T. Hryciw · M. Tang · T. Fontanie · W. Xiao

**MMS1 protects against replication-dependent DNA damage in *Saccharomyces cerevisiae***

Received: 8 August 2001 / Accepted: 4 October 2001 / Published online: 29 November 2001
© Springer-Verlag 2001

**Abstract** A series of yeast mutants were isolated that are sensitive to killing by the monofunctional DNA-alkylating agent methyl methanesulfonate (MMS) but not by UV or X-radiation. We have cloned and characterized one of the corresponding genes, *MMS1*, and show that the *mms1Δ* mutant is dramatically sensitive to killing by MMS and mildly sensitive to UV radiation. *mms1Δ* mutants display an elevated level of spontaneous DNA damage and genomic instability. Furthermore, the *mms1Δ* cells are sensitive to killing by conditions that induce replication-dependent double-strand breaks, such as treatment with camptothecin, and incubation of a *cdc2-2* strain at the restrictive temperature. *rad52Δ* is epistatic to *mms1Δ* for MMS and camptothecin sensitivity, indicating that Mms1 acts in concert with Rad52. However, unlike mutants of the *RAD52* group, *mms1Δ* cells are not sensitive to γ-rays, which induce double-strand breaks independently of DNA replication. Together these results suggest a role for an Mms1-dependent, Rad52-mediated, pathway in protecting cells against replication-dependent DNA damage.

**Keywords** Yeast · DNA replication · DNA repair · Recombination · Camptothecin

---

**Introduction**

Living cells suffer continual insult from exogenous and endogenous DNA-damaging agents. In a nondividing cell, the lesions *per se* will be of little consequence unless they interfere with transcription; however, in an actively growing cell, unrepaired DNA damage can have grave repercussions. The process of DNA replication is susceptible to inhibition by many different DNA lesions. Thus, many DNA lesions, such as alkylated bases, abasic sites, thymine dimers, and protein-DNA cross-links, can block replicating DNA polymerases (Friedberg et al. 1995). If these lesions are not repaired, or if replication cannot restart, the result is cell lethality. In addition, many mutations in genes encoding components of the DNA replication machinery also cause genetic instability. For example, *Saccharomyces cerevisiae* strains bearing certain mutant alleles of *POL3* (Pol δ), *RAD27* (FEN1), *POL30* (PCNA), and *RFA1*, *RFA2*, and *RFA3* (RFA) all accumulate recombinogenic lesions, including single-strand and double-strand breaks (SSBs and DBSs), during S phase (Tishkoff et al. 1997; Zou and Rothstein 1997; Chen et al. 1998; Merrill and Holm 1998; Chen and Kolodner 1999). Blocked DNA replication forks are thought to be unstable (Michel 2000). In helicase mutant backgrounds in *Escherichia coli*, blockage of a replication fork leads to formation of a DSB, catalyzed by the RuvABC proteins (Michel et al. 1997; Seigneur et al. 1998). In wild-type cells, it is proposed that RuvAB, together with RecBCD, catalyzes replication fork repair without actually creating a DSB, possibly by annealing the newly synthesized strands (Seigneur et al. 1998). In the rDNA array of *S. cerevisiae*, Holliday junctions frequently form during S phase, and it is thought that this is indicative of the rescuing of blocked DNA replication forks (Zou and Rothstein 1997). The exact mechanism of this Holliday junction formation during S phase is unknown, although Rad52 is known to be required (Zou and Rothstein 1997). The error-free arm of the post-replication repair...
pathway in yeast, defined by Rad6, Rad18, Mms2, Ubc13, Rad5 and PCNA (Xiao et al. 2000), is thought to facilitate error-free bypass of a replication-blocking lesion, without actually removing the offending lesion. This could be accomplished either by repriming DNA replication downstream of the lesion, followed by a recombination-mediated gap filling process, or directly with the aid of recombination proteins, as has been described in *E. coli*. Hence, the S phase-dependent formation of Holliday junctions observed by Zou and Rothstein (1997) is possibly a form of post-replication repair.

Using DNA-damaging agents to study replication-blocking lesions can be complicated by the fact that many of these agents have pleiotropic effects. Methyl methanesulfonate (MMS), for example, produces predominantly 7-methylguanine and 3-methyladenine (3MeA), but also a small percentage of O6-methylguanine and O6-methylthymine (Beranek 1990). The latter two lesions cause base mispairing, whereas 3MeA blocks DNA replication. UV light induces primarily cyclobutane pyrimidine dimers and (6-4) photoproducts. Pyrimidine dimers can block DNA polymerases (Friedberg et al. 1995), but the use of UV to study this phenomenon is complicated by the fact that these lesions are efficiently targeted by the nucleotide excision repair pathway, such that only a fraction of UV-induced DNA damage persists into S phase. An agent that causes only one type of DNA lesion is camptothecin (CPT). CPT inhibits the religation step of topoisomerase I (Top1) activity, the end result being a single-stranded DNA (ssDNA) nick with a molecule of Top1 protein bound to its 3' end (Pommier et al. 1998). This protein-bound SSB inhibits DNA replication and can be converted into a DSB by the advancing replication fork (Ryan et al. 1991; Tsao et al. 1993). Thus, CPT is an excellent reagent for studying the effects of DNA replication blocks in vivo.

One would expect a yeast mutant that is defective exclusively in the repair of replication-dependent DNA damage to be sensitive to killing by MMS but not by γ- or X-irradiation. MMS treatment directly produces 3MeA lesions and indirectly causes the accumulation of abasic sites in the genome due to the activities of the base excision repair pathway. Both 3MeA and abasic lesions inhibit DNA replication (Sagher and Strauss 1983; Larson et al. 1985) and are thus presumed to cause replication-dependent DSBs. Furthermore, such a mutant might display sensitivity to the topoisomerase I poison CPT, which also causes replication-dependent DSBs. In a search for mutants that are sensitive to simple DNA alkylating agents but show normal responses to radiation-induced DNA damage, Prakash and Prakash (1977) identified mutants belonging to five complementation groups designated mms1, 2, 4, 5, and 22 which are sensitive to MMS but not to UV or X-rays. Further analysis of mms5 has revealed it to be allelic to *MAG1*, which encodes a 3MeA DNA glycosylase (Chen et al. 1990). *MMS2* has been shown to be a member of the *RAD6* post-replication repair group (Broomfield et al. 1998). *MMS4* might act as a transcriptional (co)activator to increase cellular repair capacity during S phase (Xiao et al. 1998) and *mms4* is synthetically lethal in combination with *sgs1* (Mullen et al. 2001). The two remaining mutants have yet to be further characterized. Here we present the molecular cloning and genetic characterization of *MMS1*. The *mms1Δ* mutant strain is sensitive to killing by various agents that commonly cause replication-dependent DNA strand breaks, such as MMS and CPT, and *rad52α* is epistatic to *mms1Δ* for MMS and CPT sensitivity. However, *mms1Δ* cells are not excessively sensitive to γ-rays, and show increased recombination frequencies in an inverted-repeat assay. This sets *MMS1* apart from the *RAD52* epistasis group. We suggest that *MMS1* acts with *RAD52* to protect yeast cells from replication-dependent DNA damage, possibly SSBs or DSBs.

**Materials and methods**

*S. cerevisiae* strains

The yeast strains used in this study are listed in Table 1. WX15-1c was isolated from MD-1/FY86 diploid segregants in order to combine *mms1-1* with *ura3* for library screening. The *E. coli* strains DH5α (BRL, Gaithersburg, Md.) and NM522 (Pharmacia, Piscataway, N.J.) were used for molecular cloning and plasmid preparation.

Yeast cell culture and transformation

Yeast cells were cultured at 30°C either in a rich YPD medium or in a synthetic dextrose (SD) medium supplemented with amino acids and bases as described (Sherman et al. 1983). A dimethyl sulfoxide-enhanced method (Hill et al. 1991) was used to transform intact yeast cells. For targeted integration, plasmid DNA was digested with restriction enzymes and precipitated with ethanol prior to transformation.

Screening of a yeast genomic library

A two-step screening protocol was followed. WX15-1c cells were first transformed with a YCP50-based yeast genomic DNA library (Rose et al. 1987) obtained from Mark Rose (Princeton University, Princeton, N.J.). Ura + transformants obtained on SD-Ura selective plates were then streaked onto YPD and YPD + 0.025% MMS. Each plate carried B635 cells as a positive control and the *mms1-1* mutant as a negative control. Transformants able to grow on MMS were restreaked from YPD onto MMS plates to confirm the MMS-resistant phenotype, before being tested for plasmid cosegregation.

In the plasmid cosegregation test, cells from a transformant growing on a YPD plate were used to inoculate 2 ml of YPD. A 10-μl volume of overnight culture was used to inoculate a fresh 2-ml aliquot of YPD and the incubation was continued overnight. Cells were then diluted and plated onto YPD to isolate single colonies. About 100 colonies derived from each transformant were replica plated onto SD + Ura and SD-Ura. Colonies that grew on SD + Ura but not on SD-Ura were considered to have lost the plasmid. Several Ura + and Ura- colonies from the same transformant were streaked onto a selective MMS plate. If the Ura+ phenotype was associated with the MMS-sensitive phenotype, MMS resistance was considered to be plasmid-borne.