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RESEARCH ARTICLE

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The *Neurospora crassa mus-19* gene is identical to the *qde-3* gene, which encodes a RecQ homologue and is involved in recombination repair and postreplication repair

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Abstract An allele called *mus-19* was identified by screening temperature-sensitive and mutagen-sensitive mutants of *Neurospora crassa*. The *mus-19* gene was genetically mapped to a region near the end of the right arm of linkage group I, where a RecQ homologue called *qde-3* had been physically mapped in the *Neurospora* database. Complementation tests between the *mus-19* mutant and the *qde-3*^{RIP} mutant showed that *mus-19* and *qde-3* were the same gene. The *qde-3* genes of both mutants were cloned and sequenced; and the results showed that they have mutation(s) in their *qde-3* genes. The original *mus-19* and *qde-3*^{RIP} mutants are defective in quelling, as reported for other *qde-3* mutants. The mutants show high sensitivity to methyl methanesulfonate, ethyl methanesulfonate, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, *tert*-butyl hydroperoxide, 4-nitroquinoline-1-oxide, hydroxyurea and histidine. Epistasis analysis indicated that the *qde-3* gene belongs both to the *uvs-6* recombination repair pathway and the *uvs-2* postreplication repair pathway. The *qde-3* mutation has no effect on the integration of a plasmid carrying the *mtr* gene by homologous recombination. In homozygous crosses, the *qde-3* mutant is defective in ascospore production.

Keywords RecQ · DNA repair · Recombination · Replication

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Introduction

DNA repair is important for the maintenance of genome integrity. Cells could not survive if damaged DNA were not repaired properly, and all organisms have several DNA repair systems. In the fungus *Neurospora crassa*, many repair-deficient mutants have been isolated and characterized. Genetic and molecular studies permit the mutants to be assigned to several epistasis groups: the *uvs-6* (recombination repair), *uvs-2* (postreplication repair), *mus-38* (nucleotide excision repair I), *mus-18* (nucleotide excision repair II) and *uvs-3* (function unknown) epistasis groups (Schroeder et al. 1998; Inoue 1999). It has been shown that the *uvs-6*, *uvs-2* and *mus-38* groups correspond, respectively, to the *RAD52*, *RAD6* and *RAD3* groups in the yeast *Saccharomyces cerevisiae*. However, some mutagen-sensitive mutants still remain to be characterized. The *mus-19* mutant was isolated by screening temperature-sensitive and methyl methanesulfonate (MMS)-sensitive mutants; but the *mus-19* gene was not cloned, since its mutagen sensitivity is too moderate to allow conventional sib-selection. In the present paper, we report the mapping of *mus-19* near the RecQ homologue *qde-3* (quelling-defective). The *qde-3* gene participates in a posttranscriptional gene-silencing (PTGS) mechanism in *Neurospora* called quelling (Cogoni and Macino 1999). This gene has been physically mapped by the *Neurospora* genome project (see Galagan et al. 2003). To investigate whether *mus-19* is allelic with *qde-3*, complementation tests were performed, demonstrating that the two genes are the same. Also, a mutation in the *qde-3* gene of the *mus-19* mutant was confirmed. To avoid confusion of gene names, we unified the gene names as *qde-3* after this and the original *mus-19* is now described as *qde-3* (SA19). In this paper, the *qde-3* mutant was characterized with respect

to DNA repair. Genetic studies demonstrated that the *qde-3* gene is involved in both *uvs-2*-dependent postreplication repair and *uvs-6*-dependent recombination repair. Recombination repair functions in repairing DNA double-strand breaks (DSBs) throughout the cell cycle, whereas postreplication repair specifically fills gaps that are produced in newly synthesized strands across lesions during replication (Broomfield et al. 2001). Recombination repair and postreplication repair are generally distinguishable in epistasis analysis. The functions of the *qde-3* gene in repair at the replication fork are discussed.

Materials and methods

Strains and plasmids

The *N. crassa* strains used in this study are listed in Table 1. Strains C1-T10-37A and C1-T10-28a (Tamaru and Inoue 1989) were used as the wild-type strain. Standard *qde-3* strains, KTO-r-17A (*A qde-3*) and KTO-r-20a (*a qde-3*), were derived from a cross between AKA-1120A and C1-T10-28a. *Escherichia coli* strains DH1 and XL1-Blue were used for the amplification of plasmids. Plasmids pUC19 (MBI Fermentas) and pBluescript SK + (Stratagene) were used for general DNA manipulations. Plasmid pCB1003 (Carroll et al. 1994) carrying the *E. coli* hygromycin B resistance gene (hyg^r) was used as a vector in transformations of *N. crassa*. Plasmid pMTR::HYG (Schroeder et al. 1995), carrying the *N. crassa mtr* gene disrupted by the hyg^r gene, was used to measure the homologous integration frequency. Plasmid pCSN35, carrying the *N. crassa al-1* gene disrupted by the hyg^r gene, was kindly supplied by H. Tamaru (University of Oregon).

Media and general genetic methods

Growth media and genetic procedures for *N. crassa* were as described by Davis and de Serres (1970). Transformation of *Neurospora* was performed as described by Vollmer and Yanofsky (1986) and Tomita et al. (1993).

Table 1 Strains of *Neurospora crassa* studied in this work. The *qde-3* genotype was generated by repeat-induced point mutation (RIP)

Strain	Genotype	Source or reference
C1-T10-37A	*A*	Tamaru and Inoue (1989)
C1-T10-28a	*A*	Tamaru and Inoue (1989)
CZ272-5a	*a mus-38*	Ishii et al. (1998)
C1-T3-8a	*a al-2 pan-2 cot-1 uvs-2*	Laboratory stock
CY-10-9a	*a mei-3*	Laboratory stock
C2-T19-40A	*A al-2 pan-2 cot-1 mus-19*	Laboratory stock
C2-T109-12a	*a al-2 pan-2 mus-19*	Laboratory stock
C2-T109-R	*a mus-19*	Laboratory stock
C2-2-210a	*a al-2 ad-8 mus-19*	Laboratory stock
AKA-1120A	*A qde-3*	This study
KTO-r-17A	*A qde-3*	This study
KTO-r-20a	*a qde-3*	This study
C1-S2-1A	*A pan-2*	Laboratory stock
C3-T11-4A	*A al-2 ad-8*	Laboratory stock
QME3-1a	*a qde-3 mei-3*	This study
QMU38-2A	*A qde-3mus-38*	This study
QUS2-38A	*A qde-3uvs-2 pan-2 cot-1*	This study
QOGW-17A	*A qde-3 pan-2*	This study

Molecular analysis

Standard molecular techniques, such as mini-preparation of plasmid DNA, agarose-gel electrophoresis, restriction and ligation of DNA and Southern hybridization were carried out as described by Sambrook et al. (1989). *Neurospora* genomic DNA was isolated as described by Tomita et al. (1993).

Construction of the *qde-3* mutant

The *qde-3* gene was inactivated by exploiting repeat-induced point mutations (RIP) (Selker et al. 1987). The 3-kb *Xba*I fragment of the *qde-3* genomic DNA, which contains the entire region encoding the helicase domain, was cloned into pCB1003. Wild-type strain C1-T10-37A was transformed with this plasmid; and hygromycin-resistant transformants were isolated and crossed to strain C1-T10-28a. The resulting ascospores were randomly isolated and subcultured for 1 week. RIP-inactivated *qde-3* mutants were isolated on the basis of MMS sensitivity. The mutations of the *qde-3* gene in these strains were confirmed by Southern hybridization, using the 3-kb *Xba*I fragment as a probe.

Qualitative assay of sensitivity

Sensitivity to chemical mutagens and other chemicals was analyzed by spot tests, as described by Watanabe et al. (1997). Ethyl methanesulfonate (EMS), MMS, *tert*-butyl hydroperoxide (TBHP), *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), 4-nitroquinoline-1-oxide (4NQO), hydroxyurea (HU) and histidine were added to agar medium at final concentrations of 0.2%, 0.015%, 0.0056%, 0.5 μg/ml, 0.12 μg/ml, 1.9 mg/ml and 4 mg/ml, respectively. Conidial suspensions were spotted onto these plates and grown at 30 °C for 2 days. UV sensitivity was assayed by spotting a conidial suspension onto a plate and irradiating it at the dose of 300 J/m^2. Temperature sensitivity was tested by growing the spotted conidia at 37 °C for 2 days.

Assay of UV and MMS sensitivity

The survival of UV-irradiated or MMS-treated *N. crassa* was measured as described by Inoue and Ishii (1984). In the case of UV sensitivity, conidial suspensions (final concentration of 1×10^6 cells/ml) were irradiated at various doses of UV and aliquots were sampled and plated after appropriate dilution. In the case of MMS treatment, conidial suspensions (final concentration of 1×10^6 cells/ml) were incubated with various concentrations of MMS. After incubation at 30 °C for 1 h, aliquots were sampled and appropriate dilutions were plated. All the plates were allowed to grow at 30 °C for 3 days and the number of colonies on each plate was counted. Survival experiments were repeated at least three times.

Complementation experiment

Three heterokaryotic strains, HET01 (Q-OGW-17A + C3-T11-4A), HET02 (C2–2-210a + C1-S1-2a), and HET03 (KTO-r-17A + C2-T19-40A), were constructed by mixing conidial suspensions from two strains of the same mating type but carrying different auxotrophic markers (*ad-8* or *pan-2*) and spotting them onto Fries′ minimal agar medium containing 1.2% sucrose. Forced heterokaryons can grow on the minimal medium and produce conidia. Conidial suspensions of HET01, HET02 and HET03 were spotted onto minimal agar medium with and without MNNG at 1μg/ml.

Test of quelling

Quelling was examined as described by Cogoni et al. (1996). The wild-type orange strain and the mutant orange strain were

transformed with plasmid pCSN35. Hygromycin-resistant transformants were isolated and subcultured under fluorescent light for 1 week. Orange transformants were judged not to have undergone quelling, while white or yellow transformants were considered quelled.

Results

Chemical and temperature sensitivity

The sensitivity of the original *mus-19* mutant to a variety of chemical mutagens, other chemicals and UV was tested. Conidial suspensions were spotted on plates containing MMS, MNNG, EMS, TBHP, 4NQO, HU or histidine and the plates were incubated at 30 °C. In the UV sensitivity test, conidia were irradiated after spotting. The *mus-19* mutant showed high sensitivity to MMS and MNNG and moderate sensitivity to EMS, TBHP and histidine. Its sensitivity to UV, 4NQO and HU was similar to that of the wild type (Fig. 1). Although *mus-19* was isolated originally by screening in a MMS-sensitive, temperature-sensitive mutant, there was no difference in growth between the wild type and *mus-19* at 37 °C (data not shown).

Genetic mapping

The *mus-19* gene locus was mapped by crossing the *mus-19* mutant with strains carrying other markers. The *mus-19* gene maps to linkage group (LG)I between *aro-8* (13%) and *un-18* (4%).

Identification of the *mus-19* gene

The *mus-19* mutant showed clear, albeit modest, mutagen sensitivity. The lack of extreme sensitivity made it difficult to clone the *mus-19* gene by conventional sib-selection. By searching the *Neurospora* genome database, we found that the RecQ homologue *qde-3* is located near *mus-19* and the end of LGI (http://www-genome.wi.mit.edu/ annotation/fungi/neurospora/). Since mutants of RecQ homologues in other organisms (e.g. *S. cerevisiae*) are sensitive to mutagens, we hypothesized that the *mus-19* gene might be a RecQ homologue. To determine whether *mus-19* is allelic with *qde-3*, we conducted complementation tests. A *qde-3* mutant was generated by RIP, as described in the Materials and methods. Of 45 randomly isolated progeny, 11 isolates showed high sensitivity to MMS, although the transformant parent was not sensitive to MMS. Three of 11 isolates were hygromycin-sensitive. Southern blotting was used to confirm that the three strains have point mutations in the *qde-3* gene. Genomic DNAs from the wild type, the transformant and progeny were digested with *Hae*III and electrophoresed. In Southern hybridization using a *qde-3* DNA fragment as a probe, the transformant showed an extra band in addition to those observed in the wild-type strain (Fig. 2A), indicating that the transformant has a duplication of the *qde-3* gene. The banding patterns of progeny 20 and 29 were different from either of the parents (Fig. 2A), indicating that the nucleotide sequence of the *Hae*III restriction sites was changed by RIP. In Southern hybridization using *hyg*^{r} DNA as a probe, the band signal appeared only in the transformant (Fig. 2B). These results suggest that progeny 20 and 29 are mutationally altered in the *Hae*III restriction site of the *qde-3* gene and that introduced ectopic DNAs were lost as a result of chromosome segregation in the crosses.

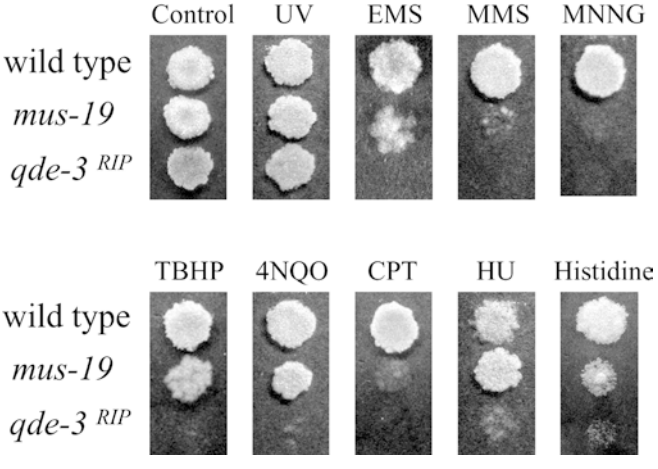

Fig. 1 Sensitivity of the original *mus-19* mutant and the *qde-3*^{RIP} mutant to chemical mutagens, other chemicals and UV. Conidial suspensions were spotted onto agar plates containing the indicated chemicals (see Materials and methods). In the case of UV, conidia were irradiated after spotting

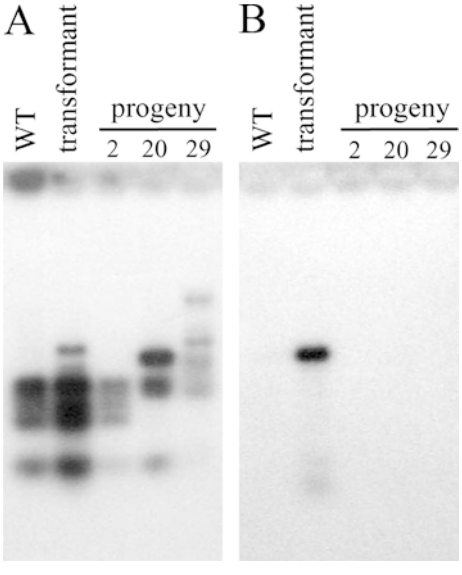

Fig. 2A, B Confirmation of RIP in the *qde-3* gene by Southern hybridization. Genomic DNAs of the wild type (*WT*), the *qde-3* duplication strain (transformant) and progeny were digested with *Hae*III and probed with the *Xba*I fragment of the *qde-3* DNA (**A**) or the hygromycin B resistance gene (**B**)

One of the mutants, AKA-1120A (progeny 20), was backcrossed with wild-type strain C1-T10-28a; and the resulting ascospores were randomly isolated. Of 40 progeny, 22 were hypersensitive to MMS, while 18 exhibited normal sensitivity, suggesting that AKA-1120A carries only the *qde-3* mutation. Homozygous crosses of the *qde-3* mutant did not produce ascospores, although development of perithecia was observed.

The sensitivity of the RIPed *qde-3* (*qde-3*^{RIP}) mutant to UV, various chemical mutagens and other chemicals was assessed in spot tests. It showed elevated sensitivity to MMS, MNNG, EMS, TBHP, 4NQO, HU and histidine, but not to UV (Fig. 1). Neither it nor the *mus-19* mutant showed a temperature-sensitive phenotype (data not shown). Inasmuch as the *qde-3*^{RIP} and *mus-19* mutants were both sensitive to MNNG, we examined whether they were able to complement one another in MNNG sensitivity. The forced heterokaryon (HET03), having nuclei with the *qde-3*^{RIP} and *mus-19* mutations, showed MNNG sensitivity (Fig. 3), indicating that the two mutations do not complement one another. The sensitivity of the *qde-3*^{RIP} mutant to mutagens was slightly greater than that of the original *mus-19* mutant (Fig. 1; data not shown), but the result indicates that *mus-19* is allelic to *qde-3*.

Identification of alterations in *qde-3* mutants

The *qde-3* genes from the *qde-3*^{RIP} and *mus-19* mutants were sequenced in order to determine the nature of each mutation. A single G-to-T substitution was found at nucleotide 3,639 in the *qde-3* open reading frame of the *mus-19* mutant. This results in a valine for glycine substitution at position 1,181 in the conserved helicase domain. While there are 139 GC-to-AT transition mutations across the duplicated region in the *qde-3*^{RIP} mutant, a G-to-A transition at nucleotide 3,237 of the *qde-3*^{RIP} generates a termination codon immediately after the DEAH box in the middle of the conserved helicase domain. Hereafter, *mus-19* is called *qde-3* and the original *mus-19* allele is now known as *qde-3* (SA19).

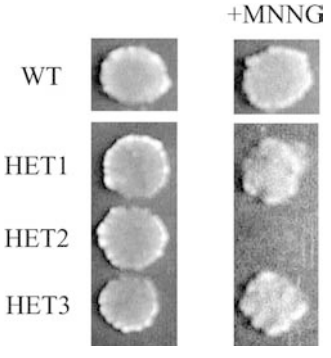

Fig. 3 Complementation test in forced heterokaryons. Three heterokaryotic strains, HET01 (Q-OGW-17A + C3-T11-4A), HET02 (C2–2-210a + C1-S1-2a) and HET03 (KTO-r-17A + C2-T19-40A), were made as described in the Materials and methods. Conidial suspensions of these strains were spotted onto a minimal agar plate or a minimal agar plate containing 1 μg *N*-methyl *N*′-nitro *N*-nitrosoguanidine (*MNNG*) /ml

Quelling test

The *albino* gene was used to test whether the *qde-3* (SA19) allele confers defective quelling. The wild type and the *qde-3* (SA19) mutant are orange. Both strains were transformed with pCSN35, which contains the *al-1* transcribed region interrupted by the *hyg*^{r} gene. Since quelling of the *al-1* gene occurs even when part of the transcribed region of *al-1* is introduced (Cogoni et al. 1996), plasmid pCSN35 could induce quelling. Hygromycin-resistant transformants were isolated and cultured under fluorescent light, and 25 of 85 transformants from the wild-type strain were yellow or white, while all 197 transformants from the *qde-3* (SA19) and from the *qde-3*^{RIP} strain were orange (Table 2).

Quantitative assay of mutagen sensitivity

The sensitivity of the *qde-3* mutant to UV and MMS was analyzed quantitatively. Conidia of the wild-type and *qde-3* strains were treated with several concentrations of MMS in 0.07 M phosphate buffer (pH 7.0) for 1 h at 30 °C or irradiated with various doses of UV. The *qde-3* mutant was twice as sensitive to MMS as the wild type, but was equally sensitive to UV (Fig. 4).

Epistasis analysis

In order to investigate the epistasis relationships between *qde-3* and other repair genes, double mutants carrying the *qde-3* mutation and other repair-deficient mutations were constructed. Three representative mutations, *mus-38*, *mei-3* and *uvs-2*, were used. These mutants are defective in nucleotide excision repair, recombination repair and postreplication repair, respectively. The *mus-38 qde-3* double mutant was more sensitive to MMS than the parental strains (Fig. 5A). In contrast, the double mutants *mei-3 qde-3* and *uvs-2 qde-3* showed the same sensitivity to MMS as the *mei-3* and *uvs-2* parental strains, respectively (Fig. 5B, C).

Table 2 Quelling frequency of the wild-type, *qde-3* (SA19) and RIPed *qde-3* strains. Fragments of the *al-1* gene were transformed into these strains. Non-orange transformants were scored; and the quelling frequency of the *al-1* gene was calculated

Strain	Quelling frequency
Wild type	25/85 (30%)
qde-3(SA19)	0/197 (0%)
qde-3^{RIP}	0/197 (0%)

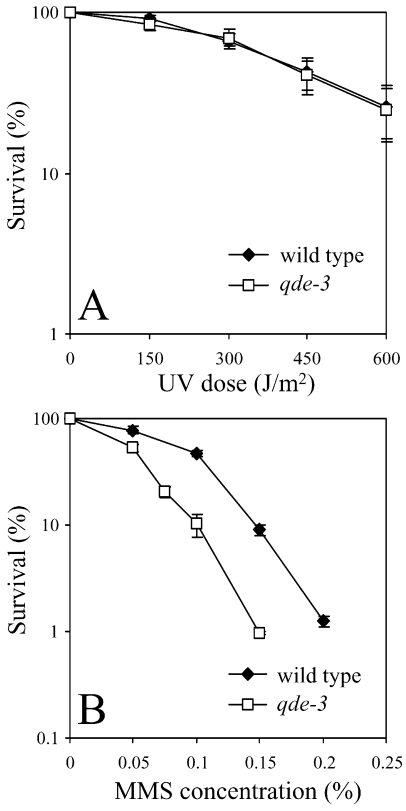

Fig. 4 Sensitivity of the wild-type (*black diamonds*) and the *qde-3* mutant (*white squares*) to UV (**A**) and methyl methanesulfonate (*MMS*) (**B**). The wild type and the *qde-3* mutant were irradiated or treated with MMS for 1 h at 30 °C. *Error bars* indicate the standard errors calculated from the data for three independent experiments

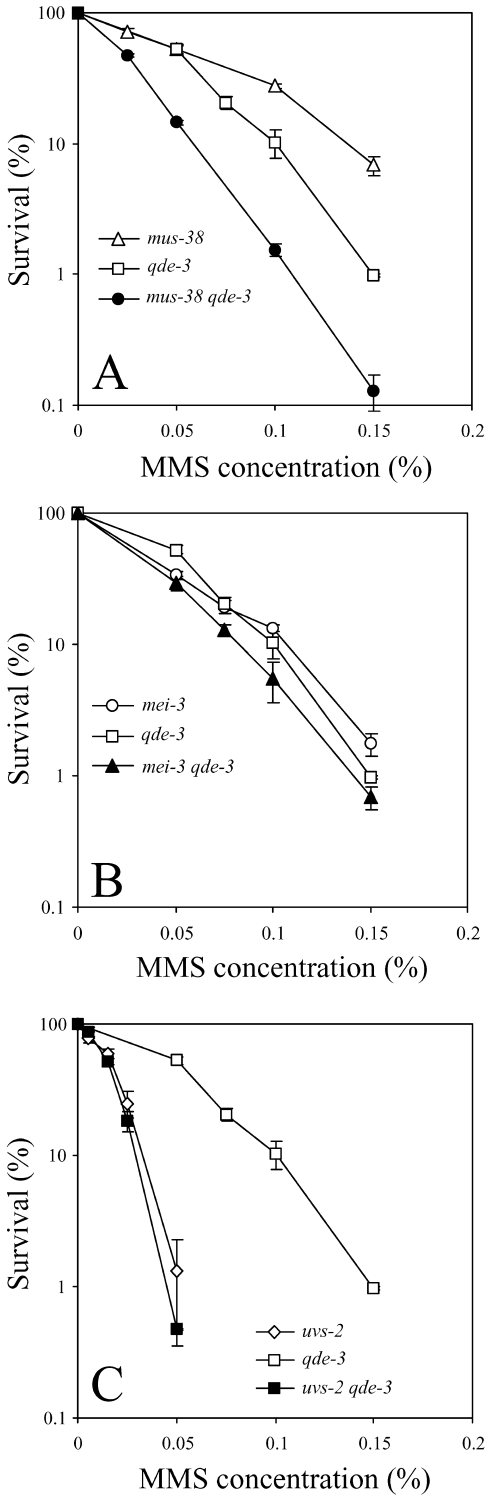

Fig. 5 Epistasis relationships between *qde-3* and *mus-38* (**A**), *qde-3* and *mei-3* (**B**) and *qde-3* and *uvs-2* (**C**). MMS treatments were investigated as described in Fig. 2. Error bars indicate the standard errors calculated from the data for three independent experiments

Targeted integration rate

Homozygous crosses of the *qde-3* mutant do not produce ascospores: perithecia develop but they are barren. Therefore, we could not measure the meiotic recombination frequency in this strain. To assess the homologous recombination frequency, the targeted integration frequency of the *mtr* gene was measured, using plasmid pMTR::HYG. The *qde-3* mutant was transformed with this plasmid after digestion with *Hin*dIII to linearize it. Hygromycin-resistant transformants were isolated and PFP resistance was measured among the hygromycin-resistant transformants. In the wild type, the targeted integration rate of *mtr* was 3.6% (Handa et al. 2000). In the *qde-3* mutant, 3.9% of the hygromycin-resistant transformants were PFP-resistant (Table 3). Therefore, there was no difference between the wild type and *qde-3*.

Discussion

This study demonstrated that the *mus-19* gene is identical to the *qde-3* gene, which encodes a RecQ homologue. *E. coli* RecQ, which has $3' \rightarrow 5'$ DNA helicase activity (Umezu et al. 1990), is a component of the RecF recombination pathway (Nakayama et al. 1984). It is the prototype of the so-called RecQ helicase family, which includes human WRN and BLM and *S. cerevisiae* Sgs1 (Gangloff et al. 1994; Ellis et al. 1995; Yu et al. 1996).

Table 3 Frequency of homologous integration of ectopic DNA in wild-type and DNA repair-deficient strains. See Materials and methods for transformants

Strain	Number of *Hyg*^{r} transformants	Number of *PFP*^{r} transformants	Targeted integration rate (%)
Wild type	338	12	3.6[a]
qde-3	223	9	3.9
mus-23	275	6	2.2[a]
mei-3	308	0	0.0[a]
mus-25	316	1	0.3[a]

[a] Handa et al. 2000

Cells that have mutations in these genes are sensitive to mutagens and show chromosomal instability. The sensitivity of the *qde-3* mutant to diverse mutagens suggests that the *qde-3* gene is required to repair various kinds of DNA damage.

The *qde-3*^{RIP} mutant was slightly more sensitive to several mutagens than the *qde-3* (SA19) mutant. This finding suggests that the loss of function in *qde-3* (SA19) is not complete, whereas *qde-3*^{RIP} is a null mutant. The *qde-3*^{RIP} mutant has many GC-to-AT mutations, including a nonsense mutation in the region of the conserved helicase domain, while *qde-3* (SA19) has only a single base substitution mutation at position 1,181 of the amino acid sequence. This single missense mutation in the *qde-3* (SA19) mutant may explain why it does not lose the *qde-3* function completely. However, as this missense mutation occurs at the position of the helicase domain that should be important for QDE3 function, it leads to multiple defects in DNA repair, ascospore production and quelling. It was reported that other *qde-3* alleles were defective in quelling but not defective in DNA repair (Cogoni and Macino 1999). In our study, *qde-3* (SA19) and *qde-3*^{RIP} both showed deficiencies in quelling and DNA repair. The difference in mutagen sensitivity between our *qde-3* mutant and the *qde-3* mutant of Cogoni and Macino may reflect allele-specific differences or effects of the genetic background.

Analysis based on sensitivity to MMS revealed that *mei-3* and *uvs-2* are epistatic to *qde-3* and that *qde-3* acts synergistically with *mus-38*. These results suggest that the *qde-3* gene belongs to both the recombinational repair and postreplication repair pathways, but not to the nucleotide excision repair pathway. In *N. crassa*, DSBs produced by ionizing radiation or radiomimetic drugs are repaired by genes belonging to the *uvs-6* epistasis group, including *mus-23*, *mus-25* and *mei-3*, which encode the homologues of *S. cerevisiae* Mre11, Rad54 and Rad51, respectively (Sakuraba et al. 2000). The epistasis relationship between *qde-3* and *mei-3* indicates that the *qde-3* gene might function with the *mei-3* gene as part of the *uvs-6* epistasis group to repair DSBs. This coincides with the result in *S. cerevisiae* that *rad52* is epistatic to *sgs1* (Onoda et al. 2001). The MMS sensitivity of *qde-3* is similar to that of *mei-3*. Wu et al. (2001) reported that human RAD51 interacts physically with the human RecQ homologue BLM. Since the amino acid sequence of the *Neurospora* QDE3 protein is most similar to that of the BLM of the five RecQ homologues in human, the QDE3 protein may interact physically with the MEI3 protein, which is the *Neurospora* homologue of yeast Rad51.

We anticipated that the recombination frequency might be altered in the *qde-3* mutant because of the involvement of *qde-3* in recombinational repair. Moreover, mutants of RecQ homologues in other organisms show aberrant recombination phenotypes (Nakayama et al. 1985; German 1993; Watt et al. 1995, 1996; Hanada et al. 1997; Yamagata et al. 1998; Onoda et al. 2000). However, the frequency of targeted integration in the *qde-3* mutant was the same as that in the wild type, suggesting that the *qde-3* mutation has no effect on homologous recombination between an introduced sequence and its host sequence. There was no conspicuous difference in total integration between the *qde-3* mutant and the wild type inasmuch as the transformation frequency was nearly the same in the two strains (data not shown).

The *qde-3* mutant developed perithecia but did not produce ascospores in homozygous crosses, suggesting that the *qde-3* gene is essential in the sexual phase. The same phenotype occurs in mutants belonging to the *uvs-6* epistasis group, including *uvs-6*, *mei-3*, *mus-23*, *mus-25* (Newmeyer and Galeazi 1978; Raju and Perkins 1978; Watanabe et al. 1997; Handa et al. 2000) and *mus-11*, which encodes the *S. cerevisiae* Rad52 homologue (Sakuraba et al. 2000). In contrast, the *uvs-2* mutant, which is epistatic to *qde-3*, is fertile in homozygous crosses (Stadler and Smith 1968). Therefore, the *qde-3* gene may work with the *uvs-6* group in meiosis.

The most notable result in this study is that *qde-3* is involved both in postreplication repair and in recombinational repair. This is the first report indicating that a RecQ homologue is involved in postreplication repair. Postreplication repair is required to fill gaps and restart DNA replication from a stalled replication fork. Since RecQ protein is required to reinitiate DNA replication after UV irradiation (Courcelle and Hanawalt 1999), one might expect the RecQ protein family to be involved in both postreplication repair and recombinational repair. Recombinational repair is a major pathway for the repair of DSBs and it is thought to be involved in the resolution of stalled replication forks (Broomfield et al. 2001). It was recently reported that BLM is required for the correct nuclear localization of RAD50-MRE11-NBS1 complexes after replication fork arrest (Franchitto and Pichierri 2002). It was also reported that BLM is localized to sites of stalled replication forks and physically interacts with Rad51 (Sengupta et al. 2003). Although we did not test whether the QDE3 protein has helicase activity, we think that involvement of the QDE3 protein in resolution of DNA structure at stalled replication forks is likely, given that the *qde-3* gene encodes a RecQ homologue. The QDE3 protein may resolve the aberrant DNA structure at a stalled replication fork,

permitting efficient processing by proteins of postreplication repair or recombinational repair. Alternatively, the QDE3 protein may resolve recombinogenic structures, such as Holliday junctions, at stalled replication forks, thus protecting the cells from abnormal recombination and inducing postreplication repair. Supporting evidence is found in the observation that UV and HU sensitivity and the cut phenotype of the *Schizosaccharomyces pombe rqh1* mutant, which is defective in a RecQ homologue, are partially suppressed by a bacterial Holliday junction resolvase (Doe et al. 2000). In vitro experiments also support this hypothesis, in that RecQ, Sgs1, BLM and WRN can unwind artificial Holliday junctions, 3′-tailed duplex DNA and some other DNA structures (Harmon and Kowalczykowski 1998; Bennett et al. 1999; Karow et al. 2000; Mohaghegh et al. 2001). We anticipate that further analysis of *qde-3* will provide insight into how RecQ-like proteins work in postreplication repair and the resolution of stalled replication forks.

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