SUPPORTING ONLINE MATERIAL

Materials and Methods

***CAN1* forward mutation assays.** All data (with the exception of the WT spectrum from Lippert *et al.* 3) were derived using isogenic derivatives of strain Δ|(-2)|-7B-YUNI300 (*MATa CAN1 his7-2 leu2-Δ::kanMX ura3-Δ trp1-289 ade2-1 lys2-ΔGG2899-2900*; ref. 18). The *rnh201Δ* strain was described previously (1). The *TOP1* ORF was deleted via transformation with a PCR product amplified from pAG25 (19). Cells were grown nonselectively at 30°C in YEP medium (1% yeast extract, 2% bacto-peptone; 2% agar for plates) supplemented with 2% dextrose and 250 μg/ml adenine. Can-R mutants were selected on synthetic complete medium lacking arginine and supplemented with 60 μg/ml canavanine. Rates of spontaneous Can-R colonies were measured as described previously (20). To generate spectra, genomic DNA isolated from independent Can-R mutants was PCR-amplified and sequenced. Rates of individual mutation types were calculated by multiplying the proportion of the mutation type in the corresponding spectrum by the total mutation rate.

***lys2* reversion assays.** All *lys2* alleles were located at the *HIS4* locus of strain YPH45 (*MATa ura3-52 ade2-101$_{oc}$ trp1Δ1*; ref. 21) and were transcribed in the same direction as the replication fork initiated at *ARS306*. The *lys2ΔBgl* mutation (9) was introduced into the *his4Δ::pTET-LYS2* allele (22) by two-step allele replacement. All *lys2ΔA746NR* alleles were under control of the native *LYS2* promoter, and construction of the *lys2ΔA746NR,(AT)$_2$* and *lys2ΔA746NR,(TC)$_3$* alleles was described previously (3). The *lys2ΔA746NR,(AG)$_4$* allele was similarly constructed using oligos 5′-GATCAAGTGAAGAGAGAGCTTAcGCAA and 5′-GATCTTGCgTAAGCTCTCTCTTCACTT; runs are underlined and lowercase letters reflect an AT to CG mutation introduced to remove a stop codon. The *RNH201* and *TOP1* genes were

deleted using PCR-amplified fragments containing dominant drug-resistant or nutritional markers as appropriate. Lys^+ rates and reversion spectra were obtained as described previously (22). For nonselective growth of *pTET-lys2ΔBgl* strains, medium was supplemented with 2 μg/ml doxycycline hyclate (Sigma) to maintain a low-transcription status.

Top1 cleavage assays. Cleavage assays were carried out as described previously (23). Briefly, custom-synthesized oligonucleotides (Midland Certified, Midland, Texas) were end-labeled prior to annealing with the cold, complementary strand at a 1:1 ratio. Reaction mixtures contained 50-200 nM of labeled DNA construct, 10 mM Tris-HCl (pH=7.5), 50 mM KCl, 1 mM EDTA, 1 mM DTT, 15 ug/mL BSA and 10% DMSO. Recombinant human Top1 (24) and/or CPT were added to 70 nM and 10 μM, respectively, as indicated. Reactions were for 1 hr at 25°C and were stopped by addition of 0.5% SDS (final concentration). In reversal assays, samples were incubated for 1 hr at 25°C before adding 0.5 M NaCl (final concentration). A small volume was withdrawn from the sample and the reaction stopped by adding 0.5% SDS (final concentration) at the specified time points (in min). Samples were analyzed on 20% denaturing polyacrylamide gels and labeled fragments detected using a PhosphoImager.

Table S1. Mutation types and rates in *lys2* frameshift-reversion assays

lys2 frameshift allele	Relevant genotype	Rate (x 10^{-9})				
		Hotspot	1-bp indels	Other	Total (CI)	Total/WT
ΔA746NR	WT* (N=90)	NA	0.14	0.81	0.95 (0.86-1.04)	
ΔA746NR,(AG)$_4$	WT (N=84)	0.65	0.47	1.92	3.05 (2.58-4.22)	1.0
	rnh201Δ (N=95)	158	<2.0	9.9	189 (178-200)	62
	rnh201Δ top1Δ (N=88)	0.16	0.59	2.11	2.86 (1.68-3.61)	0.9
ΔA746NR,(TC)$_3$	WT* (N=74)	1.43	0.19	1.17	2.79 (2.46-3.10)	1.0
	rnh201Δ (N=92)	17.7	<0.19	1.92	19.9 (15.7-22.7)	7.1
	rnh201Δ top1Δ (N=74)	<0.02	0.41	1.12	1.53 (1.34-1.89)	0.5
ΔA746NR,(AT)$_2$	WT* (N=80)	6.21	0.56	0.65	7.42 (6.96-7.57)	1.0
	rnh201Δ (N=77)	8.15	0.44	2.62	11.2 (10.3-11.9)	1.5
	rnh201Δ top1Δ (N=74)	<0.03	0.73	1.21	1.94 (1.38-5.06)	0.3
ΔBgl	WT (N=73)	0.22	1.66	1.31	3.19 (2.63-4.73)	1.0
	rnh201Δ (N=84)	5.65	0.57	0.57	6.88 (4.43-12.0)	2.2
	rnh201Δ top1Δ (N=91)	<0.05	1.05	3.74	4.80 (4.09-6.14)	1.5

Table legend

Rates of individual mutation types were calculated by multiplying the total reversion rate by the proportion of the mutation type in the corresponding spectrum. When no events of a given type were observed, the rate is designated as "<" and was calculated assuming a single event.

NA, not applicable; CI, 95% confidence interval.

*Data are from Lippert *et al*. (3).

Supplemental Figure legends

Figure S1. Mutation spectra of Can-R mutants. Nucleotides are numbered beginning with the ATG start codon. Base substitutions and indels are in red below and above the sequence, respectively; the lengths of the red bars correspond to deletion sizes.

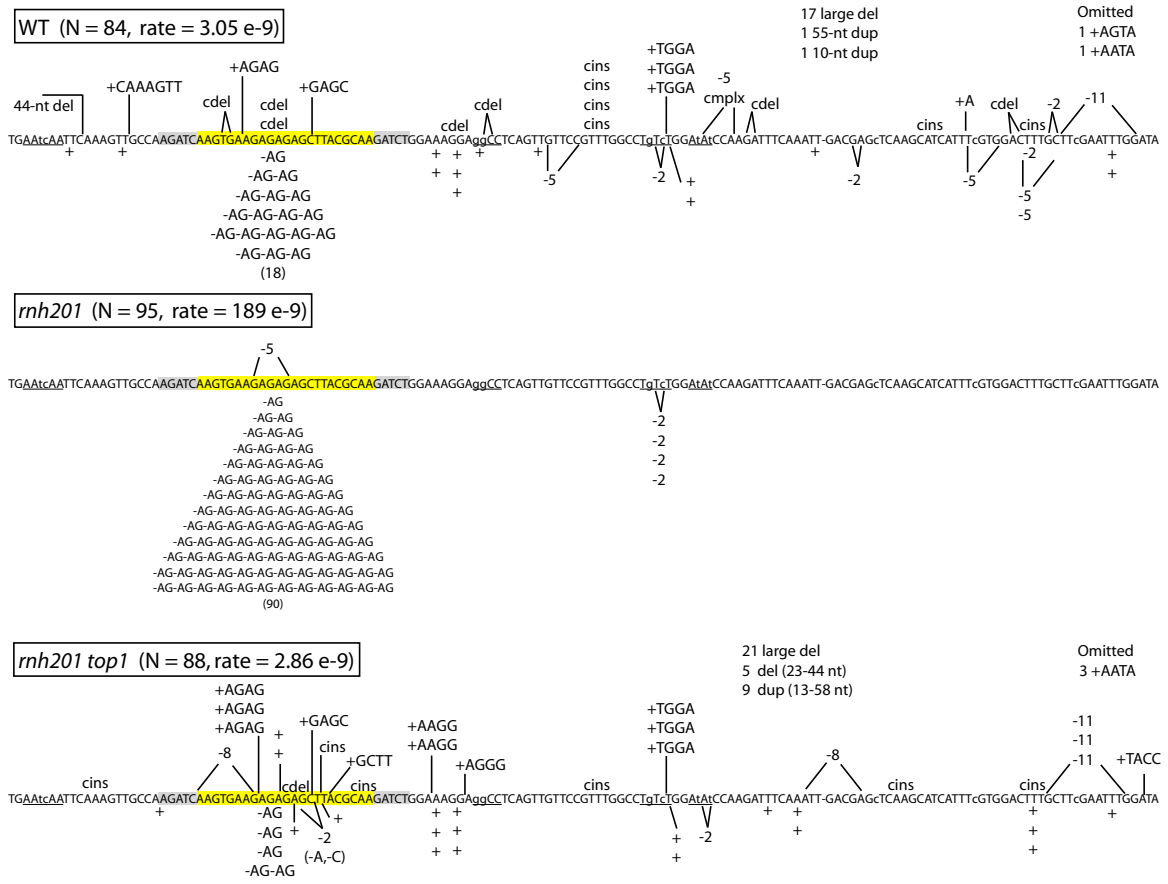
Figure S2. Reversion spectra for *lys2* alleles. In **A-C** hotspot-containing sequences transplanted from the *CAN1* locus are highlighted in yellow and sequences associated with the flanking *Bgl*II site are highlighted in gray. + or Δ correspond to the insertion or deletion, respectively, of a single bp; cins or cdel, insertions or deletions associated with one or more base substitutions; del, deletion; dup, duplication; N, number of independent revertants sequenced.

Figure S3. Human Top1 cleavage assays. A shows fragments used as substrates, with relevant dinucleotide repeats highlighted in gray. Transcribed and nontranscribed strands are designated TS and NTS, respectively; arrows indicate positions of Top1 cleavage. In **B**, the indicated strand was labeled on the 3′ end.

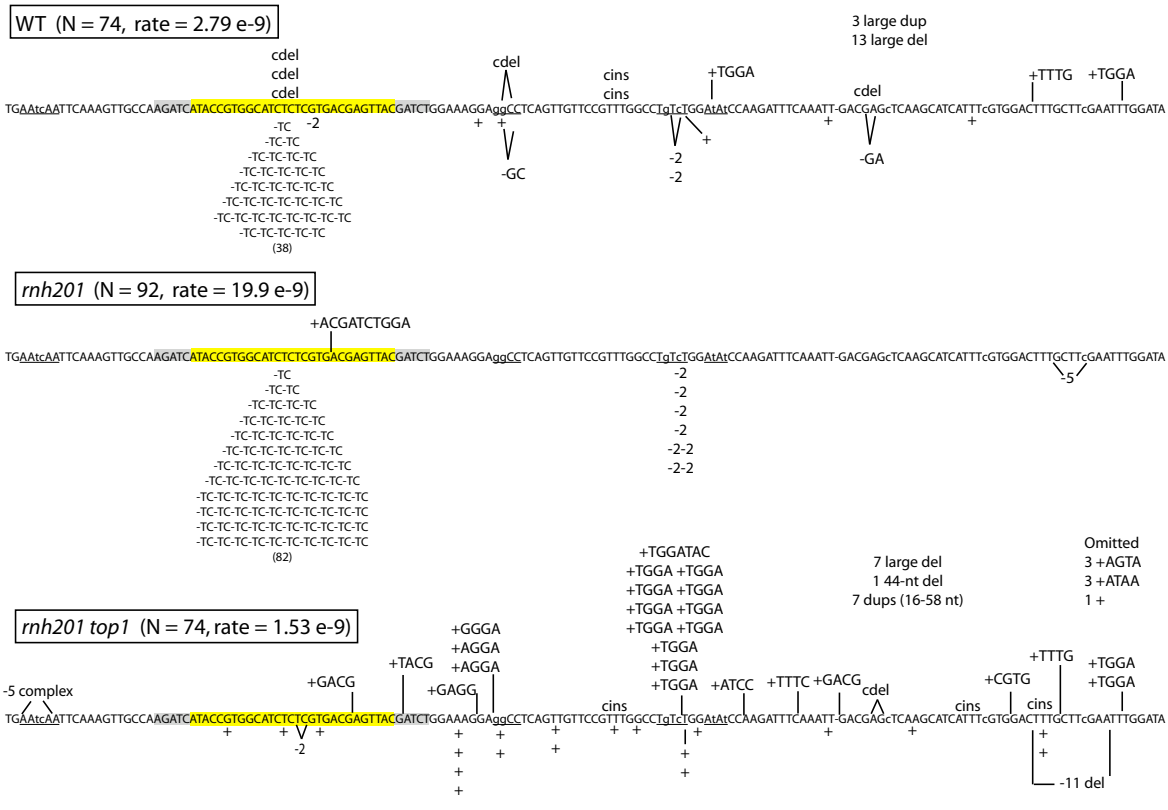
A *rnh201* (N = 185, rate = 26e-8)

B *rnh201 top1* (N = 165, rate = 18e-8)

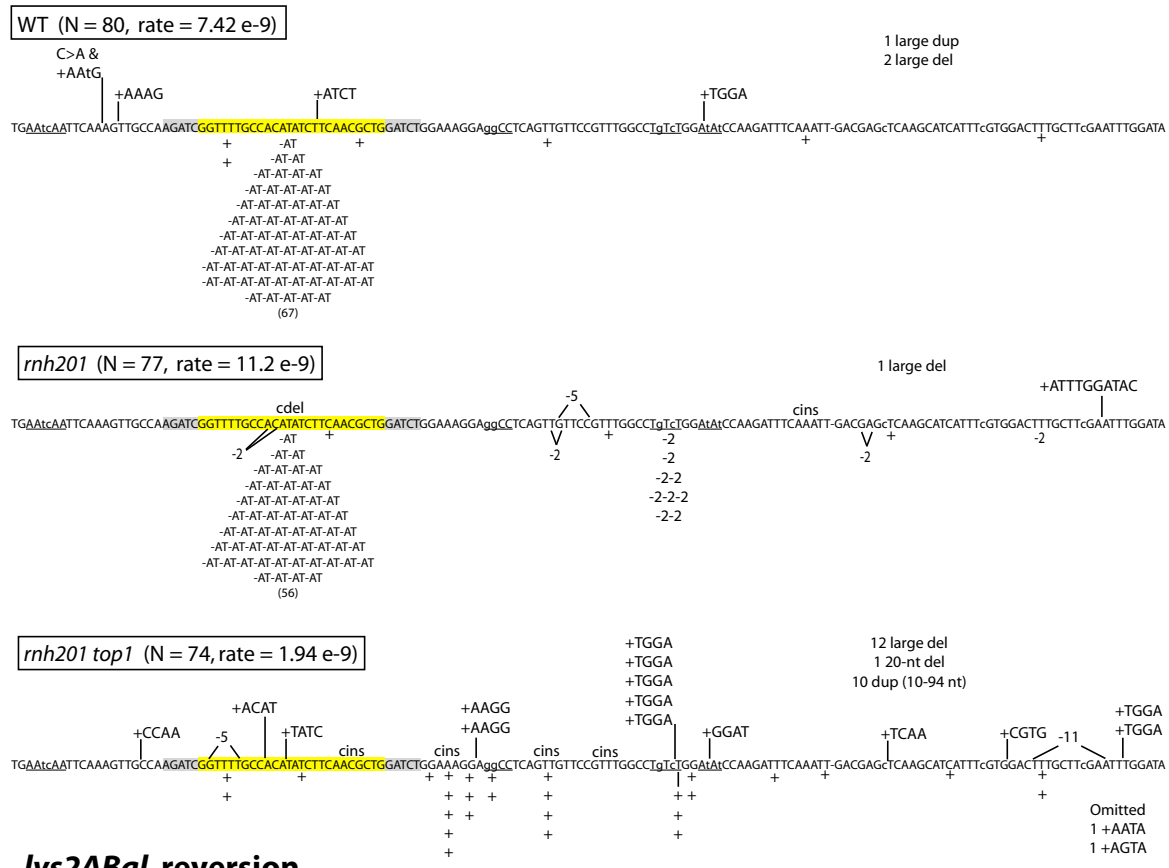
A *lys2ΔA746NR,(AG)4* reversion



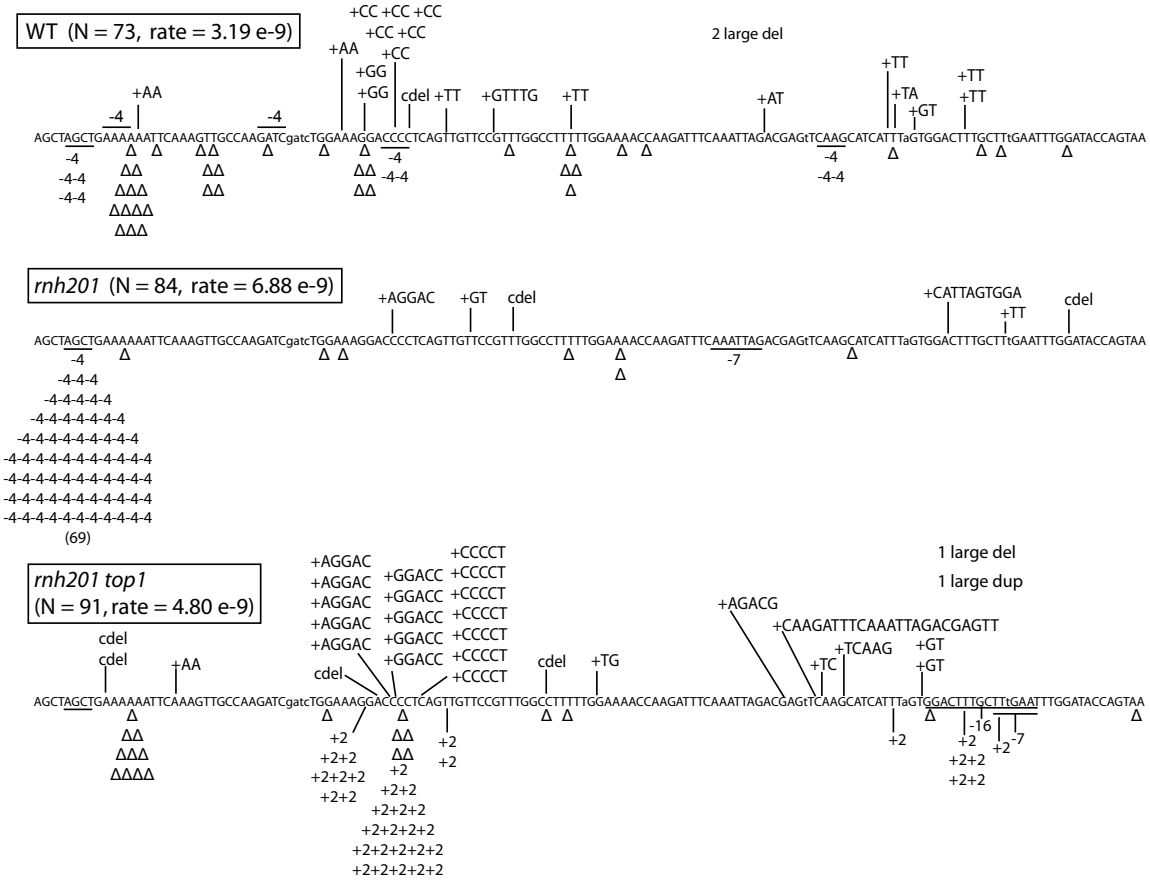
B *lys2ΔA746NR,(TC)3* reversion



C *lys2ΔA746NR,(AT)₂* reversion



D *lys2ΔBgl* reversion



A

$(AG)_4$	NTS 5′-AACGCTGAAGTGA AGAGAGAG CTTAAGCAA TS TTGCGACTTCACT TCTCTCTC GAATTCGTT-5′ a b c d
$(AT)_2$	NTS 5′-ACAAAGGTTTTGCCAC ATAT CTTCAACGCT TS TGTTTCCAAAACGGTG TA*T*A GAAGTTGCGA-5′ e f g
$(TC)_3$	NTS 5′-TTTGAAATACCGTGGCA TCTCTC GTGACGAGT TS AAACTTTATGGCACCG*T* AGAGAG CACTGCTCA-5′ h

B