in radiation-induced G2/M checkpoint control [19]. Moreover cells expressing N-terminally truncated Rint1 protein display defective radiation-induced G2/M checkpoint [19]. The homozygous deletions of *rint1* alleles in mice resulted in early embryonic death [28] with defects in the positioning of the Golgi body, centrosome amplification and chromosome mis-segregation [29]. Earlier study in human cell line suggested that Rint1 is also involved in telomere length control through Rad50 dependent recombination mechanism involving p130 which interacts specifically with the Rint1 protein [30].

In this study we report the functional characterization of fission yeast Drp1, a functional homolog of human Rint1 which is known to be involved in membrane trafficking between the ER and Golgi [31]. The fission yeast Drp1 (SPBC691.02c) is an essential protein and indispensible for its growth and survival. A temperature sensitive mutant allele of *drp1* causes genetic instability and sensitivity to DNA damaging agents. Furthermore, we showed that Rad50 and Drp1 act in the same pathway during DNA damage response. A physical interaction of Drp1 with Rad50 was observed and mutant Drp1 protein retains its ability to interact with Rad50 under non permissive conditions as well as during DNA damage response.

Table 1
Strains used in this study.

Strain	Genotype	Source
SP6	*h⁻ leu1-32*	Lab stock
NW158	*h⁺ leu1-32 ura4D18 chk1::ura4 ade6-216*	Nancy Walworth
SH271	*h⁻/h⁺ leu1-32/leu1-32 ura4D18/ura4D18 drp1::kan^R/drp1⁺ ade6-210/ade6-216*	This study
SH 318	*h⁺ leu1-32 ura4D18 rad50::ura4 ade6-216*	Jagmohan Singh
SH428	*h leu1-32 ura4D18 drp1::kan^R ade6-210* (pSP1 *Drp1-HA*)	This study
SH 457	*h leu1-32 ura4D18 rad50::ura4 drp1::kan^R ade6⁻* (pSP1 *Drp1-654-HA*)	This study
SH295	*h⁻ leu1-32 Drp1-HA::kan^R*	This study
SH393	*h⁻ leu1-32 Rad50-FLAG::Kan^R*	This study
SH398	*h leu1-32 Drp1-HA::kan^R Rad50-FLAG::Kan^R*	This study
SH441	*h⁻ leu1-32 ura4D18 drp1::kan^R ade6⁻* (pSP1 *Drp1-654-HA*)	This study
SH477	*h leu1-32 ura4D18 drp1::kan^R rad50-FLAG::Kan^R ade6-216* (pSP1 *Drp1-HA*)	This study
SH456	*h leu1-32 ura4D18 drp1::kan^R Rad50-FLAG::Kan^R ade6-216* (pSP1 *Drp1-654-HA*)	This study
SH73	*h⁻ ura4D18 Chr16[ade6-216] ade6-210*	This study
SH451	*h leu1-32 ura4D18 drp1::kan^R Chr16[ade6-216] ade6-210* (pUR19 *drp1-654*)	This study
NW1497	*h⁻ leu1-32 ura4D18 Rad22-YFP::Kan^R*	Nancy Walworth
SH603	*h⁺ leu1-32 ura4D18 drp1::kan^R ade6-216 Rad22-YFP::kan^R* (pUR19 *drp1-654*)	This study

2. Materials and methods

2.1. Strains and growth condition

S. pombe strains used in this study are listed in Table 1. Standard genetic methods were utilized for making strains as described earlier [32]. For temperature shift experiments, cells were grown to mid-log phase at 25 °C and then shifted to restrictive temperature 36° C in a water bath. To measure MMS and bleomycin sensitivity, cells were grown at 25 °C up to mid log phase, 10^7 cells were serially diluted and spotted on plates containing drugs. For UV sensitivity assay the plates were irradiated with the indicated doses of UV light and incubated at 25 °C for 3–4 days. For survival analysis cells were grown to mid-log phase, cells were further transferred to rich medium containing indicated dosage of MMS and were allowed to grow at permissive temperature for 5 h. Samples were collected, 1000 cells from each sample were plated on YEA plates and incubated at 25 °C until colonies appear. Colonies were counted and graph was plotted. To facilitate detection of Drp1 and Rad50, strains carrying Drp1-HA and Rad50-FLAG were constructed using PCR based tagging as described earlier [33,34]. All tagged strains were viable at temperatures ranging from 25 °C to 36 °C and behave exactly like the wild type cells.

2.2. Gene disruption

For *drp1* gene disruption two step gene replacement method as described by [34] was used. Plasmid containing kanamycin module was digested with *SmaI/SpeI* and was ligated at *SwaI/AvrII* giving rise plasmid pS1which completely replaces the Drp1 ORF with kanamycin resistant gene but contains upstream and downstream sequences of *drp1* gene. The pS1 plasmid was further digested with *SacII/XhoI* and a 2.2 kb fragment containing kanamycin resistant gene with *drp1* overhangs was transformed in a diploid strain. Transformants were selected by replica plating on plates containing 100 μg/ml of G418. Heterozygous diploid strain with *drp1* deletion was isolated. Deletion was confirmed by PCR using wild type *drp1* gene as a negative control.

2.3. Construction of drp1-654 mutant strain by plasmid shuffling

A library of *drp1⁻* mutants on a ura based plasmid (Drp1-pUR19) was constructed by *in vitro* mutagenesis using hydroxylamine. In short 10 μg of *drp1*-pUR19 plasmid was incubated with 500 μl hydroxylamine solution for 20 h at 37 °C in an eppendorf tube. Plasmid shuffling was performed essentially as described by [35]. DNA was purified and the mutagenized DNA was introduced in a *drp1::kan^R* haploid strain containing *drp1⁺* gene on a leucine based plasmid (pSP1-Drp1-HA). G418 resistant, leu⁺ and ura⁺ transformants, were selected. The wild type plasmid (pSP1-Drp1-HA) was shuffled out and G418 resistant ura⁺ colonies were selected. Selected strains were checked for temperature sensitivity by replica plating. The strains that were unable to form colonies at 36 °C were selected as containing *drp1* mutant gene. For identification of mutation, DNA was isolated from temperature sensitive strain, complete gene coding for *drp1* mutant was sequenced using appropriate primers and was compared with the wild-type sequence of *drp1* gene.

2.4. Mini chromosome loss assay

The minichromosome Ch16 [36] was introduced in the wild type and *drp1* deleted cells carrying mutant *drp1-654* gene on a plasmid by standard genetic techniques. To determine the rate of minichromosome loss, the cells were allowed to grow up to mid log phase in rich medium lacking adenine at 25 °C. An aliquot was removed