and other karyotypic abnormalities. These manifestations, in turn, could account for the increased frequency of cancers in BS and WS patients. Ribosomal RNA chain elongation may be slowed in WS cells, which may render RNA polymerase I more prone to pausing that could trigger the formation of double strand breaks in rDNA. Repair of such breaks by nonhomologous end-joining could result in the accumulation of deletions within the genomic rDNA array and contribute to premature aging in WS patients.

References and Notes

1. J. German, *Dermatol. Clin.* **13**, 7 (1995); N. A. Ellis, *Nature* **381**, 110 (1996).
2. S. Gangloff, J. P. McDonald, C. Bendixen, L. Arthur, R. Rothstein, *Mol. Cell. Biol.* **14**, 8391 (1994); P. M. Watt, E. J. Louis, R. H. Borts, I. D. Hickson, *Cell* **81**, 253 (1995); P. M. Watt, I. D. Hickson, R. H. Borts, E. J. Louis, *Genetics* **144**, 935 (1996).
3. N. A. Ellis *et al.*, *Cell* **83**, 655 (1995); C.-E. Yu *et al.*, *Science* **272**, 258 (1996).
4. J. Lu *et al.*, *Nature* **383**, 678 (1996); R. J. Bennett, J. A. Sharp, J. C. Wang, *J. Biol. Chem.* **273**, 9644 (1998); M. D. Gray *et al.*, *Nature Genet.* **17**, 100 (1997); J. K. Karow, R. K. Chakraverty, I. D. Hickson, *J. Biol. Chem.* **272**, 30611 (1997).
5. S. Huang *et al.*, *Nature Genet.* **20**, 114 (1998); A. S. Kamath-Loeb, J.-C. Shen, L. A. Loeb, M. Fry, *J. Biol. Chem.* **273**, 34145 (1998).
6. D. A. Sinclair, K. Mills, L. Guarente, *Science* **277**, 1313 (1997).
7. R. A. Marciniak, D. B. Lombard, F. B. Johnson, L. Guarente, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6887 (1998).
8. D. A. Sinclair and L. Guarente, *Cell* **91**, 1033 (1997).
9. L. Rong and H. L. Klein, *J. Biol. Chem.* **268**, 1252 (1993); L. Rong, F. Palladino, A. Aguilera, H. L. Klein, *Genetics* **127**, 75 (1991).
10. To construct the *sgs1*Δ-generating plasmid, 1005–base pair (bp) and 919-bp polymerase chain reaction (PCR) products corresponding to the 5′ and 3′ regions of the *SGS1* gene, respectively, were amplified from total yeast genomic DNA and cloned into pUC19. The *URA3* geneblaster fragment [E. Alani, L. Cao, N. Kleckner, *Genetics* **116**, 541 (1987)] or the *LEU2* gene was then cloned between the 5′ and 3′ PCR products, generating plasmids pPM658 and pPM668, respectively. These plasmids, when digested with the restriction enzyme Sph I and transformed into yeast, delete nucleotides from +171 to +4106 of the 4341-bp *SGS1* open reading frame. To generate the *srs2*Δ-generating plasmid, a 5.8-kb PCR product encompassing the entire *SRS2* gene was amplified from yeast genomic DNA and cloned into pUC19. The internal 3.4-kb Pst I–Cla I fragment of *SRS2* was then replaced with the *URA3* geneblaster fragment, generating pPM690. This plasmid, when digested with the restriction enzymes Sph I and Sal I, releases a linear DNA fragment that, when transformed into yeast, deletes nucleotides from +90 to +3515 of the 3520-bp *SRS2* gene. The generation of deletions was confirmed by PCR analysis of genomic DNA. All mutant yeast strains used here were derived from EMY74.7 (*MAT***a** *his3-*Δ*1 leu2-3 leu2-112 trp1*Δ *ura3-52*).
11. To generate mutations in the *SGS1* gene, we cloned the wild-type *SGS1* gene into the low-copy (*TRP1*, *CEN/ARS*) vector YCplac22, which generated plasmid pPM914. Ten micrograms of pPM914 were incubated with 1 M hydroxylamine at 75°C for up to 3 hours before precipitation with ethanol in the presence of 50 μg of bovine serum albumin and 33 mM NaCl. Precipitated DNAs were repeatedly washed with 70% ethanol and then transformed into the *srs2*Δ *sgs1*Δ double mutant strain YRP349, which carries the wild-type *SGS1* gene on the low-copy (*URA3*, *CEN/ARS*) vector YCplac33–based plasmid pPM915. Strains carrying mutant alleles of *SGS1* in pPM914 were isolated by plasmid shuffle. Trp^+ Ura^+ transformants were replica-plated onto synthetic complete medium lacking Trp and containing 5-fluoro-orotic acid (FOA) to select for the loss of the wild-type *SGS1* gene on the *URA3* plasmid. The FOA-resistant Trp^+ Ura^- transformants were then analyzed for temperature-sensitive growth. One of the isolates lacked the ability to grow at 39°C and was analyzed further. The entire *sgs1-ts* mutant gene from the plasmid conferring the ts phenotype was cloned into another plasmid that had not been treated with hydroxylamine and was shown to confer the ts phenotype in the *srs2*Δ *sgs1*Δ strain. The *sgs1-ts* mutant gene in plasmid pPM980 was then sequenced with the Thermo Sequenace kit (Amersham Pharmacia Biotech).
12. H. Qiu, E. Park, L. Prakash, S. Prakash, *Genes Dev.* **7**, 2161 (1993); S. N. Guzder, P. Sung, V. Bailly, L. Prakash, S. Prakash, *Nature* **367**, 91 (1994).
13. The incorporation of radioactivity into DNA and RNA in continuous labeling experiments, pulse-labeling of total RNA, electrophoresis and fluorographic detection of rRNA, and Northern blot analyses to examine the transcription of constitutive and inducible mRNAs and tRNAs were done as described in (*12*), except that total cellular RNA was isolated with the hot phenol method as described in *Current Protocols in Molecular Biology*, Wiley, New York, 1993), vol. 2, unit 13.12.
14. S.-K. Lee, R. E. Johnson, S.-L. Yu, L. Prakash, S. Prakash, unpublished data.
15. H. J. Himmelfarb, E. M. Simpson, J. D. Friesen, *Mol. Cell. Biol.* **7**, 2155 (1987).
16. C. Mann *et al.*, *Mol. Cell. Biol.* **12**, 4314 (1992).
17. The amounts of *TRP3*, *RAD23*, and *CDC9* mRNAs also showed no decline in the *srs2*Δ *sgs1-ts* strain at 39°C.
18. G. Knapp, J. S. Beckmann, P. F. Johnson, S. A. Fuhrman, J. Abelson, *Cell* **14**, 221 (1978); B. P. Cormack and K. Struhl, *Cell* **69**, 685 (1992).
19. For cell cycle synchronization, cells were grown in medium containing yeast extract, peptone, and dextrose (YPD). When the density reached an optical density at 600 nm (OD_{600}) of ~0.5, the cultures were harvested and resuspended in fresh YPD medium (pH 4.0). Cells were treated with α factor to a final concentration of 10 μg/ml and held for 1.5 doubling times. After confirmation of cell cycle arrest in G_1 under the microscope, α factor was washed away and the cells were resuspended in fresh YPD medium. The cultures were then split (time 0). Half of the culture was incubated at 25°C and the other half at 39°C. DNA content was determined as described [R. Nash, G. Tokiwa, S. Anand, K. Erickson, A. B. Futcher, *EMBO J.* **7**, 4335 (1988)]. Samples were taken at the indicated time points and fixed overnight at 4°C in 1 ml of 70% ethanol. The fixed cells were washed twice, resuspended in 1 ml of 50 mM sodium citrate (pH 7.0), and then treated with ribonuclease A (0.25 mg/ml) for 3 hours at 50°C. Cells were then washed and resuspended in 1 ml of 50 mM sodium citrate (pH 7.0), propidium iodide (16 μg/ml) was added and cells were incubated overnight at 4°C. The DNA content of cells was analyzed by a Becton-Dickinson FACScan flow cytometer.
20. α-factor–arrested cells were released from G_1 arrest into YPD medium containing [^{3}H]uracil. The cultures were then split and placed at 25° or at 39°C, and DNA synthesis was measured by the incorporation of radioactivity into DNA. Upon transfer to 39°C, DNA synthesis ceased rapidly in the *srs2*Δ *sgs1-ts* strain but was not affected in the *srs2*Δ *SGS1* strain.
21. S. J. Brill, S. DiNardo, K. Voelkel-Meiman, R. Sternglanz, *Nature* **326**, 414 (1987).
22. H. Yan, C.-Y. Chen, R. Kobayashi, J. Newport, *Nature Genet.* **19**, 375 (1998).

23. Supported by NIH grants GM19261 and CA80882. We thank S. J. Brill for the *SGS1* gene.

3 August 1999; accepted 21 October 1999

Posttranscriptional Gene Silencing in *Neurospora* by a RecQ DNA Helicase

Carlo Cogoni* and Giuseppe Macino

The phenomenon of posttranscriptional gene silencing (PTGS), which occurs when a transgene is introduced into a cell, is poorly understood. Here, the *qde-3* gene, which is required for the activation and maintenance of gene silencing in the fungus *Neurospora crassa*, was isolated. Sequence analysis revealed that the *qde-3* gene belongs to the RecQ DNA helicase family. The QDE3 protein may function in the DNA-DNA interaction between introduced transgenes or with an endogenous gene required for gene-silencing activation. In animals, genes that are homologous to RecQ protein, such as the human genes for Bloom's syndrome and Werner's syndrome, may also function in PTGS.

Posttranscriptional gene silencing as a consequence of transgene introduction is a broadly diffused phenomenon in plants and fungi (*1*, *2*). Introduction of double-stranded RNA (dsRNA) induces a similar phenomenon in animals (*3*). The wide occurrence of gene silencing among different organisms indicates that these phenomena may have evolved from an ancestral mechanism involved in genome protection from invading DNA (*4*) and viruses (*5*). Several models have been proposed to explain PTGS on the basis of the notion that the introduced transgenes result in the production of aberrant RNAs (aRNAs) (*2*) that are recognized as a template by host RNA-dependent RNA polymerase (RdRP). The RdRP enzyme may synthesize antisense RNA that can bind to mRNA and form dsRNAs that are targets for sequence-specific RNA degradation (*6*). These models have received experimental support. The *qde-1* gene, which encodes a cellular component of PTGS in the fungus *Neurospora crassa*, is homologous to RdRP (*7*). Moreover, the accumulation of small antisense RNA molecules correlates with the occurrence of gene

Dipartimento di Biotecnologie Cellulari ed Ematologia, Sezione di Genetica Molecolare, Università di Roma La Sapienza, Viale Regina Elena, 324, 00161 Roma, Italy.

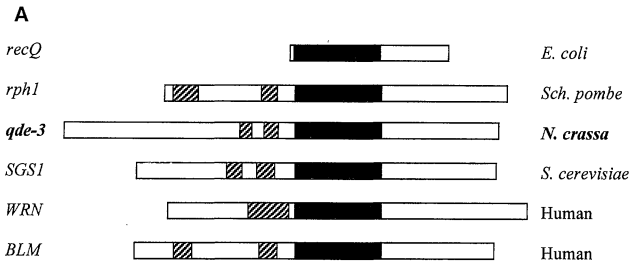
*To whom correspondence should be addressed. E-mail: carlo@bce.med.uniroma1.it

silencing in plants (*8*). It is unclear why PTGS is activated in some transgenic lines, whereas it is not activated in other lines. Gene silencing could be triggered by DNA pairing between homologous transgenes or with homologous resident genes (*9*). Such pairing, which could interfere with normal transcription, producing aRNA molecules, may occur only in some transgenic lines.

In gene silencing, also called "quelling" in *N. crassa* (*10*), three classes of quelling-defective mutants (*qde-1*, *qde-2*, and *qde-3*) have been isolated (*11*). To clone the *qde-3* gene, we used random insertional mutagenesis of an *al-1* (*albino-1*) transgenic strain showing an albino (white) phenotype as a consequence of post-transcriptional silencing of the endogenous *al-1* gene, which is involved in the biosynthesis of carotenoids (*12*). Mutation of *qde* genes releases *al-1* gene silencing, resulting in the recovery of a wild-type (orange) phenotype that can be easily selected by visual inspection. A strain (627) showing the recovery of *al-1* gene expression was isolated. By using a heterokaryon complementation analysis, we found that strain 627 belongs to one of the three previously identified *qde* complementation groups, *qde-3*. To isolate the *qde-3* gene, we obtained (by plasmid rescue) genomic DNA from strain 627 flanking the insertion site (*13*). Two genomic cosmids were isolated by using the flanking sequences as a probe and were found to complement the *qde-3* mutants, resulting in restoration of *al-1* gene silencing that was visible as the appearance of a white phenotype. Furthermore, a 9-kb Sph I fragment derived from the cosmids complemented *qde-3* mutants. This DNA fragment was sequenced, revealing a long open reading frame of ~6 kb that contains two putative introns identified by splicing consensus sequences and mapped by reverse transcriptase–polymerase chain reaction (RT-PCR) (*14*). To demonstrate that the putative 6-kb open reading frame is coincident with the *qde-3* gene, we mapped the insertion site of the tagging plasmid in the *qde-3* mutant strain 627. The tagging plasmid was inserted immediately downstream from the second intron of the *qde-3* gene, within the 3′-terminal acceptor site.

The putative QDE3 protein deduced from the *qde-3* nucleotide sequence contains 1955 amino acids. The encoded QDE3 polypeptide has a calculated molecular weight of 216,612 daltons. Using the predicted QDE3 peptide in a BLASTP search of amino acid sequence databases (*15*), we identified homologies with several peptides belonging to the family of RecQ DNA helicases. Homology is restricted to a 350–amino acid domain located in the center region of the polypeptide (residues 875 through 1228). This domain is coincident with the seven helicase domains that are strongly conserved among the RecQ helicases in organisms ranging from *Escherichia coli* to humans (Fig. 1). The *qde-3* helicase domain shows the highest similarity with the *Sgs1* protein of *Saccharomyces cerevisiae* (54% identity) and the *Rqh1* protein of *Schizosaccharomyces pombe* (55% identity). Among RecQ proteins, however, QDE3 appears to belong to a subfamily of proteins that are considerably larger than the *E. coli* prototype (Fig. 1A). Related proteins belonging to this subfamily include three human genes [*BML* (Bloom's syndrome gene) (*16*), *WRN* (Werner's syndrome gene) (*17*), and *RecQ4* (*18*)] and yeast genes *Sgs1* (*19*) from *S. cerevisiae* and *Rqh1* (*20*) from *Sch. pombe*. Other regions of the QDE3 protein, like the proteins of the human and yeast subfamilies, are rich in charged and polar amino acids, and the NH_2-terminal region contains acidic domains (Fig. 1A).

The yeast Sgs1p and the murine WRN interact with DNA topoisomerases (*19*, *21*). To test for an interaction between QDE3 and topoisomerases in *Neurospora*, we assayed the sensitivity of several *qde-3* mutants to the type II topoisomerase inhibitor, etoposide, and to the type I topoisomerase inhibitor, camptothecin (*22*). Etoposide, used at high concentration (10-fold higher than that generally used), did not show an inhibitory effect on either mutant or wild-type strains, indicating that *Neurospora* cells have low sensitivity to this drug. In camptothecin sensitivity assays, three *qde-3* mutant strains [627 and two ultraviolet (UV) irradia-



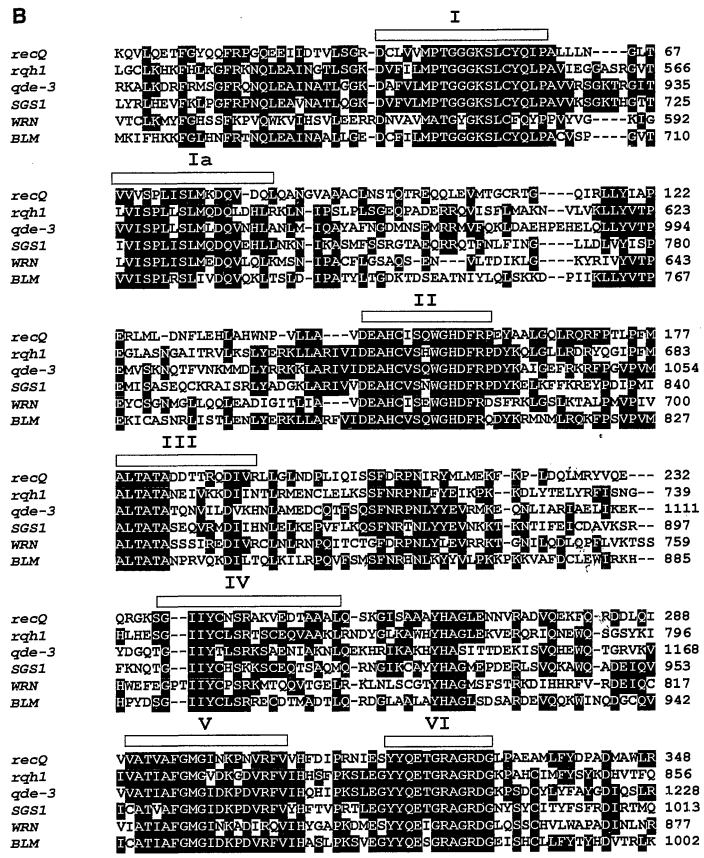


Fig. 1. *qde-3* belongs to the RecQ DNA helicase family. (**A**) Schematic representation of the members of the RecQ DNA helicase family. The names of the gene products and the organisms are shown. The solid areas indicate the conserved helicase domains. Acidic domains are shown as shaded boxes. (**B**) Amino acid sequence alignment of the helicase domains of the members of the RecQ DNA helicase family (*27*). The gene product names and the positions of amino acid residues are shown. Identical residues are shown in black; dashes indicate spaces introduced to maintain sequence alignment. Boxes above the sequences indicate the positions of seven helicase domains (I, Ia, II, III, IV, V, and VI).

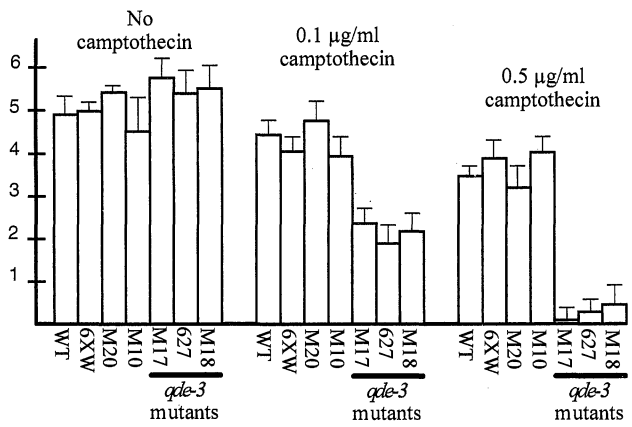


Fig. 2. Sensitivity of *qde-3* mutants to type I topoisomerase inhibitor, camptothecin. Three *qde-3* mutants (strain 627, M17, and M18), a wild-type strain (WT), an *al-1* silenced strain (6XW), a *qde-1* mutant strain (M20), and a *qde-2* mutant strain (M10) were assayed for sensitivity to camptothecin. Strains were grown in liquid cultures in the presence of different concentrations of camptothecin as indicated. For each mutant strain tested, the mass (in grams) of dried mycelia after 48 hours of growth is shown. Error bars indicate SD.

tion–induced mutants, M17 and M18] showed a dramatic increase of sensitivity to the inhibitor (Fig. 2). By contrast, the control strain (6XW), which has the same genetic background as the *qde-3* mutants, and the *qde-1* (M20) and *qde-2* (M10) mutant strains did not show increased sensitivity to camptothecin. Thus, the reason for increased sensitivity of *qde-3* strains to the type I topoisomerase inhibitor camptothecin is probably a consequence of mutations within the *qde-3* gene.

The fact that the *qde-3* gene encodes a putative DNA helicase suggests a role for this gene in the activation step of gene silencing. A model for *qde-3* function shows that the QDE3 DNA helicase could unwind double-stranded DNA, which may be required for DNA-DNA interactions between transgenic repeats. In addition, the DNA-pairing model proposes that DNA interaction between transgenes may induce changes in methylation or chromatin structure (or both), producing an "altered state" that could result in aRNA production (*9*). It has been proposed that DNA helicase or topoisomerase complexes may be involved in chromatin remodeling (*23*). The fact that QDE3 probably interacts with topoisomerases in vivo may suggest that QDE3 may have also a role in chromatin changes required for aberrant transcription. Alternatively, it has been proposed (*3*) that aRNAs could be dsRNAs produced from transgenic inverted repeats (IRs). The ability of RecQ helicases to process cruciform DNA structures (*24*) may indicate that QDE3 could be involved in resolving transgenic IR cruciforms to allow transcription of dsRNAs.

Eukaryotic RecQ DNA helicases have been generally implicated in DNA repair and in regulating recombination (*16*, *17*, *20*). Our findings suggest that a specific RecQ helicase could be involved in a function other than DNA recombination and repair. In fact, in *Neurospora*, QDE3 seems to be specialized in gene silencing, because we found that the mutation in the *qde-3* gene is sufficient to impair quelling, although at least another *recQ* homologous gene is present in *Neurospora* (*25*). Moreover, *qde-3* mutant strains and a wild-type strain showed the same ability to repair DNA damage induced by several mutagens (*26*).

The fact that RecQ-like protein is involved in gene silencing in *Neurospora* has begun to help us to understand the PTGS phenomenon. It also presents the obvious opportunity to test whether homologous *recQ* genes may be implicated in gene silencing in other organisms, especially in plants. This new function of a RecQ protein may also contribute to a deeper understanding of the biology of the *recQ* gene family and its function in higher eukaryotes, including humans.

References and Notes

1. A. Depicker and M. Van Montagu, *Curr. Opin. Cell Biol.* **9**, 373 (1997).
2. C. Cogoni and G. Macino, *Trends Plant Sci.* **2**, 438 (1997).
3. M. K. Montgomery and A. Fire, *Trends Genet.* **14**, 255 (1998).
4. M. A. Matzke and A. J. Matzke, *Cell. Mol. Life Sci.* **54**, 94 (1998).
5. K. D. Kasschau and J. C. Carrington, *Cell* **95**, 461 (1998).
6. J. A. Lindbo, L. Silva-Rosales, W. M. Proebsting, W. G. Dougherty, *Plant Cell* **5**, 1749 (1993); M. Wassenegger and T. Pélissier, *Plant Mol. Biol.* **37**, 349 (1998).
7. C. Cogoni and G. Macino, *Nature* **399**, 166 (1999).
8. A. J. Hamilton and D. C. Baulcombe, *Science* **286**, 950 (1999).
9. O. Voinnet, P. Vain, S. Angell, D. C. Baulcombe, *Cell* **95**, 177 (1998); M. Stam, J. N. M. Mol, J. M. Kooter, *Ann. Bot.* **79**, 3 (1997).
10. C. Cogoni *et al.*, *EMBO J.* **15**, 3153 (1996).
11. C. Cogoni and G. Macino, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 10233 (1997).
12. The *N. crassa al-1* silenced strain 6XW, used in insertional mutagenesis, and *qde* mutant strains were described previously (*11*). The general growth medium was composed of Vogel's minimal medium with 2% sucrose in agar plates; heterokaryon analysis and maintenance of stock cultures were performed as described (*11*). Plasmid pMXY2 containing the *Neurospora* selectable marker benomyl was used for insertional mutagenesis. Insertional transformants were selected on minimal medium containing benomyl (1 mg/ml). Release or restoration of *al-1* gene silencing after transformation or heterokaryon formation was evaluated by visual inspection of conidia color: wild-type conidia were orange, whereas white to yellow conidia were indicative of *al-1* gene silencing.
13. Cloning of the DNA region flanking the integrated plasmid was achieved with a plasmid rescue approach. Chromosomal DNA (100 ng) extracted from strain 627 was digested with Bgl II restriction enzyme, and the derived restriction fragments were ligated in a reaction volume of 0.2 ml in order to favor intramolecular ligation events. The DNA was ethanol precipitated and used to transform *E. coli*. Chromosomal DNA flanking the integration site was isolated with the enzymes Bgl II or Sal I, and the resulting restriction fragment was used as a probe on a *N. crassa* genomic cosmid library. Two positively hybridizing cosmids (6E8 and 54D7), containing 30-kb inserts, were introduced by transformation into insertional mutant *qde-3* strain 627 and into an UV irradiation–induced *qde-3* mutant strain. The subcloning of restriction fragments from genomic library clones was performed in pBluescript SK plasmid (Stratagene).
14. To map the intron positions, we used total RNA as the template in RT reactions containing, as primers, an oligonucleotide 5′-ATGGTTGACTTGATCCAG-3′ to map the first intron or an oligonucleotide 5′-TGGAACTTCGTTTCTTGG-3′ for the second intron. The two cDNA reactions were subsequently amplified by PCR with the oligonucleotide pair 5′-GACTACAGCCGGCAACTG-3′ and 5′-GATGTGAGGAAGGCTCTC-3′ or the oligonucleotide pair CGAGCACGGTGGCGTGTG-3′ and 5′-CAGGGTGGAAAGTTCTTG-3′, respectively. The resulting PCR fragments were inserted in TA cloning vector (Invitrogen) and sequenced.
15. The nucleotide sequence of the *qde-3* gene was determined with Taq FS DNA polymerase and fluorescent dideoxy terminators in a cycle-sequencing method, and the resulting DNA fragments were electrophoresed and analyzed with an automated Applied Biosystems 373A DNA sequencer. The nucleotide and derived amino acid sequences were analyzed with MacMolly Tetra programs. Protein comparisons were carried out by BLASTP, and the ClustalW program [J. D. Thompson, D. G. Higgins, T. J. Gibson, *Nucleic Acids Res.* **22**, 4673 (1994)] was used for alignments. The *qde-3* gene sequence has been deposited with GenBank (accession number AF205407).
16. N. A. Ellis *et al.*, *Cell* **83**, 655 (1995).
17. C.-E. Yu *et al.*, *Science* **272**, 258 (1996).
18. S. Kitao *et al.*, *Genomics* **54**, 443 (1998).
19. S. Gangloff, J. P. McDonald, C. Bendixen, L. Arthur, R. Rothstein, *Mol. Cell. Biol.* **14**, 8391 (1994).
20. E. Stewart, C. R. Chapman, F. Al-Khodairy, M. A. Carr, T. Enoch, *EMBO J.* **16**, 2682 (1997).
21. M. Lebel and P. Leder, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 13097 (1998).
22. Topoisomerase inhibitors were added in different concentrations to 100-ml minimal medium liquid cultures. Mycelia were harvested after 48 hours of growth at 30°C in shaking incubators. Sensitivity to the inhibitors was evaluated as the effect of growth inhibition on dried mycelia weight.
23. M. Duguet, *J. Cell Sci.* **110**, 1345 (1997).
24. R. K. Chakraverty and I. D. Hickson, *Bioessays* **21**, 286 (1999).
25. A. Pickford, C. Cogoni, G. Macino, unpublished data.
26. The sensitivity of *qde-3* mutants to increasing doses of methyl methansulfonate (MMS), hydroxyurea (HU), mitomycin C (MC), and UV irradiation was tested. MMS, HU, and MC were added directly to conidia suspensions as described [C. Ishii, K. Natamuran, H. Inoue, *Mol. Gen. Genet.* **288**, 33 (1991)], and UV irradiation was carried out as described [H. Inoue, T. M. Hong, F. J. deSerres, *Mutat. Res.* **80**, 27 (1981)].
27. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

28. A special thanks to G. Azzalin for skillful technical assistance. We also thank G. Coruzzi and A. Pickford for revising the manuscript. Supported in part by grants from the Istituto Pasteur Fondazione Cenci Bolognetti, from the Ministero dell' Università e della Ricerca Scientifica e Tecnologica, and from the European Union BIOTECH program.

29 July 1999; accepted 10 November 1999

References and Notes

[8] **A Species of Small Antisense RNA in Posttranscriptional Gene Silencing in Plants**
Andrew J. Hamilton; David C. Baulcombe
Science, New Series, Vol. 286, No. 5441. (Oct. 29, 1999), pp. 950-952.
Stable URL:
http://links.jstor.org/sici?sici=0036-8075%2819991029%293%3A286%3A5441%3C950%3AASOSAR%3E2.0.CO%3B2-S

[11] **Isolation of Quelling-Defective (qde) Mutants Impaired in Posttranscriptional Transgene-Induced Gene Silencing in Neurospora crassa**
Carlo Cogoni; Giuseppe Macino
Proceedings of the National Academy of Sciences of the United States of America, Vol. 94, No. 19. (Sep. 16, 1997), pp. 10233-10238.
Stable URL:
http://links.jstor.org/sici?sici=0027-8424%2819970916%2994%3A19%3C10233%3AIOQ%28MI%3E2.0.CO%3B2-E

[17] **Positional Cloning of the Werner's Syndrome Gene**
Chang-En Yu; Junko Oshima; Ying-Hui Fu; Ellen M. Wijsman; Fuki Hisama; Reid Alisch; Shellie Matthews; Jun Nakura; Tetsuro Miki; Samir Ouais; George M. Martin; John Mulligan; Gerard D. Schellenberg
Science, New Series, Vol. 272, No. 5259. (Apr. 12, 1996), pp. 258-262.
Stable URL:
http://links.jstor.org/sici?sici=0036-8075%2819960412%293%3A272%3A5259%3C258%3APCOTWS%3E2.0.CO%3B2-J

LINKED CITATIONS

[21] **A Deletion within the Murine Werner Syndrome Helicase Induces Sensitivity to Inhibitors of Topoisomerase and Loss of Cellular Proliferative Capacity**

Michel Lebel; Philip Leder

Proceedings of the National Academy of Sciences of the United States of America, Vol. 95, No. 22. (Oct. 27, 1998), pp. 13097-13102.

Stable URL:

http://links.jstor.org/sici?sici=0027-8424%2819981027%2995%3A22%3C13097%3AADWTMW%3E2.0.CO%3B2-1