cessed for mRNA in situ hybridization with *4311*, *flt-1*, and *flk-1* riboprobes as described (*28*). Embryos or organs were stained with X-gal as described (*25*), and no background staining was detected in all control tissues. Sections were counterstained with nuclear red.

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Requirement of the Yeast *RTH1* 5′ to 3′ Exonuclease for the Stability of Simple Repetitive DNA

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Simple repetitive DNA sequences are unstable in human colorectal cancers and a variety of other cancers. Mutations in the DNA mismatch repair genes *MSH2, MLH1,* and *PMS1* result in elevated rates of spontaneous mutation and cause a marked increase in the instability of simple repeats. Compared with the wild type, a null mutation in the yeast *RTH1* gene, which encodes a 5′ to 3′ exonuclease, was shown to increase the rate of instability of simple repetitive DNA by as much as 280 times and to increase the spontaneous mutation rate by 30 times. Epistasis analyses were consistent with the hypothesis that this *RTH1*-encoded nuclease has a role in the *MSH2-MLH1-PMS1* mismatch repair pathway.

Defects in DNA mismatch repair in *Escherichia coli* and yeast result in an increase in the instability of simple repetitive DNA sequences (*1*, *2*). Tumors from hereditary nonpolyposis colorectal cancer (HNPCC) kindreds contain frequent alterations in the length of microsatellites (*3*), and mutations in any of the four known human mismatch repair genes, *hMSH2*, *hMLH1*, *hPMS1*, and *hPMS2*, are associated with most of these cancers (*4*, *5*). HNPCC patients account for 3 to 6% of colorectal cancers each year (*6*). Colorectal cancers also occur in patients without a strong family history of the disease, and these too display microsatellite instability (*3*, *5*, *7*). However, a large proportion of the sporadic tumor cell lines with microsatellite instability do not contain mutations in the four mismatch repair genes, which suggests that other genes may contribute to microsatellite instability and cancer (*5*).

In eukaryotes, long-patch DNA repair of both base mismatches and loops has bidirectional excision capability (*8*); hence, 5′ to 3′ and 3′ to 5′ exonucleases may play a role in the repair process. The *RTH1* gene of *Saccharomyces cerevisiae* encodes the yeast counterpart of the mammalian 45-kD 5′ to 3′ exonuclease required for lagging-strand DNA synthesis in reconstituted systems (*9*, *10*). After the ribonuclease H1–catalyzed cleavage of primer RNA one nucleotide 5′ of the RNA-DNA junction, the 5′ to 3′ exonuclease removes the remaining monoribonucleotide of the RNA primer (*10*). A null mutation of *RTH1* (*rth1*Δ) causes conditional lethality at 37°C; mutant cells are blocked in nuclear division and have a morphology typical of mutants defective in DNA replication. At the permissive temperature, rates of spontaneous mitotic recombination are elevated in the *rth1*Δ strain, and sensitivity to the alkylating agent methyl methanesulfonate is enhanced (*9*). Here, we show that the *rth1*Δ mutation increases the instability of simple repetitive sequences.

Poly(GT) repeat sequences ranging in size from 10 to 30 repeats are present in most eukaryotes, including humans (*11*). To examine the effect of *RTH1* on the stability of poly(GT) repeats, we used two types of low-copy centromeric plasmids. Plasmid pSH91 contains an in-frame 33–base pair (bp) poly(GT)$_{16}$G insertion within the coding sequence of a modified *URA3* gene (*2*). Alterations of the tract that produce an out-of-frame mutation produce Ura$^-$ cells that are resistant to 5-fluoroorotic acid (5-FOA). Plasmid pSH31 contains an out-of-frame 29-bp poly(GT)$_{14}$G insertion within the coding sequence of the β-galactosidase gene (*12*). Alterations of the tract that restore the correct frame produce blue colonies on medium containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). To monitor frame shifting within the chromosome, we used yeast strain SH75, in which an out-of-frame 29-bp poly(GT)$_{14}$G sequence containing a β-galactosidase construction similar to that in pSH31 has been integrated into the yeast genome at the *LYS2* locus (*12*).

The *rth1*Δ mutation increased tract instability in all three systems. In the two plasmid-based systems, we observed rates of tract alteration in the *rth1*Δ mutant that were 100 to 280 times the wild-type rate (Table 1). Tract instability within the chromosome also increased in the *rth1*Δ strain, but its magnitude (43 times the wild-type rate) was less than that in the plasmid systems. This result is consistent with the observation that tract instability is less pronounced in the chromosome than in the plasmids in the *pms1* mutant (*2*) (Table 1). The rate of tract alteration in the *rth1*Δ strain was one-half to one-third the rate in the isogenic strains that contained mutations in *MSH2*, *MLH1*, or *PMS1* (Table 1). The *PMS1* and *MLH1* genes act in the same pathway of mismatch repair, because double mutants show microsatellite instability similar to that of single mutants (*2*). Conversely, a double mutant carrying a *pms1* mutation in combination with a *pol2* mutation, which abolishes the 3′ to 5′ proofreading exonuclease activity of DNA polymerase ε, exhibits mutation rates that are the product of the relative mutation rates of *pol2* and *pms1* single mutants (*13*); this finding indicates that mismatches that accumulate in the absence of *POL2* proofreading activity are removed by the mismatch repair system.

To determine whether *RTH1* functions in concert with *MSH2*, *MLH1*, and *PMS1*, we examined tract instability in the double mutants *rth1*Δ*msh2*Δ, *rth1*Δ*mlh1*Δ, and *rth1*Δ*pms1*Δ. We found that the *rth1*Δ mutation in combination with any of the mismatch repair mutations resulted in rates of tract instability that were about three times those of isogenic strains defective in a single mismatch repair gene. These observations suggest that in addition to playing a role in the *MSH2-MLH1-PMS1* mismatch repair pathway, *RTH1* effects mismatch removal by another minor repair pathway.

Previous studies have suggested that the *MSH2-MLH1-PMS1* pathway of mismatch repair primarily corrects insertions or deletions of one or two units of dinucleotide repeats (*2*). To determine the types of repeat alterations generated in the *rth1*Δ strains, we sequenced plasmid DNA that contained tract alterations from 5-FOA–resistant colonies or from blue colonies on X-Gal plates (Table 2). With the exception

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of one 14-bp deletion, the *rth1*Δ strains showed only insertions of one or two repeat units. In contrast, the *msh2*Δ, *mlh1*Δ, and *pms1*Δ strains and the *rth1*Δ*msh2*Δ double mutant showed both insertions and deletions of one or two repeat units (*2*) (Table 2). The bias for additions in the *rth1*Δ strain is consistent with a role of *RTH1* exonuclease in the removal of short insertions from the newly synthesized DNA strand. It is possible that the RTH1 protein affects the recognition by the mismatch repair proteins of small loops in the newly synthesized strand. The correction of deletions of one or two repeat units in the *rth1*Δ strain implies that there is an as yet unidentified 3′ to 5′ exonuclease activity involved in mismatch repair that performs this function.

Mutations in *MSH2*, *MLH1*, and *PMS1* cause a marked increase in spontaneous mutation rates (*14*). We found that the rate of spontaneous forward mutation to canavanine resistance (*can1*^{r}) in the *rth1*Δ strain is about 30 times the rate in the isogenic wild-type strain (Table 3). A similar increase in mutation rate occurs in the *msh2*Δ strain. As was observed for tract length instability (Table 1), the *rth1*Δ*msh2*Δ double mutant exhibited a somewhat higher mutation rate than that of the *rth1*Δ and *msh2*Δ single mutants (Table 3). The requirement of yeast *RTH1* for the stability of DNA repeats raises the possibility that mutations in the human *RTH1* counterpart that produce defects in mismatch repair contribute to colorectal and other cancers that are associated with microsatellite instability (*15*).

Table 1. Effect of the *rth1*Δ mutation on the stability of poly(GT) tracts. Isogenic *rth1*Δ strains were generated by transformation of the respective wild-type or mismatch repair defective strains with plasmid pR2.10 (*17*). Alterations of tract length were monitored and rates of tract instability were determined as in (*18*). FOA^r, resistance to 5-FOA; β-GAL, β-galactosidase.

Strain	Genotype	Tract instability detection method	Tract location	Rate of tract instability (±SD)	Rate relative to wild type
MS71	Wild type	FOA^r	Plasmid	$7.1\ (\pm0.1) \times 10^{-6}$	1
YRTH29	*rth1*Δ	FOA^r	Plasmid	$7.1\ (\pm0.2) \times 10^{-4}$	100
YRP27	*msh2*Δ	FOA^r	Plasmid	$1.8\ (\pm0.5) \times 10^{-3}$	254
YRP23	*mlh1*Δ	FOA^r	Plasmid	$1.9\ (\pm0.4) \times 10^{-3}$	268
AMY101	*pms1*Δ	FOA^r	Plasmid	$2.2\ (\pm0.5) \times 10^{-3}$	310
YRTH33	*rth1*Δ*msh2*Δ	FOA^r	Plasmid	$5.9\ (\pm1.4) \times 10^{-3}$	831
YRTH30	*rth1*Δ*mlh1*Δ	FOA^r	Plasmid	$5.7\ (\pm1.1) \times 10^{-3}$	803
YRTH32	*rth1*Δ*pms1*Δ	FOA^r	Plasmid	$7.3\ (\pm1.7) \times 10^{-3}$	1028
MS71	Wild type	β-GAL	Plasmid	$2.9\ (\pm0.2) \times 10^{-6}$	1
YRTH29	*rth1*Δ	β-GAL	Plasmid	$8.2\ (\pm1.0) \times 10^{-4}$	283
YRP27	*msh2*Δ	β-GAL	Plasmid	$2.6\ (\pm0.2) \times 10^{-3}$	897
YRTH33	*rth1*Δ*msh2*Δ	β-GAL	Plasmid	$8.7\ (\pm2.2) \times 10^{-3}$	3000
SH75	Wild type	β-GAL	Chromosome	$2.1\ (\pm0.9) \times 10^{-5}$	1
YRTH38	*rth1*Δ	β-GAL	Chromosome	$9.1\ (\pm2.0) \times 10^{-4}$	43
MS89	*pms1*Δ	β-GAL	Chromosome	$1.8\ (\pm0.5) \times 10^{-3}$	86

Table 2. Types of poly(GT) tract alterations generated in the *rth1*Δ strain. Plasmids isolated from 5-FOA–resistant (pSH91) or blue (pSH31) colonies were propagated in *E. coli*, and the poly(GT) tracts were sequenced with the use of double-stranded DNA and the "−40" primer as in (*12*).

Strain	Genotype	Tract location	Number of tracts sequenced	Number of tracts with base pair deletions (−) or additions (+)				
				−4	−2	+2	+4	Others
MS71	Wild type	pSH91	19	0	2	13	0	4*
YRTH29	*rth1*Δ	pSH91	35	0	0	29‡	5	1†
YRTH29	*rth1*Δ	pSH31	17	0	0	17	0	0
YRP27	*msh2*Δ	pSH91	18	0	12	6	0	0
YRTH33	*rth1*Δ*msh2*Δ	pSH91	29	1	8	16	4	0

*The four other alterations observed in the wild-type strain were −14, −10, +14, and +14. †The other alteration observed in the *rth1*Δ strain was −14. ‡The lack of tracts with deletions of one or two repeat units in the *rth1*Δ mutant compared with the *msh2*Δ and *rth1*Δ*msh2*Δ mutants is highly significant as determined by χ^2 analyses ($P \ll 0.005$).

Table 3. Effect of the *rth1*Δ mutation on the rate of spontaneous forward mutation to canavanine resistance (*can1*^{r}). Strains were identical to those in Table 1, except that they did not carry the poly(GT) plasmid. Mutation rates were determined as in (*19*).

Strain	Genotype	Rate of forward mutation to *can1*^{r} (±SD)	Rate relative to wild type
MS71	Wild type	$3.8\ (\pm1.0) \times 10^{-7}$	1
YRTH29	*rth1*Δ	$1.2\ (\pm0.3) \times 10^{-5}$	32
YRP27	*msh2*Δ	$1.3\ (\pm0.3) \times 10^{-5}$	34
YRTH33	*rth1*Δ*msh2*Δ	$4.7\ (\pm0.9) \times 10^{-5}$	124

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17. Plasmid pR2.10 was used to make the genomic deletion mutation of *RTH1* by gene replacement (*9*). The *RTH1* gene was replaced by the *URA3* gene. Cells in which the integrated *URA3* gene was subsequently lost by recombination between the flanking *HisG* sequences were selected for on media containing 5-FOA. Deletion mutations were confirmed by Southern (DNA) blot analyses.
18. Yeast strain MS71 (*MATα ade5-1 his7-2 trp1-289 ura3-52 CAN1*^{s}) and isogenic deletion strains were transformed with plasmid pSH91 or pSH31 to monitor alterations of plasmid-borne DNA repeats. Yeast

strain SH75 [*MAT***a** *ade6 arg4-17 his4-Sal trp1 tyr7-1 ura3 lys2*::p(GT)$_{14}$G β-GAL] and isogenic deletion strains were used to monitor repeat alterations in the chromosome. To monitor repeat alterations in pSH91, we grew 19 independent 100-μl cultures, each starting from ~10 5-FOA–sensitive cells, in yeast extract–peptone-dextrose (YPD) media for each strain. Cultures were grown to ~10^5 to 10^6 cells before being plated onto medium containing 5-FOA. Rates of tract alteration were determined from the number of 5-FOA–resistant colonies by means of the method of the median (*16*). To monitor tract alterations in pSH31 and in the chromosome, we grew 20 independent 100-μl cultures, each starting from ~10 cells, in YPD for each strain. Cultures were grown to ~10^3 to 10^4 cells before being plated onto medium containing X-Gal. The rate of tract alteration was determined from the number of cultures with no blue colonies (*16*).

19. For each strain, 19 independent 100-μl or 500-μl cultures were grown in YPD. Cells were then plated onto arginine-deficient medium containing canavanine. Rates of forward mutation at the *CAN1*^{s} locus were determined from the number of canavanine-resistant colonies by means of the method of the median (*16*).

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High Concentrations of Toxaphene in Fishes from a Subarctic Lake

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Concentrations of toxaphene and other organochlorine compounds are high in fishes from subarctic Lake Laberge, Yukon Territory, Canada. Nitrogen isotope analyses of food chains and contaminant analyses of biota, water, and dated lake sediments show that the high concentrations of toxaphene in fishes from Laberge resulted entirely from the biomagnification of atmospheric inputs. A combination of low inputs of toxaphene from the atmosphere and transfer through an exceptionally long food chain has resulted in concentrations of toxaphene in fishes that are considered hazardous to human health.

Lake Laberge (61°11′N, 135°12′W) is located in southern Yukon Territory, Canada. Although the town of Whitehorse has grown along the Yukon River upstream of the lake, Lake Laberge and its watershed are symbols of pristine wilderness for Canadians (*1*). In 1991, a routine survey of contaminants in fishes from Yukon lakes revealed that lake trout (*Salvelinus namaycush*) and burbot (*Lota lota*) from Lake Laberge were contaminated with amounts of toxaphene and other lipophilic contaminants several-fold greater than in the same species from other subarctic and arctic lakes and rivers (*2*, *3*). As a result of the high toxaphene concentrations, fish consumption advisories were issued by Health Canada (*4*), and the commercial, sport, and native subsistence fisheries were closed.

Toxaphene was once used in North America as a piscicide (fish-killing agent) to remove rough fish species and as an agricultural pesticide (*5*). Although Canada and the United States discontinued its use in the early 1980s, it is currently used in Eurasia and Central America and is carried by long-range atmospheric transport to subarctic and arctic regions (*6*). As a result, elevated concentrations of toxaphene have been found in upper-trophic-level biota from the Arctic (*7*), despite their distance from the original source.

One possible cause for the contamination of fishes in Laberge was surreptitious dumping. It is known that one lake in northern central Yukon was treated with toxaphene in 1963 (*8*). Alternatively, the elevated concentrations of toxaphene could be attributed to biomagnification through the food chain because concentrations of persistent chlorinated organics such as toxaphene increase from prey to predator (*9*) and are highest in fish from lakes with the longest food chains (*10*). Significant biomagnification of lipophilic contaminants occurs at subarctic and arctic latitudes, where aquatic organisms must survive winters lasting several months (*11*). Here, we analyzed food-chain organisms, water, and sediments of Yukon lakes for toxaphene to distinguish between the "surreptitious dumping" and "food-chain length" hypotheses.

Traditional studies of contaminant biomagnification in lacustrine food webs have used discrete trophic classifications based on inferred feeding behavior or analyses of stomach contents (*9*, *10*). However, interpretation is complicated for species such as lake trout that may be piscivorous, omnivorous, or insectivorous in different lakes, feeding on varying combinations of terrestrial and benthic insects, other fish, or plankton (*12*). Stable nitrogen isotope ratios ($\delta^{15}N$)

$$\delta^{15}N = [(^{15}N/^{14}N_{sample} \div {}^{15}N/^{14}N_{atmos.\ nitrogen}) - 1] \times 1000$$

have been used as an index of trophic level for freshwater organisms (*13*–*16*). This ratio increases an average of 3 to 5 per mil from prey to predator (*13*, *16*). Stable $\delta^{15}N$ values in fish represent the integral of several months of feeding, thereby providing a continuous measure of trophic position (*17*). Recently, Cabana and Rasmussen (*18*) demonstrated that $\delta^{15}N$ in lake trout muscle is correlated with food-chain length across a large number of lakes. We hypothesized that $\delta^{15}N$ would be directly correlated with contaminant concentrations in individuals varying in trophic position.

Large-volume water samples from six lakes on the Yukon River system and ^{210}Pb-dated sediment core slices from Laberge, Fox, and Kusawa lakes (*19*) were used as surrogate measures for toxaphene inputs

Table 1. Surface and maximum fluxes of toxaphene (in nanograms per square meter per year) and the median dates of slices in sediment cores from lakes in Yukon Territory [YT; this study (*21*)], Northwest Territories [NWT; (*26*)], and two lakes treated with toxaphene in Alberta [AB; (*25*)], Canada. Peanut and Chatwin fluxes were not corrected for sediment focusing, and the mean dates of these slices are given.

Lake	Surface flux	Date	Maximum flux	Date
Laberge-2, YT (61°11′N, 135°12′W)	180	1991	270	1978
Laberge-3, YT	32	1991	340	1973
Fox, YT (61°14′N, 135°28′W)	180	1992	260	1983
Kusawa, YT (60°20′N, 136°22′W)	24	1989	140	1974
Far, NWT (63°42′N, 90°40′W)	310	1986		
Hawk, NWT (63°38′N, 90°42′W)	500	1985		
Amituk, NWT (75°03′N, 93°48′W)	140	1980		
Hazen, NWT (81°45′N, 71°30′W)	120	1986		
Peanut, AB (54°01′N, 114°21′W)	2.3×10^4	1985	1.0×10^5	1962
Chatwin, AB (54°15′N, 110°51′W)	1.7×10^4	1990	5.1×10^5	1963

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