mmol of reactive functionality per gram of beads for immobilization of hydroxymethyl **1** or **2**. Samples of both PS-1 and PS-2 beads were fixed to the bottom of a petri dish and examined with a fluorescence microscope (340 nm excitation and 510 ± 40 nm emission filters). Figure 3A shows the eyepiece view in which PS-1 (slightly greenish) and PS-2 (slightly bluish) fluoresce weakly in the absence of cognate peptides. When P1 is added, however, the fluorescence of PS-1 (but not PS-2) dramatically increases (by ~300%) (Fig. 3B). The enhanced fluorescence of PS-1 can be reduced to its original level by washing with $CHCl_3$, and PS-2 can be made to fluoresce by adding P2 (an increase in fluorescence of 70%) (Fig. 3C). Although low millimolar peptide concentrations could thus be detected visually with PS-1 and PS-2, the more sensitive chemosensor PS-1 could detect P1 concentrations as low as 1 μM by quantitative analysis of a single bead image obtained with the microscope and a charge-coupled device camera.

These experiments demonstrate the sequence-selective optical detection of peptides by a small molecule chemosensor and should be readily extended to biological (and other) sensing applications given recent advances in peptide recognition by synthetic receptors in water (*14*–*16*) and in the preparation of synthetic peptide-binding receptor libraries (*20*). In particular, our simple prescription for peptide-binding small molecules and the ready synthetic accessibility of isophthalic acid–diamine–based receptors clearly indicates one way to make other chemosensing molecules having different peptide-binding properties and selectivities. It should also be straightforward to integrate the methods described here with combinatorial synthesis to prepare solid-state libraries containing many different kinds of chemosensors that could be screened to find compounds having particular sensing properties or used in chemosensing devices incorporating a large, diverse array of selective sensing elements (*21*).

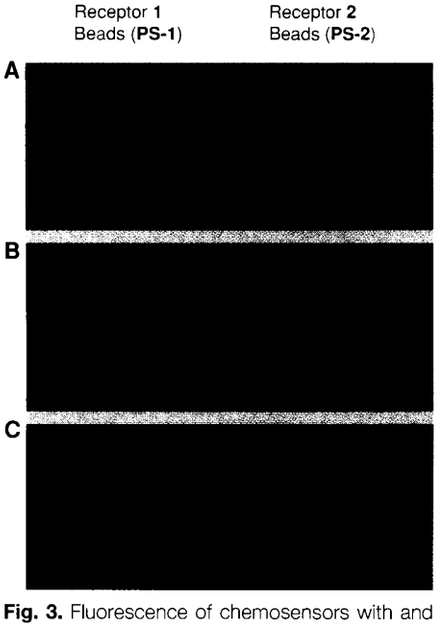

Fig. 3. Fluorescence of chemosensors with and without their cognate peptides: (**A**) absence of peptide, (**B**) presence of 5 mM peptide P1, and (**C**) presence of 5 mM peptide P2.

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Expansion and Length-Dependent Fragility of CTG Repeats in Yeast

Catherine H. Freudenreich, Sara M. Kantrow, Virginia A. Zakian*

Expansion of DNA trinucleotide repeats (TNRs) is the causative mutation in a growing number of human genetic diseases. Large expansions of a CTG tract were obtained and shown by genetic and physical assays to be length-dependent sites of chromosome breakage in *Saccharomyces cerevisiae*. Deletion of *RAD27*, which encodes a nuclease involved in Okazaki fragment processing, caused length-dependent destabilization of CTG tracts and a substantial increase in expansion frequency. The genetic assay described here can be used to evaluate other factors that induce TNR expansion or chromosome fragility in humans.

Expansion of TNRs has been shown to cause human genetic diseases, including myotonic dystrophy, Huntington's disease, several ataxias, and fragile X syndrome (*1*). As a TNR tract expands, the probability of further expansion increases. For example, at the myotonic dystrophy locus, the normal allele size of 5 to 37 CTG/CAG repeats (hereafter called CTG) is relatively stable, but when tracts reach ~50 repeats, they are much more likely to expand in the next generation (*1*). TNR DNA forms stable hairpins that cause replication pausing in vitro (*2*, *3*); it has been proposed that replication pausing promotes expansion or contraction in vivo by replication slippage (*4*). The five known loci of expanded CCG repeats in the human genome all share the additional feature of being fragile sites. Fragile sites, defined as gaps or breaks in condensed human chromosomes, are typically assayed by exposing tissue culture cells to reagents that slow replication or bind

Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA.

*To whom correspondence should be addressed. E-mail: vzakian@molecular.princeton.edu

DNA (5). At least some fragile sites also cause chromosome breakage in humans (6).

Expansion and fragility of TNRs are thought to result from perturbations of DNA replication or chromatin structure or both, which are features that can be mimicked in yeast and other organisms. However, TNRs in model systems, including yeast, have so far failed to exhibit the dramatic and frequent expansions that occur in human disease, perhaps because expansions are rare in wild-type cells (*7*, *8*). We wanted to identify a length-dependent phenotype for CTG tracts so that genes or conditions that affect TNR expansion could be isolated. To determine whether CTG tracts are fragile sites in yeast, we took advantage of the fact that a double-strand break (DSB) between two direct repeats increases the rate of recombination between the repeats, with concomitant deletion of the intervening sequences (9). Strains were constructed with a CTG tract and a *URA3* gene between two direct repeats (Fig. 1A). Recombinants were identified by elimination of *URA3*, which generates a cell that can grow in the presence of the drug 5-flouroorotic acid (FOA). If the CTG tract increases chromosome breakage, strains with a CTG tract between the repeated regions will generate FOA-resistant (FOA^R) cells at an elevated rate as compared with a strain without the CTG tract (Fig. 1A).

To make the direct repeat substrate, we transformed a yeast strain containing a tract of 130 CTG repeats (CTG-130) within the yeast *LYS2* locus on chromosome II (*8*, *10*). Five of the 33 Lys^+ transformants had expanded CTG tracts with tract sizes ranging from ~170 to ~250 repeats (Fig. 1B) (*10*, *11*). To establish that the increase in band size was due to expansion of the CTG tract, we digested the DNA with 14 different restriction enzymes, including enzymes with sites within *LYS2* and *URA3* and enzymes that cut variants of CTG. In each case, only fragments expected for expansion of the CTG tract were obtained (Fig. 1B) (*11*). Digestion with Bbv I, which cuts CTG repeats, eliminated all hybridization (*11*). In previous studies of TNRs in model systems such as *Escherichia coli*, yeast, human cells in culture, and mice, deletions were much more common than expansions, and expansions that occurred were usually small and present in only a subset of cells (*7*, *8*). Thus, some aspect of the transformation process, such as recombination near the tract, appeared to increase the generation or recovery of expansions.

To evaluate whether a long CTG tract could act as a fragile site in yeast, we determined the rate of recombination between the repeated *LYS2* sequences in five strains with tract sizes of 0 to 250 repeats, using fluctuation analysis (*12*). A substantial tract length–dependent increase in the rate of generating FOA^R colonies was observed: Compared to the control, the rate was ~5-fold greater for CTG-130, ~9-fold greater for CTG-180, ~27-fold greater for CTG-200, and ~53-fold greater for CTG-250 (Fig. 1C). Southern (DNA) blot analysis showed that the structure of the *LYS2* locus in FOA^R colonies was as expected (*11*). To ensure that the increase in recombination was not due to a second site mutation, we introduced another recombination sub-

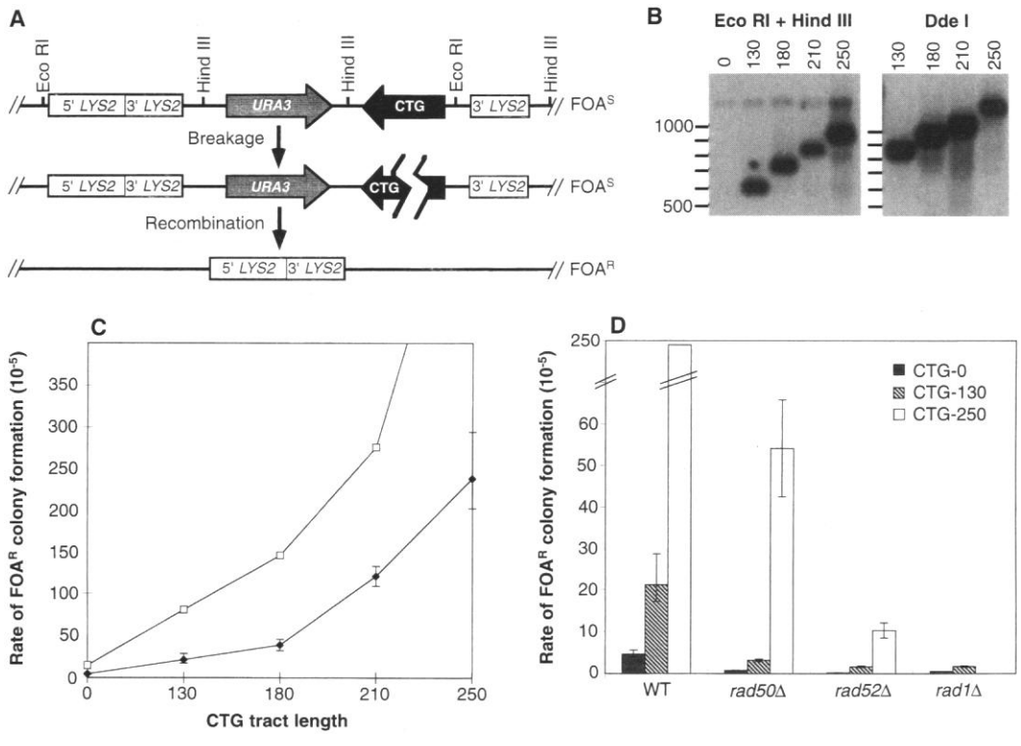

Fig. 1. Assay for chromosome fragility. (**A**) Structure of the modified *LYS2* locus is drawn approximately to scale except for 5′ *LYS2*. The *URA3* gene (1.17 kb) and CTG tract (390 to 750 bp) were placed between a full-length copy of the *LYS2* gene and a 700-bp 3′ fragment of the *LYS2* gene to create a 700-bp nontandem duplication of 3′ *LYS2*. Starting strains are FOA-sensitive (FOA^S) because they contain the *URA3* gene. If breakage occurs in the 2.1-kb region between the duplicated segments of *LYS2*, recombination can occur between them, thereby eliminating the intervening sequences to generate a FOA^R cell. A control strain lacking the CTG tract was constructed as above, except that the duplicated segments of *LYS2* are 1.1 kb and the distance between them is 1.7 kb. Because of these differences, the recombination rates in the control strain are probably overestimated compared to the strains containing CTG tracts. (**B**) DNA was prepared from Lys^+ transformants (*10*) for the control strain, a CTG-130 tract strain, and transformants with expanded CTG tracts (tract size is indicated above each lane). DNA was digested with Eco R I and Hind III or Dde I, which are expected to yield fragments of 590 bp and 850 bp,respectively, for CTG-130; run on a 1% agarose gel; blotted to nylon membrane; and hybridized to a CTG probe. Recognition sites for Eco RI and Hind III are indicated in (A); Dde I cuts the region at 22 locations. (**C**) Fluctuation analysis was performed (*12*) to determine the rate of FOA^R colony formation for the control strain and four CTG-tract strains (diamonds). The average of two (CTG-180 and CTG-210) or three (CTG-0, CTG-130, and CTG-250) experiments is shown. Error bars indicate the range of values obtained. Fluctuation analysis was also done using colonies grown on media containing 0.1 M HU (squares). The CTG-250 + HU sample value of 670 × 10^{-5} is off the chart. (**D**) The rate of generation of FOA^R colonies is shown for CTG-0, CTG-130, and CTG-250 strains in the wild-type, *rad50Δ*, and *rad52Δ* backgrounds, and for CTG-0 and CTG-130 strains in the *rad1Δ* background. Values are the average of two (*rad50Δ*, *rad52Δ*, and *rad1Δ*) or three [wild-type (WT); Fig. 1C] experiments (*12*). Error bars indicate the range of values obtained. For wild-type CTG-250, the range is 202 to 294 × 10^{-5}. Double hatch marks indicate a discontinuity in the *y* axis.

strate of repeated sequences flanking a *URA3* gene at a different chromosomal location into an FOA^R derivative of the CTG-250 strain and into the parent strain. The rate of generation of FOA^R cells was the same and was very low in both strains (*11*).

In human cells, growth in folate- or thymidine-deprived media slows replication and increases the breakage of expanded CCG tracts (*5*). In yeast, hydroxyurea (HU) slows DNA replication by lowering nucleotide pools (*13*). A fluctuation analysis of strains grown on plates containing HU showed that the rate of FOA^R colony formation was increased about threefold for the control and for each CTG tract strain (Fig. 1C). Therefore, as with human cells, perturbation of DNA replication in yeast increases the likelihood of chromosome breakage. In *E. coli*, stalled replication forks are preferential sites for DSBs (*14*). If long TNR tracts cause pausing during DNA synthesis, breakage of stalled replication forks could explain the observed chromosome fragility. The effect of HU shows that the recombination assay can be used to detect conditions that increase TNR-induced chromosome breakage.

If CTG tract-mediated recombination proceeds via a DSB, then recombination should depend on genes required for DSB repair. The fluctuation analysis was repeated using strains lacking genes involved in DSB repair (*12*, *15*): Rad52p is required for repair of DSBs by either gene conversion or single-strand annealing, Rad1p is required for single-strand annealing, and DSBs are processed more slowly in *rad50Δ* strains (*9*). The rate of generating FOA^R colonies was decreased substantially and to about the same extent in both the *rad52Δ* and *rad1Δ* strains (Fig. 1D). The rate was also decreased in the *rad50Δ* background, although less dramatically. These data indicate that the FOA^R colonies arise by repair of a DSB that occurs near or at the CTG tract, and that repair occurs mainly by single-strand annealing.

The structure of the chromosome with the CTG tract was determined by pulsed-field gel electrophoresis (*16*). To increase the fraction of broken chromosomes, a *rad50Δ* strain grown in HU was used. Southern hybridization revealed that the *rad50Δ* CTG-250 strain contained intact chromosome II (825 kb) as well as two fragments with the size (~470 and ~355 kb) and hybridization properties expected for breakage in the vicinity of *LYS2* (Fig. 2). The 470- and 355-kb fragments were not detected in the control strain (Fig. 2). These data provide direct physical evidence that a CTG-250 tract increased breakage of chromosome II within a few kilobases of the tract. The CTG tract at the myotonic dystrophy locus in humans has not been observed to be a fragile site (*17*). However, given the considerable conservation in both chromatin structure and enzymes involved in DNA replication and repair between yeast and humans, it is likely that long CTG tracts also act as fragile sites in humans. Because conditions that increase chromosome fragility are different for different expanded sequences (*5*), the appropriate growth conditions for inducing breaks at CTG tracts may not have been tested, or CTG tracts may break at a frequency lower than that detectable by standard cytological techniques.

The length-dependent breakage of CTG tracts in yeast provides a genetic assay for genes or conditions that increase tract expansions or fragility by testing their effects on the generation of FOA^R colonies. In yeast, deletion of *RAD27*/*RTH1* causes decreased stability of dinucleotide repeats and an increase in duplication mutations between short direct repeats (*18*). The mammalian homolog of *RAD27*, *FEN1* (flap

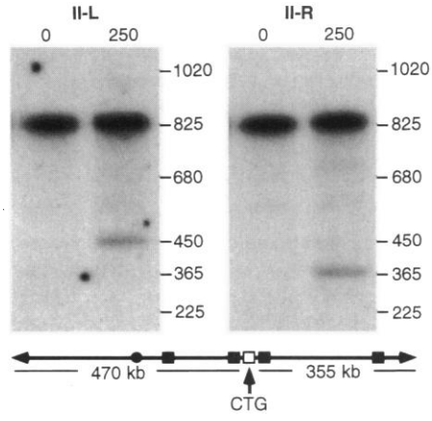

Fig. 2. Pulsed-field gel analysis of chromosome breakage. *rad50Δ* CTG-0 and CTG-250 strains were grown in media containing 0.05 M HU, and chromosomes were isolated and separated by pulsed-field gel electrophoresis (*16*). The gel was blotted to nylon membrane and hybridized to probes either to the left (II-L) or right (II-R) (black boxes) of the CTG tract (white box). The centromere is represented by a black circle. Breakage of the 825-kb chromosome II near the *LYS2* locus will produce bands of ~470 kb (left fragment) and ~355 kb (right fragment). The gel was probed sequentially with *TLC1* (~160 kb left of *LYS2*), *RAD16* (1.5 kb left of *LYS2*), *TKL2* (1.5 kb right of *LYS2*), and *RIF1* (~280 kb right of *LYS2*). The data for the *TLC1* (left) and *RIF1* (right) probes are shown. Size markers in kilobases were determined from the positions of intact yeast chromosomes on the ethidium bromide–stained gel. Broken chromosomes were also observed in the *rad50Δ* CTG-250 strain in the absence of HU, but the fraction was smaller (*11*). Longer exposures revealed additional bands that appear to be fusions of the breakage products (*11*).

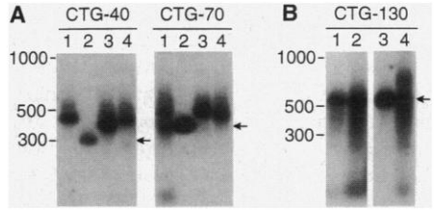

Fig. 3. Decrease in CTG tract stability by *RAD27* deletion. Genomic DNA was prepared from *rad27Δ* strains of the indicated tract length, digested with Eco RI and Hind III, separated on a 1% agarose gel, blotted to nylon membrane, and hybridized to a CTG probe. Size markers are given in base pairs. (**A**) Three representative expansions of the CTG-40 and CTG-70 tracts in *rad27Δ* strains are shown. In each panel, lane 2 is the CTG tract from wild-type strains (arrows). Estimated tract sizes are 83, 70, and 77 repeats for the CTG-40 expansions (lanes 1, 3, and 4), and 100, 100, and 83 repeats for the CTG-70 expansions (lanes 1, 3, and 4). For CTG-70, lane 1 shows a mixed population in which about half of the cells had an expansion and about half did not. (**B**) DNA was prepared from *rad27Δ* CTG-130 strains grown at 25°C (lane 1) or 30°C (lanes 2 and 4). Lane 3 contains DNA from a wild-type CTG-130 strain grown at 30°C. The expanded tract in lane 4 is ~177 repeats. The spot of low-molecular-weight hybridization at the bottom of the gel for the *rad27Δ* strains grown at 30°C (lanes 2 and 4) is probably due to degradation of the tract, because it was also present in undigested DNA (*11*).

Table 1. Increased frequency of expansions and contractions in a *rad27Δ* strain. PCR (*20*) was performed on individual colonies to determine the percentage of expanded, contracted, or unchanged tract lengths for both CTG-40 and CTG-70 strains in wild-type or *rad27Δ* backgrounds after growth at 30°C. Expansion or contraction was defined as having ≥30% of the tract expanded or shortened, respectively. Because cells grew for ~25 generations, a minimum estimate of the expansion rate for the *rad27Δ* CTG-70 strain is 10^{-2} per colony per generation. Examples of expansions are shown in Fig. 3.

Strain	Number of colonies examined	Expansion (%)	Contraction (%)	Unchanged (%)
Wild-type CTG-40	200	0.5	1	98.5
rad27Δ CTG-40	100	18	10	72
Wild-type CTG-70	104	0	6	94
rad27Δ CTG-70	46	33	46	22

endonuclease), is involved in processing the 5′ ends of Okazaki fragments (*19*). Comparison of wild-type and *rad27Δ* strains by means of the direct repeat recombination assay suggested an increase in the frequency of generating FOA^R colonies in the *rad27Δ* background (*11*). To assess tract length quantitatively, tract size was determined with the polymerase chain reaction (PCR) in individual colonies grown at 30°C from both wild-type and *rad27Δ* strains carrying either a CTG-40 or CTG-70 tract (Table 1) (*15*, *20*) and was confirmed by Southern hybridization in a subset of colonies (Fig. 3A) (*20*). Expansions and contractions were rare in the wild-type background. However, in the *rad27Δ* strains, expansions and contractions were frequent (Table 1; see Fig. 3A for six examples). Compared to the wild type, the frequency of expansions and contractions of CTG-40 in the *rad27Δ* strain were 36- and 10-fold greater, respectively. For CTG-70, the expansion frequency was at least 33-fold greater and the contraction frequency was 8-fold greater. Expansions were also detected in a CTG-130 *rad27Δ* strain by Southern hybridization (Fig. 3B, lane 4), but the frequency was not determined because tracts longer than CTG-130 were difficult to amplify with PCR.

The instability of the CTG tract in *rad27Δ* strains increased with increasing tract length, because the CTG-hybridizing bands became more heterogeneous in length as tract size increased (Fig. 3) (*11*). In addition, the instability of CTG tracts in the *rad27Δ* strain increased with higher temperature: For CTG-130 at 25°C there was little change in tract length (Fig. 3B, lane 1), whereas tract heterogeneity was evident at 30°C (Fig. 3B, lanes 2 and 4). The increased frequency of expansions and contractions seen in *rad27Δ* cells was specific, because large or frequent expansions (or both) were not observed when other genes involved in DNA metabolism such as *MLH1* (mismatch repair), *REV3* (repair polymerase), or *RAD1*, *RAD50*, *RAD51*, or *RAD52* genes (recombination) were mutated (*11*). Because Rad27p is normally involved in processing the 5′ ends of Okazaki fragments, these data suggest that unprocessed Okazaki fragments can lead to tract expansion and contraction. Extension at the 3′ end of an Okazaki fragment is predicted to displace the 5′ end of the adjacent Okazaki fragment to generate a 5′ single-stranded tail or flap that is normally cleaved by Rad27p (*19*). A flap of CTG repeats could fold into a hairpin structure, which might then be ligated to the 3′ end of the preceding Okazaki fragment to create an expansion (*21*).

Both genetic and physical assays indicated that CTG tracts are length-dependent sites of breakage in yeast and that a condition that slows DNA replication (the presence of HU) increased fragility. We have identified a mutation (*rad27Δ*) and a condition (transformation) that allow the recovery of large and homogeneous expansions of CTG repeats in yeast, similar in size to disease-causing expansions in humans. The increased frequency of CTG tract expansion in a strain lacking Rad27p suggests that humans with mutations in the *RAD27* homolog *FEN1* might be predisposed to expansion of TNRs, because *FEN1* is highly conserved between yeast and humans (*22*). The recombination assay described here could be used to test other sequences, conditions, or gene products suspected of inducing chromosome fragility or TNR expansion.

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10. *Saccharomyces* strain YPH500L CTG-130, described in (*8*), contains the *URA3* gene and CTG-130 tract replacing most of the *LYS2* coding sequence. YPH500L CTG-130 was transformed with a Pvu I–Pvu II fragment of plasmid pSK1 containing the *URA3* gene 3′ to the *LYS2* gene. Transformants in which the 5′ *LYS2* fragment had been replaced with the entire *LYS2* gene were selected on complete defined medium lacking lysine (YC-Lys) media, to generate strains with the 700-bp 3′ portion of the *LYS2* sequence repeated on either side of *URA3*-CTG. Lithium acetate transformations were done as in (*23*). DNA was prepared from Lys^+ transformants and analyzed by Southern blots for correct replacement and CTG tract length. Expansions must have occurred during or soon after transformation, because the vast majority of cells in the transformation colony had the expansion (Fig. 1B). Although we were unable to identify any single parameter of the transformation protocol that enhanced expansion, expansions were obtained in three of five independent transformations.
11. C. H. Freudenreich, S. M. Kantrow, V. A. Zakian, data not shown.
12. For fluctuation analyses, cells were plated for single colonies on YC-Lys plates or YC-Lys plates containing 0.1 M HU. Colonies (13 for wild-type experiments 1 and 2 and the HU experiment; 10 for all others) were cored out with a Pasteur pipette and resuspended in water, and a portion was plated on FOA plates. Rates of FOA^R colony formation were determined by the method of the median (*24*). Values from starting colonies that had shortened tracts, as determined by Southern hybridization, were dropped.
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15. The *RAD50*, *RAD52*, and *RAD1* genes were deleted from the CTG-tract or control strains by homologous recombination using PCR-generated fragments of the *HIS3* gene flanked by sequences 5′ and 3′ to the respective gene (*25*). *RAD27* was deleted with plasmid pMRrad27ΔTRP1 (*26*). Transformants were selected on YC-His (grown at 30°C) or YC-Trp (grown at 25°C) and checked for correct insertion and deletion by Southern hybridization. *rad27Δ* cells are viable at 25°C, grow more slowly than wild-type cells at 30°C, and, in at least some strain backgrounds, arrest in late S/G_2 when grown at 37°C (*26*, *27*). Unless otherwise indicated, *rad27Δ* strains were constructed and maintained at 25°C to minimize the *rad27Δ* phenotype.
16. For pulsed-field gel analysis, isogenic strains lacking a CTG tract or with the CTG-250 tract were grown to early log phase in YC-Ura media to maintain the tract, then switched to complete media containing 0.05 M HU and grown for about two more doublings (~18 hours). Cells were harvested, and chromosomes were prepared as in (*28*) and separated in a Bio-Rad apparatus. The 355- and 470-kb breakage products were observed in three of three experiments.
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