Saccharomyces cerevisiae RAD27 complements its Escherichia coli homolog in damage repair but not mutation avoidance

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In eukaryotes, the flap endonuclease of Rad27/Fen-1 is thought to play a critical role in lagging-strand DNA replication by removing ribonucleotides present at the 5’ ends of Okazaki fragments, and in base excision repair by cleaving a 5’ flap structure that may result during base excision repair. Saccharomyces cerevisiae rad27Δ mutants further display a repeat tract instability phenotype and a high rate of forward mutations to canavanine resistance that result from duplications of DNA sequence, indicating a role in mutation avoidance. Two conserved motifs in Rad27/Fen-1 show homology to the 5’→3’ exonuclease domain of Escherichia coli DNA polymerase I. The strain defective in the 5’→3’ exonuclease domain in DNA polymerase I shows essentially the same phenotype as the yeast rad27Δ strain. In this study, we expressed the yeast RAD27 gene in an E. coli strain lacking the 5’→3’ exonuclease domain in DNA polymerase I in order to test whether eukaryotic RAD27/FEN-1 can complement the defect of its bacterial homolog. We found that the yeast Rad27 protein complements sensitivity to methyl methanesulfonate in an E. coli mutant. On the other hand, Rad27 protein did not reduce the high rate of spontaneous mutagenesis in the E. coli tonB gene which results from duplication of DNA. These results indicate that the yeast Rad27 and E. coli 5’→3’ exonuclease act on the same substrate. We argue that the lack of mutation avoidance of yeast RAD27 in E. coli results from a lack of interaction between the yeast Rad27 protein and the E. coli replication clamp (β-clamp).

Key words: complementation, Escherichia coli, flap endonuclease, PolI 5’→3’ exonuclease, RAD27, Saccharomyces cerevisiae

RAD27, a yeast homolog of mammalian FEN-1, belongs to a family of evolutionarily conserved 5’→3’ exonucleases and 5’-flap endonucleases that play important roles in DNA replication, repair and recombination (Friedberg 1991; Prakash et al. 1993; Reagan et al. 1995; Lieber 1997). The nuclease activity of Rad27/Fen-1 removes RNA primers made during lagging-strand DNA synthesis (Bambara et al. 1997). In Escherichia coli and other bacteria, the removal of RNA primers is performed by 5’→3’ exonuclease activity of DNA polymerase I (polI), encoded by the polA gene (Konrad and Lehman 1974; Kornberg and Baker 1992), which is a homolog of Rad27/Fen-1 nuclease (Robins et al. 1994).

The in vivo role of 5’-flap endonuclease in preserving the integrity of the genome is underscored by RAD27 deletion mutants. The rad27 null mutant strains arrest in G2/M at 37°C, grow slowly at 30°C, and exhibit increased plasmid loss (Reagan et al. 1995). The mutant deficient in E. coli polA 5’→3’ exonuclease also exhibits temperature-sensitive lethality (Konrad and Lehman 1974). These phenotypes suggest the importance of flap-endonuclease for DNA replication.

Yeast rad27 mutants and E. coli polA 5’→3’ exonuclease mutants also display DNA repair defects and a chromosome instability phenotype. The rad27 strains are highly sensitive to the DNA damaging agent methyl methanesulfonate (MMS), but are only moderately sensitive to ultraviolet irradiation and show insensitivity to gamma irradiation (Reagan et al. 1995; Xie et al. 2001).
The mutant strains of the polA 5′→3′ exonuclease also exhibit increased sensitivity to MMS (Glickman et al. 1973).

In assays that measure chromosome instability, rad27 deletion strains are associated with di- and trinucleotide repeat instability (Johnson et al. 1995), and a strong mutator phenotype (Tishkoff et al. 1997; Johnson et al. 1995; Kokoska et al. 1998). The mutator phenotype is characterized by a 50-fold increase in the CAN1 gene mutation rate, with a bias toward duplication mutations. The duplication mutation phenotypes, together with one-base addition (plus frameshift) mutations, can also be seen in E. coli polA 5′→3′ exonuclease mutants (Nagata et al. 2002). Recently, a haploinsufficiency of fen-1 in mice was shown to lead to rapid tumor progression, where tumors from mice showed microsatellite instability (Kucherlapati et al. 2002). Thus, Fen-1 protein in humans is assumed to reduce the induction of genetic diseases characterized by a trinucleotide repeat expansion, such as myotic dystrophy, Huntington’s disease, and fragile X syndrome (Schweitzer and Livingston 1998; White et al. 1999). Based on the duplication/addition mutator phenotype, Nagata et al. (2002) hypothesized that sequence expansion would occur in the nascent DNA during lagging-strand synthesis, forming a mismatch bulge, and PolI would bind and recognize this bulge in the nascent DNA during Okazaki fragment processing to remove the mismatch structure using the 5′→3′ exonuclease.

The wide range of defects observed in rad27/fen-1/ polA 5′→3′ exonuclease strains in DNA replication, recombination, repair and chromosome instability suggests that flap endonuclease has partly or completely different functions in each of these processes. Alternatively, each of these processes may be enzymatically performed by interactions with different proteins, such as DNA polymerases for DNA replication, recombinases for recombination, and repair enzymes for DNA repair. To explore this issue, we have characterized the ability of yeast RAD27 to complement a variety of defects in chromosome metabolisms associated with E. coli polA 5′→3′ exonuclease mutants.

At first, we examined the ability of yeast RAD27 to complement the sensitivity of the E. coli PolI 5′→3′ exonuclease-defective strain KK103 (polA107) to MMS. As shown in Fig 1, the sensitivity of KK103 to MMS was partially complemented in the presence of pWKS30 carrying yeast RAD27, where RAD27 is expressed from an authentic yeast promoter. Under this condition, we confirmed the expression of the yeast RAD27 gene in E. coli using a RAD27-lacZ fusion plasmid (data not shown).

We next assessed the ability of RAD27 to complement genomic instability in the E. coli polA107 strain using the tonB gene as a marker. As shown in Table 1 (fifth column), expression of RAD27 in the polA107 strain did not reduce the spontaneous mutation rate to the level shown by the polA strain. A duplication/addition mutator phenotype of polA107 was not modified by the presence of RAD27 (Table 1). An investigation of plus frameshifts involved the recovery of 36 plus frameshift sites among 72 ColB mutants isolated from the KK103/pRAD27 strain. Twelve were associated with a run of bases, whilst the remaining 24 were not. Twenty-seven plus frameshift sites in the KK103/pRAD27 strain occurred in a GC sequence, and 9 in an AT sequence. The results are essentially the same as in the KK103 strain (Nagata et al. 2002), in which run vs non-run was 6:14 and GC vs AT was 16:4 (data not shown, but available as Table S1 at the following web site: http://www.biology.tohoku.ac.jp/~kazuo/ohnishi/). Among 19 duplications in the KK103/pRAD27 strain, which ranged in size from 2 to 23 bp and with the majority being 2 bp in length, one duplication (3097–3104) occurred 7 times at sites flanking a repeated sequence. We observed the same duplication among ColB mutants from the KK103 collection. These sequence characteristics of duplication/addition in KK103/pRAD27 are essentially the same as the characteristics of duplication/addition in KK103 (data not shown, but available as Table S2 at the following web site: http://www.biology.tohoku.ac.jp/~kazuo/ohnishi/). The results

![Fig. 1. Sensitivity of E. coli polA107 cells carrying the yeast RAD27 gene to MMS. The E. coli K12 strain KK103 (polA107 sig219::Tn10) (Nagata et al. 2002) and its parental pol+ strain KK1 (Wang et al. 1996) were transformed with a yeast RAD27 plasmid named pGO3, a derivative of the vector pWKS30 (Wang and Kushner 1991). Exponential cells were washed in a phosphate buffer (Akasaka and Yamamoto 1991) and treated with MMS in a phosphate buffer at 37°C for 30 min. The figure shows the average survival of pol+ (○), polA107 (□), polA107/pRAD27 (●) and polA107/vector (○) after exposure to MMS.](image-url)
indicate that although yeast Rad27 protein is a homolog of 5’→3’ exonuclease, it cannot play a role in mutation avoidance in a heterogeneous cellular environment.

In this study, we found that expression of RAD27 from the authentic promoter was able to partially rescue sensitivity to MMS (Fig. 1). The high level of spontaneous mutation in polA107, with a bias toward duplication/addition mutations, was not reduced by the expression of yeast RAD27 (Table 1). The yeast RAD27 cannot ameliorate the strong bias toward duplication/addition mutations in polA107. Thus, it is evident that yeast RAD27 can complement a repair defect but cannot complement mutation avoidance in E. coli polA107.

Recent studies have shown that the expression of human Fen-1 protein in yeast rad27Δ cells can correct a number of mutant phenotypes, including sensitivity to MMS and a high level of spontaneous mutation (Hansen et al. 2000; Greene et al. 1999), and that expression of the 5’→3’ exonuclease domain of E. coli PolI in yeast rad27Δ cells could complement sensitivity to MMS and mutator phenotypes (Sun et al. 2002). In these cases, however, the extent of the complementation was partial. The inability of human Fen-1 protein or E. coli PolI protein to completely complement the sensitivity to MMS or mutation avoidance in yeast rad27A may be a result of differences in the ability of the Fen-1 protein or PolI protein and the Rad27 protein to undergo interaction and stimulation by the yeast PCNA protein, a platform for replication and repair (Hansen et al. 2000; Greene et al. 1999). Thus, human Fen-1, yeast Rad27 and E. coli PolI 5’→3’ exonuclease act on the same substrate, but there are differences in interaction between PCNA (or bacterial β-clamp) and Fen-1/Rad27/PolI.

The partial complementation of repair by RAD27 in the polA107 strain indicates that freely existing Rad27 in E. coli can repair part of the damage from MMS, which prompted us to assume that, in yeast, base excision repair is partly performed by free Rad27 protein, and the remaining damage is processed by Rad27 protein which has interacted with PCNA. The interaction of yeast Rad27-PCNA (Klungland and Lindahl 1997; Gary et al. 1999) or E. coli PolI-β-clamp (Lopez de Saro and O’Don-
n pall 1991) has been demonstrated. Furthermore, it is known that there exist PCNA-dependent and PCNA-independent flap endonuclease-mediated repair pathways (Liu et al. 2004). Thus in E. coli, freely existing Rad27 can process part, but not all, of the damage due to MMS, since Rad27 cannot interact with E. coli β-clamp.

Concerning the increase in spontaneous duplication/addition mutations in E. coli with a defective 5′→3′ exonuclease of DNA PolI, Nagata et al. (2002) proposed that, during lagging-strand synthesis, sequence expansion occurs in the nascent DNA during DNA replication, which will cause a mismatch bulge in the nascent DNA and lead to a duplication/addition mutation after the next round of replication. When PolI binds and recognizes the mismatch bulge in the nascent DNA, the 5′→3′ exonuclease affects nascent DNA mismatch removal by flap nuclease. During the processing of the mismatch bulge in the nascent DNA, the PolI-β-clamp complex can act as a 5′ flap nuclease. The above interpretation can explain the inability of yeast RAD27 to reduce the high rate of spontaneous mutagenesis in polA107 strains, since Rad27 cannot interact with E. coli β-clamp.

In this experiment, the expression of yeast RAD27 was controlled by the authentic yeast promoter. Even though we confirmed the expression of the yeast RAD27 gene in E. coli, the possibility that the amount of Rad27 in E. coli was not large enough to repair the damage from MMS and reduce duplication/addition mutations cannot be eliminated.

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