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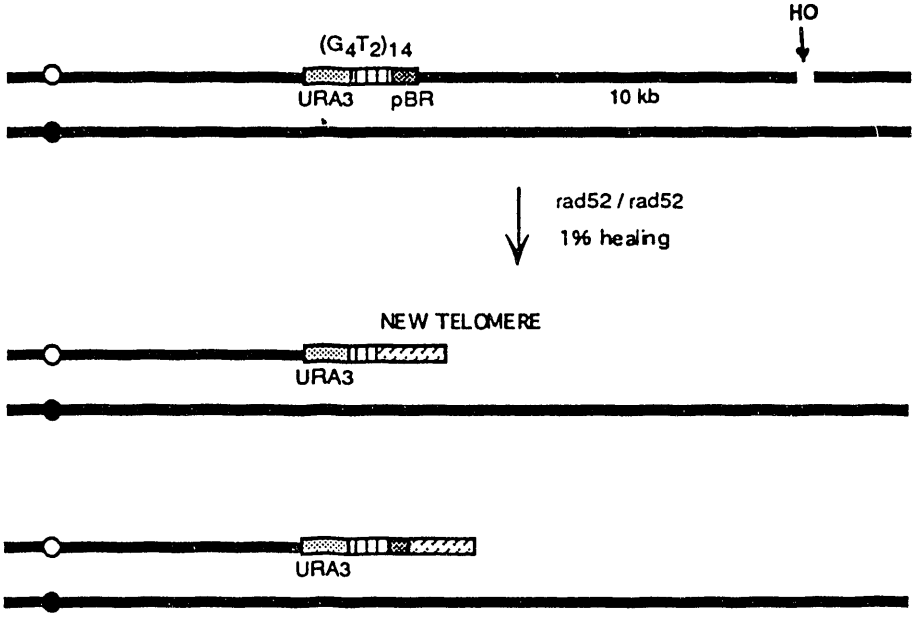
Broken chromosomes must either be repaired or lost. The break separates part of the chromosome, containing a telomere, from the rest, containing a centromere. While the centromere-containing fragment can properly segregate, the broken end will be progressive y deg.aded. The acentric fragment cannot segregate and will also be degraded. In diploids, the loss of a chromosome to produce a monosomic cell can (sometimes) be tolerated. Broken chromosomes are most .eadily repaired by homologous recombination, in which the broken end(s) recombine with the intact homologous chromosome. If recombination is prevented, chromosomes can also be repaired in other ways.

We have centered our attention on two alternative non-homologous mechanisms of repair: 1) the acquisition of a new telomere, and 2) repair of broken chromosomes by non-homologous joining of broken chromosome ends. In both cases, we create a double-strand break at a defined chromosomal location in yeast cells. The break is created by the site-specific HO endonuclease in cells that carry the *rad52* mutation to prevent repair of a double-strand break by homologous recombination. In diploid cells, we can recover cells that contain a terminally deleted, "healed" chromosome that has acquired a new telomere. In haploid cells, we can recover cells in which the double-strand break has been repaired by re-joining the broken ends, usually accompanied by a deletion.

I. De novo telomere formation.

The procedure we have used to recover terminally deleted chromosomes is illustrated below:



The double-strand break is created at the mating-type locus *MAT*, using a galactose-inducible HO endonuclease gene, by placing cells on galactose medium. Approximately 10 kb more proximal to the centromere, we have inserted a set of sequences to act as a possible "default location" for new telomere formation. At this location are the URA3 gene, which must be maintained during the selection, 13 repeats of G_4T_2 sequences and a small segment of pBR322. Previous studies have shown that the G_4T_2 sequences, the telomere sequences from Tetrahymena, can be used as a site to which the more variable $G_{1\text{-}3}T$ sequences of Saccharomyces can be added. Terminally deleted chromosomes are the predominant type of diploids that are Ura^+ and Thr^-. Those that carry terminal deletions are readily identified by CHEF gel electrophoresis, in which one can see a new chromosome band smaller than chromosome I.

DISCLAIMER

Southern blot analysis of these terminally deficient, healed chromosomes showed that they had all acquired new telomeres and that the healing events were all very close to or within the G_4T_2 sequences adjacent to *URA3*. This event is not very efficient; only between 0.2% and 1% of the cells repair the chromosome in this way; the rest of the cells lose the broken chromosome. (There seems to be some strain dependence in the actual frequency of these healing events). When the same experiment was done in a strain that was identical except that the insert had only *URA3* and pBR322 sequences, there were no healing events. This means that one cannot recover new telomeres at random chromosomal sites, but rather they must be added near some internal telomere-like sequence. However, further restriction site analysis of these healed chromosomes showed that in approximately half of the cases, the new telomere sequences were added at some distance from the G_4T_2 sequences (a BamH1 site between the G_4T_2 repeats and the pBR322 sequences was retained).

These results confirm previous studies by Blackburn and Szostak's labs that new telomere sequences could be added at non-telomeric locations near G_4T_2 repeats. However in our case we have shown that these events can occur not with transformed, linearized DNA but with broken chromosomes that have their normal chromatin structure and when the break is very far (10 kb) from the eventual site of joining. When we place an artificial HO cut site closer (1 kb away) to the inserted "default" sequences, the efficiency of recovering repaired chromosomes with a new telomere rises from about 0.2% to 2%.

Further restriction site analysis and DNA sequencing (see below) showed that there were several distinct sites, near but not within the G_4T_2 repeats, to which new telomeres could be added (see Fig. 1A). This is in agreement with several previous studies that examined new telomere formation on tranformed DNA; but in none of the previous studies were such newly formed telomeres actually sequenced. We have undertaken that analysis by ligating an oligonucleotide onto the T4 exonuclease-treated yeast DNA (to "blunt-end" the telomeric DNA) and then isolating the newly formed telomere by PCR amplification of the region between the *URA3* gene and the new telomere sequences. The results of this analysis are shown in Fig. 2. We find that new telomere sequences containing the yeast $G_{1\text{-}3}T$ repeats can be added to G,T, rich sequences, the longest of which is 9 bp (GGGTGTGGT) and the shortest of which is a single GT pair.

We believe that these data and those discussed below make it highly unlikely that new telomere sequences are being added by some *RAD52*-independent recombination mechanism. First, the very short GG or GT or GGGTG sequences at which new telomeres are added are too short to be used as recombination substrates. The 9 bp region is also likely to be much too short to be used for standard, homologous recombination. Second, such G,T segments occur at many locations along the 10 kb interval between the break and the sites of healing. Healing events depend on the nearby presence of telomere-like sequences even though the addition occurs at a distance. This result would be expected for a telomerase, which can in some way recognize telomere regions, but is not a feature expected for recombination. A third, compelling argument against recombination comes from an inspection of the healing events themselves.

Despite a great deal of work on yeast telomeres, there is yet no direct evidence that new telomeres in yeast are added by the kind of RNA-dependent DNA polymerase (telomerase) that has been documented in ciliates and in humans. Experiments in vitro with Tetrahymena telomerase show that it can add new sequences at a distance from G_4T_2 repeats, as we have seen. But what remains a mystery is how a template-directed telomerase can add a variable $G_{1\text{-}3}T$ sequence instead of a regular G,T array. One experiment with human telomerase suggests that telomerase template RNA can be modified to create mistakes in telomere addition. Whether yeast telomerase is designed to make many "mistakes" is unclear. An alternative is that yeast has many different template RNAs that can associate with telomerase so that each short stretch of telomere is added by a different template. Our data suggest that the way in which the telomerase first associates (base pairs?) with the site of addition is primer-dependent, so that particular sequences are preferentially added to some sites of new telomere addition.

Among the healing events at the GGGTGTGGT site are three classes: A) three events appear to have occurred at the end of this segment. In these three cases the sequences that are added are all normal $G_{1\text{-}3}T$ sequences, but unrelated to each other. This is what has been seen for sequences directly added to G_4T_2 repeats after transformation of linearized DNA. B) One event apparently involved "chewing back" most on the segment to add to the last first two GG. C) five events appear to have added new telomere sequence onto the end of this sequence, after removing the terminal T. What is striking about all five of these independent events is that the first 11 nucleotides that were added are identical (GTGTGGGTGTG), after which the sequences diverge from each other to the unpredictable $G_{1\text{-}3}T$ pattern seen with the other new telomeres. This is a striking result, that suggests that some sequences may act as primers for new telomere addition by "setting the frame" of the template, so that the next sequences that are added are always the same. One must presume, then, that the sequences added do not, in turn, oblige the telomerase to continue to add "in frame."

We have searched the database for the same GGGTGTGG sequence that is used here as a preferential site; we find no evidence that this sequence must be followed by GTGTGGGTGTG, as this sequence is found adjacent to the first sequence only 3/48 times. The infrequency in the data base with which the conserved added sequence is found following the addition site also argues against a recombinational addition of these sequences.

To explore this matter further, we have carried out a series of similar analyses, but varying the sequences in the 100 bp adjacent to the G_4T_2 segment. We have created three more such sequences, illustrated in Figure 1 and 3. In construct BX1, the circularly permuted sequence GGTGGGTGT was inserted in place of as the preferentially used GGGTGTGGT sequence discussed above. This original preferred site was inserted approximately 90 bp away from the G_4T_2 sequences, to see if it would be used preferentially when it was further away. In addition, we also included two repeats of the human telomere sequence TTAGGG as a possible addition site. As seen in Figure 3A, among 13 healing events analyzed, three used this same sequence at its more distant location. One retained the distal T and proved to be identical to one of the previous examples (class A, Figure 2) for the first 12 bp. The two other new telomeres were apparently added after the removal of the distal T (as in class B, Figure 2); in both cases the same GTGTGGGTGTG was added as before, after which the sequences again diverged from each other and the previous examples. This result substantiates the conclusion that there is a highly preferential addition of the first 12 bp of new telomere sequence when the primer sequence is GGGTGTGG.

When we look at the other 10 healing events with construct BX1, four of them are added to the complete circularly permuted site. However, in this case, there is no distinctive order to the first 10 nucleotides added. There were also two additions at the ends of either the first or second TTAGGG repeat. In both of these cases, exactly the same first 20 nucleotides were added before the sequences diverged.

In construct BX2 (Figure 3B), the original preferred site was replaced by TGGTGGTGG (a $G_{1\text{-}3}T$ sequence that is in fact never found within authentic telomeres). This sequence was preferentially used as a site for new telomere addition, although the overall proportion of healing events in the sequences distal to G_4T_2 (as opposed to with the Tetrahymena sequences) dropped from nearly 50% to 35%. Surprisingly, of eight new telomeres added to the end of this segment, three had the same GTGTGGGTGTG sequence that was exclusively added to GGGTGTGG, though here the addition site is TGGTGGTGG. The remaining five events at this site also had a perfectly conserved 13 bp sequence before the $G_{1\text{-}3}T$ sequences diverged from each other: GTGTGTGGGTGTG.
This sequence is identical to the first conserved, added sequence except for the addition of an additional GT at the beginning. We also note that one of the remaining sequences added in BX2 had apparently "trimmed back" TGGTGGTGG to TGGTGG and then added the same GTGTGGGTGTG sequence we have noted above.

In construct BX3 (Figure 3C), the preferential site of addition was replaced by another circularly permuted version: TGTGGTGGG. Surprisingly, new telomeres inserted distal to G_4T_2 were the least frequent, about 25%. Nevertheless, of 9 events we sequenced, seven were at or immediately adjacent to the 9 bp G,T sequence. However, four of these events actually added to a G separated by a C from the TGTGGTGGG sequence. In these four cases, there was no fixed order to the first 10-13 nt added. Among the remaining three healing events, two of the three have the same first 10 nt: TGTGGGTGTG, which would be identical to the 11 bp conserved sequence GTGTGGGTGTG if the addition site had experienced the removal of a G before the new sequences were added.

We interpret these results to indicate that telomerase can add new telomere sequences to very short G,T regions, but only if there is a longer, telomere-like region lying more proximally and within about 100 bp. Obviously, there are many short G,T rich sequences in the 10 kb intervening between the cut end of the DNA and the place where healing occurs, yet, by themselves, they are insufficient to facilitate new telomere formation. The requirement for this internal telomere-like region can be explained in several ways. First, it is possible that telomere addition requires exonucleolytic digestion of the broken DNA until the appropriate sequences are exposed. Physical monitoring and genetic experiments we have carried out argue that there is extensive 5' to 3' degradation, but little if any 3' to 5' degradation of HO-cleaved DNA. This degradation proceeds in both directions from the HO cut in rad52 strains; moreover, we have observed that degradation can continue for more than 10 kb, leaving a long 3' ended single strand. Perhaps some exonuclease digestion must be slowed or stopped before new telomere addition is possible. Perhaps, too, that proteins binding to the G_4T_2 repeats act to retard the exonuclease and thus allow new telomere formation in its vicinity, at any G,T-containing sequence. Alternatively, perhaps the telomerase must both bind to a telomere-like region itself and then independently locate a primer sequence to which it can begin adding new $G_{1\text{-}3}T$ sequences. In either case, many aspects of this process are unexplained. For example, if degradation is primarily by 5' to 3' exonuclease, how is the second, single strand discarded before new telomere formation occurs?

Several laboratories have recently suggested that the protein Rap1 plays a very important role in maintenance of telomere length and perhaps in new telomere formation. When we inspect the sequences that are added, we find that there are, at random intervals, Rap1 binding sites (GGTGTGTGGGTGT) in the new telomere sequences. This site does not exist at the sites that are preferentially used to create new telomeres, but a Rap1 binding site is created at the junction of the new sequences that are added in BX2-AC3,AC5, BB4, BB5 and CC1A. There is no such site within or overlapping the most frequently added sequence GTGTGGGTGTG .

There has also been a recent report of the purification of protein in yeast that binds preferentially to the human telomere repeat sequence, TTAGGG. This site was used 2/13 times in BX1. Whether the TTAGGG-binding protein is required for those additions is not known and would be difficult to determine, as the protein is essential.

Our finding that new telomeres can be created within approximately 100 bp of the Tetrahymena repeats shows that the kinds of healing events that have been observed with transformed, naked DNA into yeast also occur when a chromatin-containing chromosome is broken and repaired. Moreover, healing can occur at a significant distance from the site of cleavage. Perhaps most significantly, we find that the sequences that are added are highly biased by the primer sequence to which they are added. At many sites, especially those that are short (1-5 bp) there is no special order to the sequences that are added, though one can make some alignments if one assumes that the site of actual addition of new telomeres was within some of these G,T segments. But for the addition sites that had longer stretches of G,T (or TTAGGG) there does seem to be a highly restricted set of sequences that are first added. After the first 11-13 (or in once case 20 bp) that are added, each telomere sequence diverges. The predominant sequences added to these different sites of addition are summarized in Figure 4.

We believe that these results can be interpreted in terms of the way an RNA-dependent DNA polymerase can add new telomere sequences in Saccharomyces. First, some arrangements of $G_{1-3}T$ establish a "frame" in which the new sequences added are always the same for 11-13 bp. Other permutations of $G_{1-3}T$ do not have this property. In fact, half of the uses of TGTGGTGGG proved to be additions to the adjacent CG. TTAGGG also seems to have the property of dictating what sequences should follow, based on two examples both of which are surprisingly the same for the first 20 nt. Obviously for this last case the additions are highly unlikely to have arisen by any sort of recombination mechanism, given that TTAGGG is not found immediately adjacent to yeast telomeric sequences.

We are still working on an explicit model for the way telomerase acts in these situations, but our general conclusion is that yeast telomerase has one or more RNA templates that are either degenerate or highly flexible. When presented with Tetrahymena sequences onto which new sequences are to be added, no particular "frame" of the template is selected. But some permutations of TG,TGG,TGGG apparently align the telomerase RNA in such a way that the next sequences are dictated. However, when the same or another telomerase then elongates this newly added (GT)GTGTGGGTGTG sequence there is apparently no such restriction. Were yeast telomerase RNA analogous to Tetrahymena template RNA, this would mean that the bases 5' to those actually used as template do not align perfectly with the sequences that were just added. From that point on, the characteristically variable $G_{1-3}T$ is added (though in our view the consensus sequence for what is added is $(TG)_{1-6}TG_{2-3}$).

Our prediction is that the telomerase RNA will contain the sequence complementary GTGTGTGGGTGTG. It is possible that the variability is all generated from this sequence by the kind of slippage that Blackburn's group has generated in Tetrahymena by inserting an extra A into the template sequence. The same distortion could have arisen by an ancient alteration of the telomerase protein binding to a fixed template.

We will attempt to look for telomerase RNA among the small nuclear RNA population by using several versions of the consensus and adjacent sequences as oligonucleotide probes.

II. Deletion formation by non-homologous joining of broken chromosome ends.

By using haploid strains that are unable to repair a DSB at *MAT* by homologous recombination with its donors, we can recover strains that have repaired the DSB by nonhomologous means. We have used three different approaches. First, we have used *HO rad52 swi1 MAT*α strains in which HO expression occurs only rarely, but those cell that attempt to switch will die. At a frequency of about 1% these cells "throw off" sterile derivatives that have been shown to be deletions of part of the *MAT*α1 gene. More rarely, these strains also give rise to a-like maters, which are larger deletions that remove both *MAT*α1 and *MAT*α2. Alternatively, we have used *rad52* strains carrying a galactose-inducible HO gene and recovered survivors that were either sterile or a-like. Finally, we have used Rad^+ and Rad^- "donorless" strains either carrying the galactose inducible gene or meiotic spore colonies carrying the normally expressed HO gene. In these cells, the DSB cannot be repaired because the homologous donor sequences at *HML* and *HMR* have been removed. Repair is not efficient; between 0.2% to 2% of cells are recovered in this way and the rest die. The differences may be strain specific.

To analyze the sterile (*mat*α1) and a-like (*mat*α1 *mat*α2) DNA in the *MAT* region, we have used PCR amplification and DNA sequencing of the amplified product. For all three methods of selecting deletions, the sizes of deletions were variable and almost all of them had different end-points. If one imagines that the sequences were re-joined by base-pairing of annealed 3' ended single strands, one finds that most of the deletions have very limited amounts of possible complementarity at the joints. An example is shown in Figure 5 and the complete results are presented in Table 1. In the first set we examined (Strain BW330; Table 1A), deletions ranged in size from 26 bp to 697 bp. Most of these deletions exhibit only 1-2 bp of possible complementarity out of the 10 bp flanking the junction point; five have only 2 possible base pairs, three have 3 possible base pairs and one has 6/12.

The results from the *rad52* donorless strains are similar, except that the sizes of the deletions were significantly smaller (17-34 bp) (Figure 5B and 5C). Possible the size of the deletion correlates with the

level of expression of HO endonuclease, but it may be a strain difference. We are still examining this question. Interestingly, when we examined a set of deletions from an isogenic donorless strain that was Rad$^+$, we found that 6/6 deletions were very small (1-3 bp). Whether there is a different mechanism in Rad$^+$ cells remains to be established. We also recovered a few additions of sequence that can be explained if the ends of the HO-cleaved DNA are partly "filled in" before ligation takes place.

These results demonstrate that yeast, like mammals, harbors a mechanism to repair chromosome breaks by the formation of nonhomologous deletions. Very similar results for both mammalian cells and *S. pombe*, a distantly related fungus, were reported at the EMBO meeting on recombination last summer.

Comparison of repair of DSB created by HO cleavage and those created by mechanical stress.

A decade ago we demonstrated that yeast cells carrying a tandemly duplicated dicentric linear chromosome would undergo apparent chromosome breakage and rearrangement (by homologous recombination). Since that time several labs have shown that one can construct chromosomes with a conditional centromere that is "off" when the region is strongly transcribed and functional when transcription ceases. Thus it is possible to generate a population of dicentric chromosomes and study their breakage and healing. We have collaborated with Dr. Kerry Bloom (Univ. of North Carolina) to compare the kinds of non-homologous deletions created by HO with those near centromere regions in *rad52* strains. Bloom's lab isolated a number of strains that contained deletions of some or all of the conditional centromere (which is entirely contained in a 900 bp BamH1 fragment). We have used PCR amplification and DNA sequencing to examine the deletions that were created in this way (Table 2). To our surprise, all of the deletions were large (233-555 bp) and all of them removed the entire centromere region (elements I,II and III) even though removing a small part of element III would be sufficient to eliminate centromere function and abolish the dicentric condition. The deletions tend to remove an equal amount of DNA on either side of the three *CEN* elements.

When we inspect the junctions of these deletions, they show the same low degree of possible overlapping complementarity that was found at *MAT* (Table 2).

For completeness, we are isolating *MAT* deletions in this same strain, to be certain that the sizes of the deletions are indeed bigger at the conditional *CEN*. This result raises an interesting possibility that we wish to pursue. It has generally been assume that dicentric chromosomes actually break - pulled apart by opposing centromere movement. However, a consideration of the tensile strength of DNA and the possible forces that can be exerted on a centromere by the single microtubule to which it is attached raises the possibility that the chromosome cannot actually be "snapped." If this is the case, then chromosome breakage might in fact be mediated by a nuclease that attacks DNA that has been tightly stretched. This might explain why the entire centromere region is removed. We are designing experiments to investigate this question further. For example if one uses genetic and physical tests to monitor the location of the "breaks" in the interval between the two centromeres, are they clustered very close to the two *CEN*s or can they arise anywhere in the 40 kb interval?

Future research

We are continuing these studies. In addition, however, our results have also prompted us to think about an alternative way to look at the way true mechanical breaks in DNA are repaired. For this purpose we wish to look at the repair of DSB created by ionizing radiation. The design of the experiment is relatively simple: we will use a rad52 strain that carries an XhoI linker insertion in the *MAT*α2 gene. A *mat*α2::XhoI *MAT*α1 strain is sterile. This strain will be irradiated and survivors will be mated to a *MAT*α tester strain to select for diploids by complementing markers. Only a strain that has mutated *MAT*α1 to *mat*α1 will be able to mate, as it will be a-like in its mating behavior (*mat*α2::XhoI matα1). We will then analyze the mutations in the matα1 region by selectively amplifying this segment, using a PCR primer that is specific for the XhoI-containing DNA and a primer that is distal to *MAT*. Thus, we will have a set of X-ray induced deletions with which to compare HO- induced and *CEN*-induced breaks. The construction of appropriate strains is now beginning.

Table 1. **Non-homologous deletion repair events after HO-induced cleavage at *MAT*, when there is no homologous repair of the *MAT* gene. Sizes of deletions are indicated under the name of the derivative(s). Small deletions of *MATα* produce *matα*1 (sterile) derivtives. Larger deletions such as B17 and A3 delete both *MATα*1 and *MATα*2 and create a-like cells.**

A. BW STRAIN 330 DELETIONS (*HO MATα rad52 swi1*)

A5/BW9	AATGCAGC	ACGGAATA	G30	GTCTTGTC	TTCTCTGC
62 bp	AATGCAGC	AGAGTGGT	162 bp	GTCTTGTC	TGTACATT
	GGTTTTGT	AGAGTGGT		CTACACAA	TGTACATT
D2/BW1	GCTGAAGA	ATGGCACG	F32	GCTTTTAG	AGCATATT
139 bp	GCTGAAGA	TGTGTTTG	284 bp	GCTTTTAG	AGCTATCC
	TGGGAAGA	TGTGTTTG		CAGTCATA	AGCTATCC
B28/BW3	TCTCTGCT	CGCTGAAG	A3	CTACTTCTT	TTAACCT
547 bp	TCTCTGCT	ACCTTCGG	655 bp	CTACTTCTT	CCTATTT
	TGCTAGTT	ACCTTCGG		ATATCGATT	CCTATTT
B17/BW6	ATGTTTCA	AAACATTA	B13	CTGCTCGC	TGAAGAAT
697 bp	ATGTTTCA	TATAGAGT	173 bp	CTGCTCGC	TGGTCGTG
	TTTGGCCT	TATAGAGT		ATAGAGTG	TGGTCGTG
D11	TTGGCTATA	CGGGACGG			
486 bp	TTGGCTATA	AACAAAAT			
	GGCCACATA	AACAAAAT			

B. Strain KK411 (*HO hmrΔ hmlΔ MATα* Rad$^+$) and KK412 (*HO hmrΔ hmlΔ MATα rad52*)

411-1	CTTCGCGC	AACAGTAT	411-13	ACTTCGCG	CAACAGTA
1 bp	CTTCGCGC	ACAGTATA	411-18	ACTTCGCG	AACAGTAT
	TTCGCGCA	ACAGTATA	1 bp	CTTCGCGC	AACAGTAT
411-7	CTTCGCGC	AACAGTAT	421-1	ACTTCGCG	CAACAGTA
411-15	CTTCGCAC	AGTATAAT	3 bp	ACTTCGCG	CAGTATAA
3 bp	CGCGCAAC	AGTATAAT		TCGCGCAA	CAGTATAA
411-12	TACTTCGC	GCAACAGT	421-6	TATGGGAC	TACTTCGC
2 bp	TACTTCGC	AACAGTAT	17 bp	TATGGGAC	TAATTTTA
	CTTCGCGC	AACAGTAT		CAACAGTA	TAATTTTA
421-7	ACTACTTC	GCGCAACA	421-8	ATGGGACT	ACTTCGCG
25 bp	ACTACTTC	CTGGTTTT	30 bp	ATGGGACT	CTGGTTTT
	TATAAACC	CTGGTTTT		TATAAACC	CTGGTTTT
421-19	CAGCACGG	AATATGGG			
421-20	CAGCACGG	TATAAACC			
34 bp	TATAATTT	TATAAACC			

Table 1, cont.

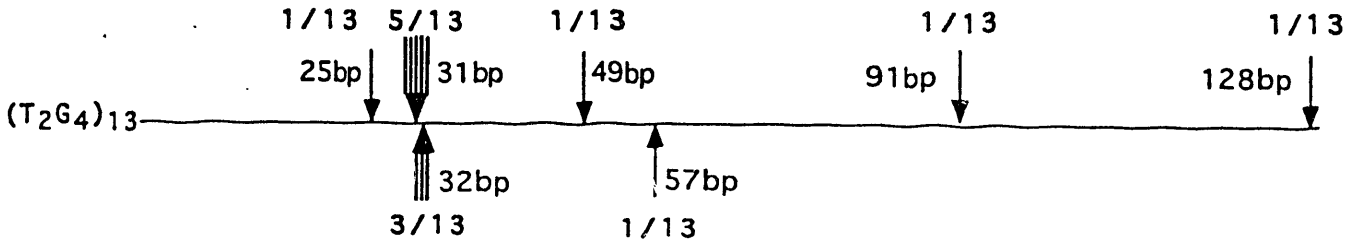
C. XW157 (*HO hmrΔ hmlΔ MAT***a** Rad^{+})

A3	AAATAAAC	GTATGAGA	a2	CTTTCCGC	AACAGTAA
81 bp	AAATAAAC	GTATAATT	18 bp	CTTTCCGC	AACCCTGG
	CCGCAAGA	GTATAATT		STTTTATA	AACCCTGG

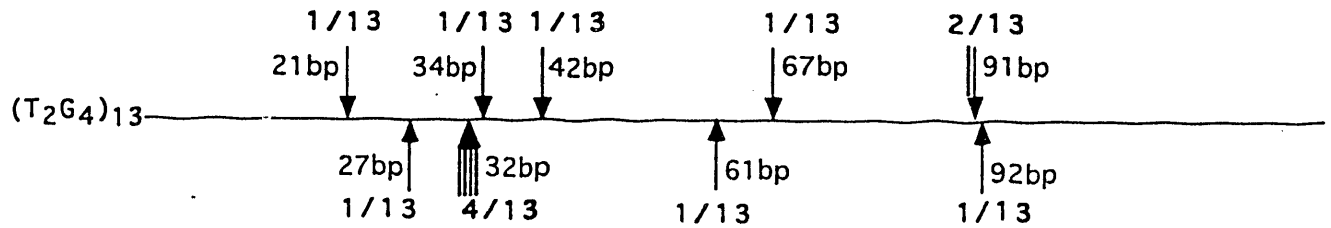
Table 2. Deletions that delete a conditional (galactose-regulated) CEN3 sequence inserted near HIS4 on chromosome III. When cells are shifted from galactose to glucose, this conditional centromere becomes active and creates a dicentric chromosome. In a *rad52* strain, most cells die under this circumstance. Survivors have small deletions that remove at least part of the centromere. In all cases examined, the deletions removed all of the three main elements of the centromere (I, II, and III) even though a small deletion of element III (the region closest to the other centromere and presumably closest to any chromosome break) should be sufficient to rescue the chromosome.

7.03	TATACTTT	AACGTCAA
234 bp	TATACTTT	AATGGTAT
	TTTCATTG	AATGGTAT
7.04	GTACAAAT	AAGTCACA
233 bp	GTACAAAT	CAACTTAA
	TGGTAAAG	CAACTTAA
7.06	TCTTATTC	AAATGTAA
349 bp	TCTTATTC	AAACAAAA
	GAAGTAAT	AAAGAAAA
7.19	TTCTTATT	CAAATGTA
369 bp	TTCTTATT	CAACTTAA
	TGGTAAAG	CAACTTAA
7.28	CATAACCA	CTTTAACT
382 bp	CATAACCA	CAATAGAA
	TTATTATT	CAATAGAA
7.40	CAAATTAA	CAACCATA
555 bp	CAAATTAA	TTGAAAAA
	GGTAATGA	TTGAAAAA
7.54	ACAAAAAA	TTGTTAAT
7.59	ACAAAAAA	TTGGTAAA
331 bp	AGAAAAAG	TTGGTAAA
7.61	TATACC	TCTATAC
293 bp	TATACC	TCAATAG
	TATTAT	TCAATAG
7.64	TTTGTATT	ACTTCTTA
385 bp	TTTGTATT	ACAGTAAA
	GCAACTTA	ACAGTAAA

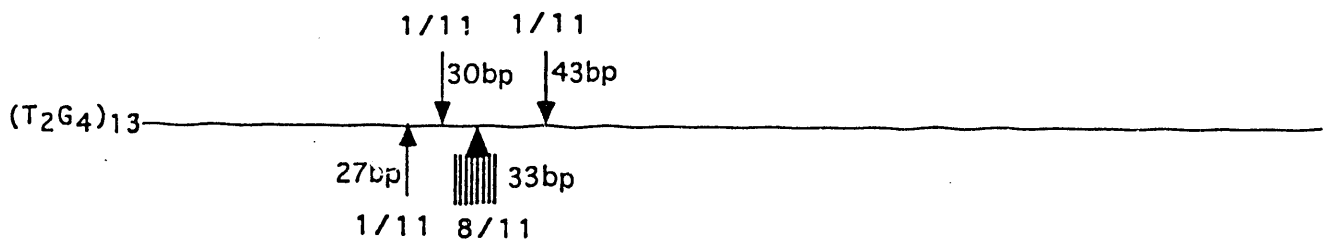
A. BX healing events



B. BX1 healing events



C. BX2 healing events



D. BX3 healing events

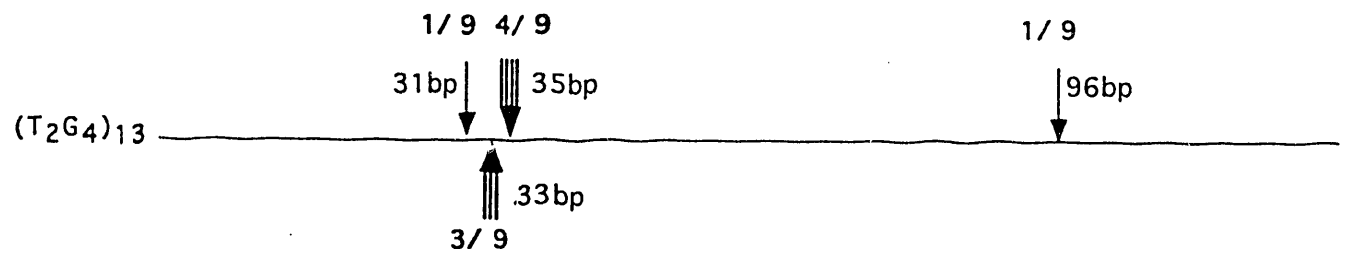


Figure 1. Position of healing events that occur at some distance from the $(T_2G_4)_{13}$ repeats. The number of independent healing events in each case are shown.

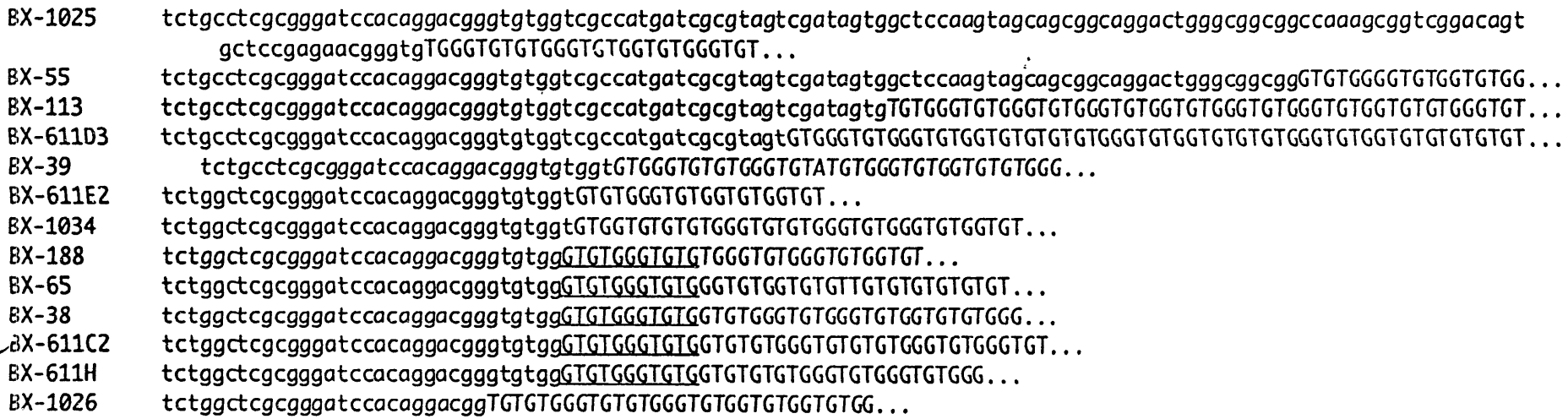


Figure 2. New telomere DNA added at a distance from the G4T2 repeats. the original DNA sequence in this region is shown in lower case letters. New telomere sequence is shown in capital letters. A highly ordered sequence that is added on to one particular primer is underlined.


A
BX1-40 aaagagatcgatagcgagcttagggttagggagcgccatgcctcaacgggtgtggtGTGTGGGTGTGGGTGTGGGTGT..
BX1-75 aaagagatcgatagcgagcttagggttagggagcgccatgcctcaacgggtgtggGTGTGGGTGTGGGTGTGGTGT...
BX1-32 aaagagatcgatagcgagcttagggttagggagcgccatgcctcaacgggtgtggGTGTGGGTGTGTGTGTGGGTGTG..
BX1- 7 aaagagatcgatagcgagcttagggttagggTGTGTGGGTGTGGTGTGTGGTGTGTGT..
BX1-64 aaagagatcgatagcgagcttagggTGTGTGGGTGTGGTGTGTGGGTGTGGTGTGGG...
BX1-70 ggatccactggacggtgggtgtcgccaaagagTGTGTGGGTGTGGGTGTGGTGTGGGT..
BX1-47 ggatccactggacggtgggtgtcgTGTGGGTGTGGTGTGTGTGTGGGTGT...
BX1-53 ggatccactggacggtgggtgtGGGTGTGGGTGTGGGTGTGTGTGTGGGTGT...
BX1-60 ggatccactggacggtgggtgtGGGTGTGTGGGTGTGGGTGTGGTGTGTGGG...
BX1-65 ggatccactggacggtgggtgtGGTGTGTGTGGGTGTGGGTGTGGTGTGGG...
BX1-55 ggatccactggacggtgggtgtGTGGGTGTGGTGTGTGGGTGT...
X1-52 ggatccactggacggtgTGGGTGTGTGGGTGTGGGTGTGGGTGT...
X1-49 ggatccactggTGTGTGGGTGTGGGTGTGGGTGTGGTGTGGGTGT...
B
BX2-BC1 ggatccacaggactggtggtggcgcaatctagTGTGTGTGGGTGTGGGTGTGGTGTGTGGGTGTGGTGTGGGTGTGGTGTGGGTGT...
BX2-AC3 ggatccacaggactggtggtggGTGTGTGGGTGTGTGGGTGTGTGGGTGTGGGTTTGGGTGTGGTGTGTGGTGTGG-URA3
BX2-AC5 ggatccacaggactggtggtggGTGTGTGGGTGTGGTGTGGGTGTGTGTGTGGGTGTGGTGTGTGGG...
BX2-BB4 ggatccacaggactggtggtggGTGTGTGGGTGTGGGTGTGGTGTGTGGGTGTGTGGG...
BX2-BB5 ggatccacaggactggtggtggGTGTGTGGGTGTGGGTGTGTGGGTGTGGTGTGGTGTGGTGTGGGTGTGGTGTGTGGGTGT...
BX2-CC1A ggatccacaggactggtggtggGTGTGTGGGTGTGGTGTGGTGTGGGTGTGTGGGTGTGGGTGTGGTGTGG...
BX2-BC5B ggatccacaggactggtggtggGTGTGGGTGTGGTGTGGGTGTGTGTGGGTGTGGTGTG...
BX2-CA3 ggatccacaggactggtggtggGTGTGGGTGTGGTGTGTGGGTGTGGG...
BX2-CB3 ggatccacaggactggtggtggGTGTGGGTGTGGTGTGGGTGTGGTGT...
BX2-CC3A ggatccacaggactggtggGTGTGGGTGTGGGTGTGGGTGTGGTGTGTGTGGGTGTGGTGTGTGTGTGGGTGTGT...
BX2-AC4 ggatccacaggactggGTGTGGTGTGTGTGGGTGTGGGTGTGGTGTGTGT...
BX3-CA2 ggatccaaaggactgtggtgggcgccaatctagaatattgaaagacgctctactgacccatgcgcactctttatttttggagggtGTGGTGTGTGGG...
BX3-AB3 ggatccacaggactgtggtgggcgTGTGTGGGTGTGGGTGTGGGTGTGGGTGTGTGGGTGTGGGTGTGGG...
BX3-CA4A ggatccaaaggactgtggtgggcgTGGTGTGTGGGTGTGGTGTGTGGGTGTGTGGGTGTGGTGTGTGTGTGGG...
BX3-CA5A ggatccaaaggactgtggtgggcgTGTGGGTGTGGGTGTGGGTGTGGTGTGTGTGGGTGTGT...
BX3-BC6 ggatccaaaggactgtggtgggcgTGTGGTTGTGGTGTGTGGTGTGTGGGTGTGTGTGG...
BX3-BC8 ggatccacaggactgtggtgggTGTGGTGTGTGGGTGTGGGTGTGGGTGTGGGTGTGGTGTGT...
BX3-DB3 ggatccaaaggactgtggtgggTGTGGGTGTGTGGGTGTGTGGGTGTGTGGGTGTGG...
BX3-BC9 ggatccaaaggactgtggtgggTGTGGGTGTGGTGTGTGGGTGTGTGGGTGTGTGGGTGTGGGTGT...
BX3-DB5 ggatccaaaggactgtggtgTGTGTGGGTGTGGTGTGTGGGTGTGGTGTGGGTGTGG...

Figure 3.

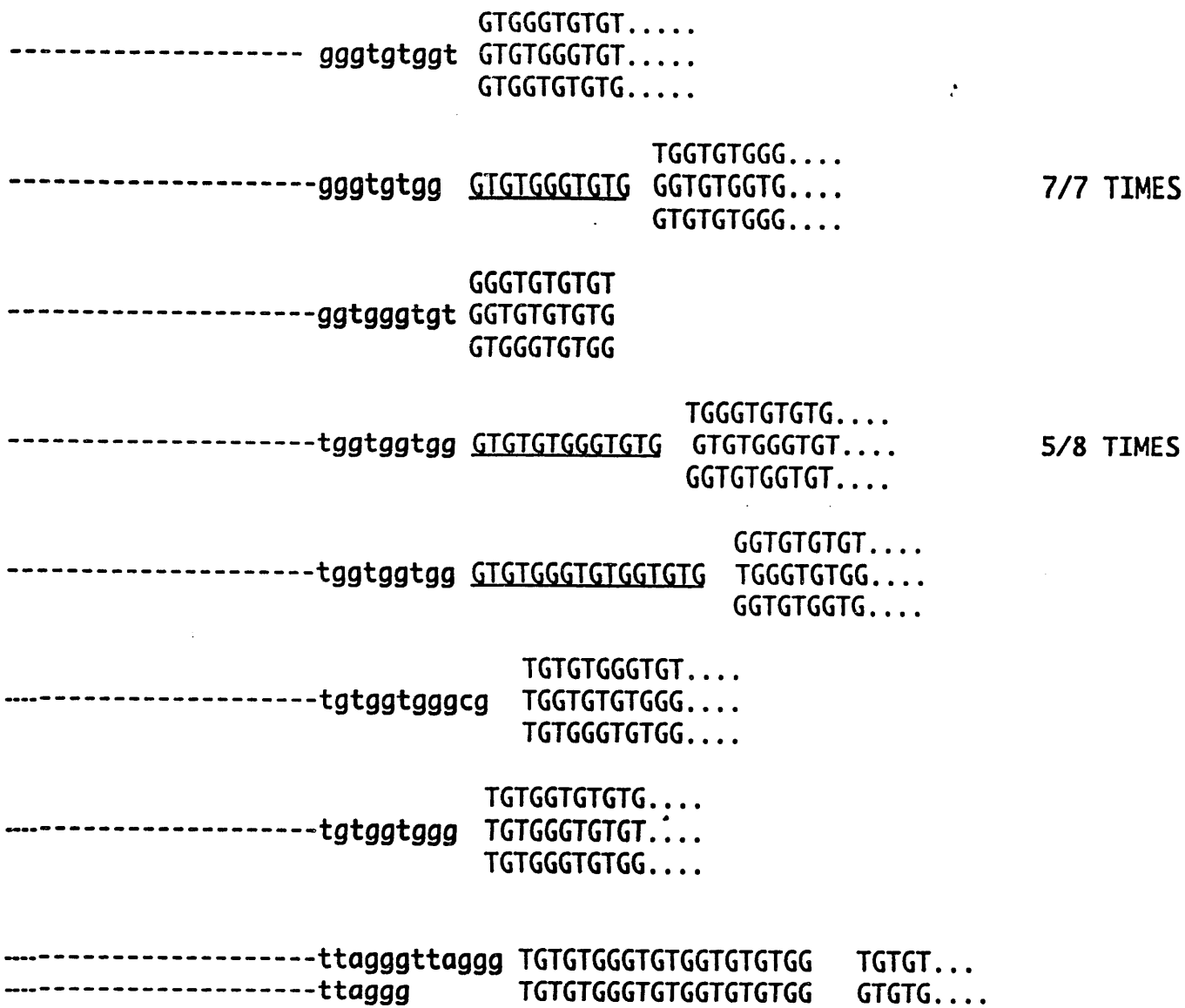


Figure 4. Preferred telomere additions at certain primer sites

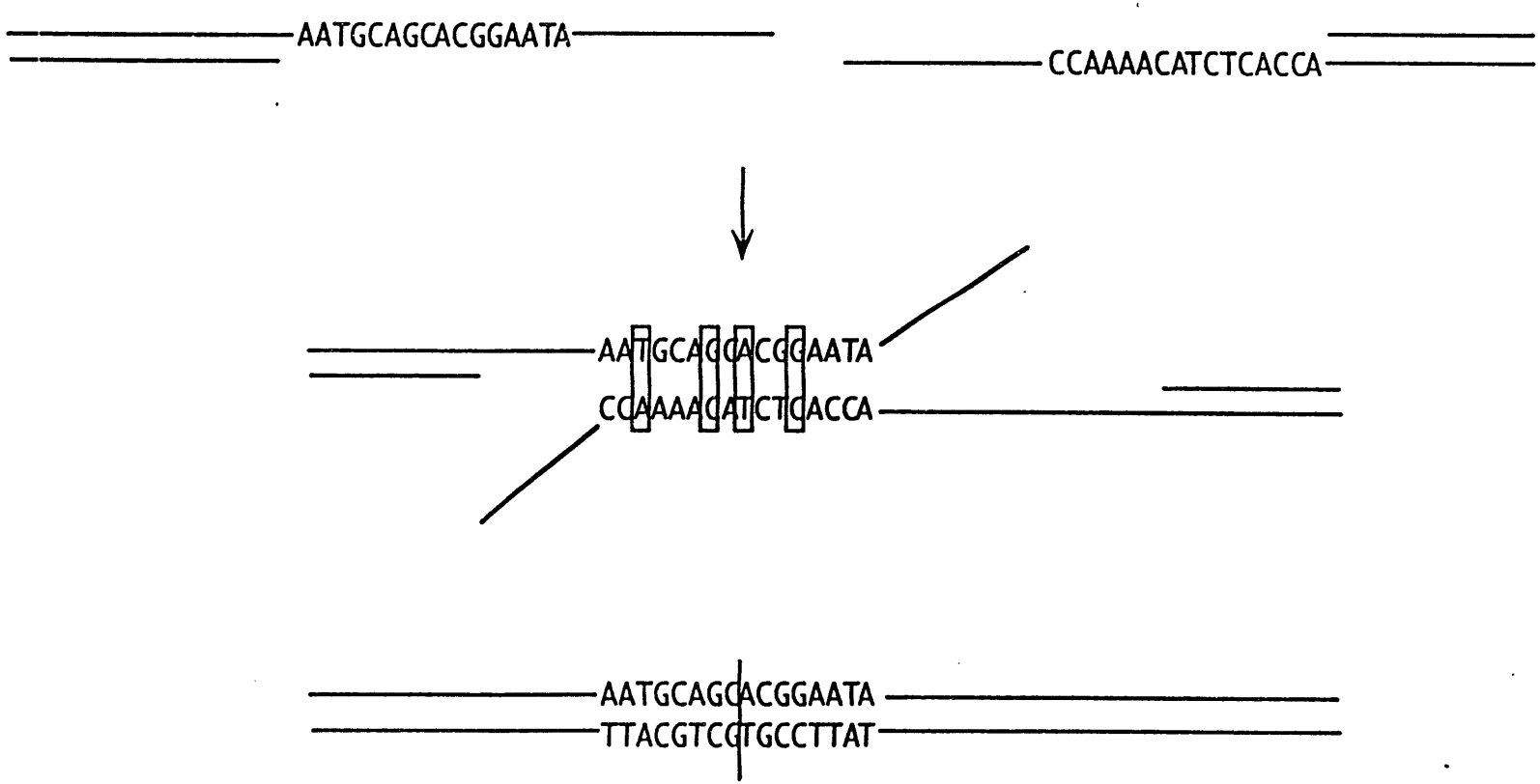


Figure 5. Nonhomologous deletions formed after HO-induced breaks at *MAT* in a *rad52* haploid strain.

END

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