Note

A Function for Subtelomeric DNA in *Saccharomyces cerevisiae*

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ABSTRACT

The subtelomeric DNA sequences from chromosome *I* of *Saccharomyces cerevisiae* are shown to be inherently poor substrates for meiotic recombination. On the basis of these results and prior observations that crossovers near telomeres do not promote efficient meiosis I segregation, we suggest that subtelomeric sequences evolved to prevent recombination from occurring where it cannot promote efficient segregation.

IN the yeast *Saccharomyces cerevisiae*, subtelomeric DNA is distinctly different from the rest of the chromosomal DNA in that it is repetitive and contains relatively few genes (Louis 1995). While subtelomeric sequences comprise ~7% (~25 kb × 32 ends/12,000 kb) of the genome, their function is unknown. During meiosis, homologs pair and segregate to reduce the chromosome number by half. Reciprocal recombination (crossing over) between homologs is essential for segregation. However, crossovers near the ends of *S. cerevisiae* chromosomes fail to promote efficient segregation (Ross *et al.* 1996) and might prevent it if they induce crossover interference (Su *et al.* 2000), a process that could prevent the occurrence of functional crossovers elsewhere on that chromosome. It also has been suggested that recombination near Drosophila chromosome ends fails to promote efficient segregation requiring utilization of the distributive segregation system (Carpenter 1973; Rasooly *et al.* 1991; Moore *et al.* 1994). Subtelomeric sequences from *S. cerevisiae* appear to exhibit an absence of meiotic double-strand-break sites (Klein *et al.* 1996; Gerton *et al.* 2000) and undergo little reciprocal recombination (Steensma *et al.* 1989; Goldman and Lichten 1996; Su *et al.* 2000). In this report, the low rate of recombination near the ends of chromosome *I* from *S. cerevisiae* are shown to be an inherent property of the subtelomeric sequences and not a telomere position effect (TPE). The few crossovers that occur are shown to produce crossover interference. As high levels of meiotic crossing over near chromosome ends could impair chromosome segregation, we suggest that subtelomeric DNA functions to prevent these crossovers from occurring. Therefore, subtelomeric regions help to ensure that crossovers occur where they promote segregation.

Subtelomeric regions exhibit low levels of transcription and late DNA replication (Ferguson and Fangman 1992; Pryde and Louis 1997). Low levels of transcription near telomeres may be due in some part to a well-characterized TPE that was first revealed by an epigenetic repression of expression of genes inserted within or near telomeres (Gottschling *et al.* 1990; Pryde and Louis 1997; Wyrick *et al.* 1999). Late replication is also due to a TPE, but one that appears to involve a mechanism independent of the genes required to repress transcription (Ferguson and Fangman 1992; Pryde and Louis 1997). Investigation of meiotic recombination on the left end of chromosome *I* revealed that crossovers were negligible in the endmost 4–9 kb and then increased with distance from the telomere (Figure 1, A and B; Su *et al.* 2000). Depending on the composition of the subtelomeric region, similar gradients were observed for a TPE on transcription (Renauld *et al.* 1993).

The mechanism that keeps rates of meiotic recombination low in subtelomeric regions is unknown but could be due to either telomere proximity or properties inherent in subtelomeric DNA sequences. If the low rate of recombination is caused by telomere proximity, it might be related to the TPE on transcription or replication or it could be the result of the sequestration of telomeres into the bouquet array at meiotic leptotene (Trelles-Stricken *et al.* 1999). Such sequestration could prevent these sequences from participating in meiotic pairing and recombination.

To test whether low levels of meiotic recombination at the ends of chromosomes were the result of telomere proximity, genetically marked subtelomeric sequences

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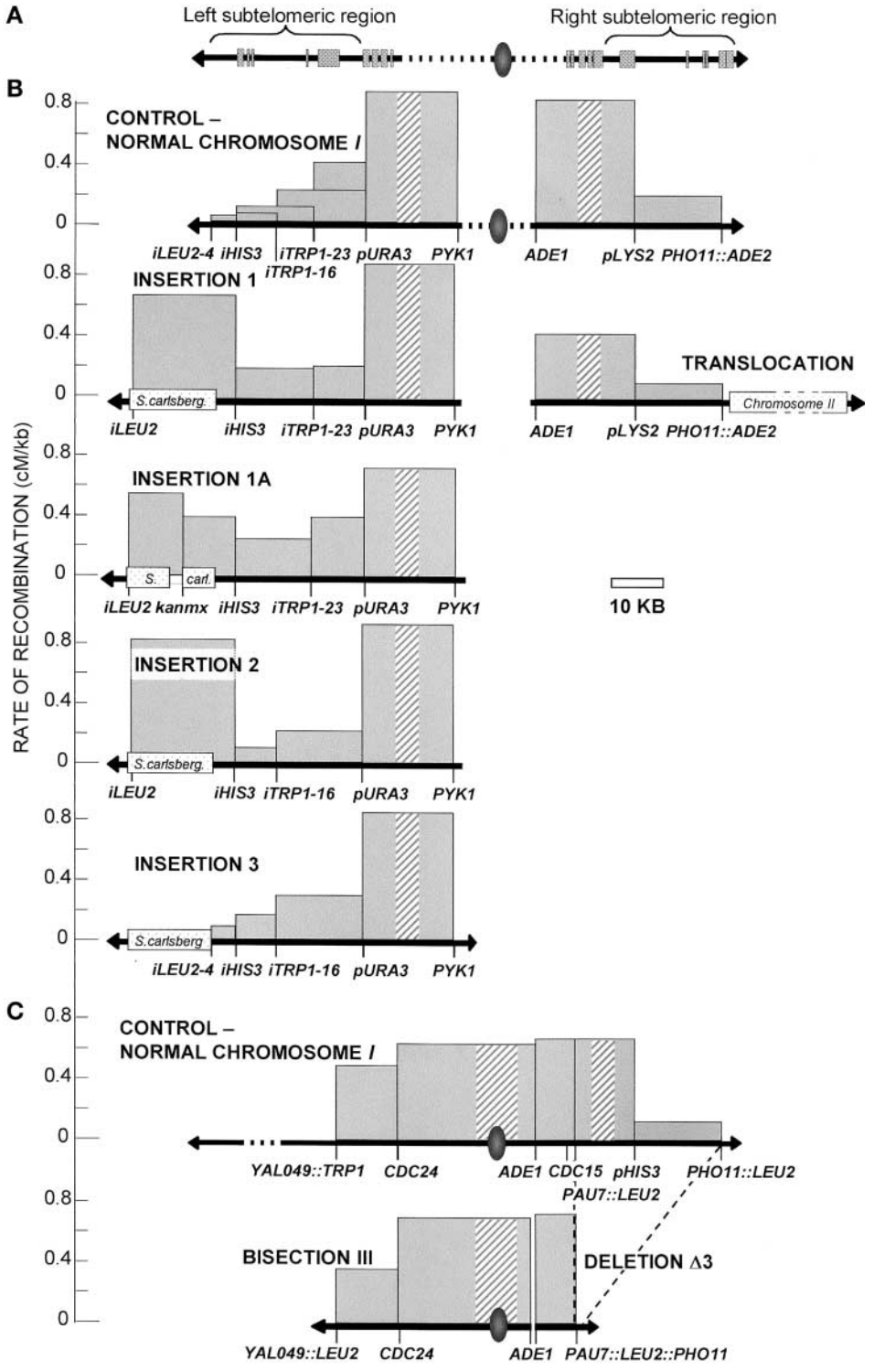

FIGURE 1.—Low rates of meiotic reciprocal recombination in subtelomeric regions of chromosome *I* are not due to a TPE. Recombination rates (centimorgans per kilobase) are from Table 1 for the noted heterozygous marker pairs in strains containing normal copies of chromosome *I* and those containing homozygous constructs that either separated the telomere from the marked subtelomeric region or moved a telomere to a new location in the middle of the chromosome. (A) Physical map of the subtelomeric regions of chromosome *I* showing location of open reading frames (shaded regions). (B) The left telomere was separated by inserting 15.5 kb of *S. carlsbergensis* (*S. carlberg.* or *S. carl.*) DNA (INSERTIONS 1, 2, and 3). The *S. carlsbergensis* DNA was further subdivided by inserting *kanmx* on one homolog (INSERTION 1A). The right telomere was separated using chromosome *I*-chromosome *II* reciprocal translocation II (TRANSLOCATION) (KABACK *et al.* 1999), which contains a breakpoint ~1 kb to the right of the *PHO11* gene (total length 800 kb). (C) A telomere was placed adjacent to YAL049 using chromosome *I*, bisection III (KABACK *et al.* 1999), and adjacent to *CDC15* using deletion Δ3 (BARTON *et al.* 1997). Dashed lines denote deletion borders. Arrows indicate telomeres and ovals indicate centromeres. Dashed regions on chromosome maps and hatched regions of the bar graphs denote large physical distances that are not near telomeres and are not drawn to scale.

from chromosome *I* were moved away from their natural telomere either by inserting ~15 kb of *S. carlsbergensis* DNA at the left end or by reciprocally translocating a large fragment from chromosome *II* to the right end (Figure 1B). Reciprocal recombination was analyzed by tetrad analysis. The results (Table 1) showed only a small increase with respect to the controls in the left subtelomeric region, *iLEU2-4-iHIS3-iTRP1-16(23)-pURA3* (INSERTION 1, 1A, 2, and 3), and a small decrease with respect to the controls in the right subtelomeric region, *pLYS2-PHO11::ADE2* (TRANSLOCATION). The average total amount of recombination in the combined gene-poor *iLEU2-4-iHIS3-iTRP1-16(23)-pURA3* region was almost identical in the insertions and the controls (6.1 ± 1.6 cM *vs.* 5.2 ± 1.1 cM). The lower rate of recombination in the *pLYS2-PHO11::ADE2* interval

of chromosome *I* in the translocation is consistent with an effect due to chromosome-size-dependent control of reciprocal recombination (Kaback *et al.* 1992). In all strains examined, the internal control intervals either were the same (*pURA3-PYK1*, control for the insertions) or showed the expected decrease (*ADE1-pLYS2*, control for the translocation) due to the large increase in chromosome size (Kaback *et al.* 1992). Thus, any TPE on meiotic recombination must be minimal and certainly does not affect most of the subtelomeric DNA.

Next, reciprocal recombination was examined within the translocated telomere proximal *S. carlsbergensis* DNA, which originally came from the middle of chromosome *III* (*iLEU2-iHIS3*). The results showed that the rate was relatively high and about equal to that found in the middle of chromosome *I* (Kaback *et al.* 1989b). The *S. carlsbergensis* DNA insert was divided with an additional marker (kanmx; INSERTION 1A) into telomere proximal and distal halves. The telomere proximal half (*iLEU2-kanmx*) underwent recombination at a slightly higher rate compared to the telomere distal half (*kanmx-iHIS3*), consistent with idea that telomere proximity has little if any appreciable effect on meiotic recombination rates.

In these experiments, the intervals adjacent to the *S. carlsbergensis* insert, *iLEU2-4-iHIS3*, *iHIS3-iTRP1-23*, and *iHIS3-iTRP1-16*, showed a very small increase in recombination rates compared to the controls. While these results might be due to telomere removal and a small TPE, it is more likely that they are due to recombination events initiated in the actively recombining *S. carlsbergensis* DNA and resolved in the adjacent *S. cerevisiae* DNA. Note the absence of an observable increase in the more distal *iTRP1-pURA3* interval. Irrespective of any small increase, recombination rates in these intervals were still very low and substantially less than elsewhere on chromosome *I* (Kaback 1989).

To further rule out a TPE, telomeres were placed adjacent to two regions that normally recombine at high rates and reciprocal recombination was measured (Figure 1C). Chromosome *I* bisection III places a cloned telomere from an artificial chromosome adjacent to YAL049 (Kaback *et al.* 1999). Deletion Δ3 places the natural chromosome *IR* telomere adjacent to *PAU7*, ~2 kb from *CDC15* (Barton *et al.* 1997). Genes adjacent to the chromosome *IR* telomere have been shown to be under the control of a *SIR*-gene-dependent TPE (Barton *et al.* 1997; A. B. Barton and D. B. Kaback, unpublished results). Nevertheless, rates of reciprocal recombination in both new telomere proximal intervals, YAL049*::TRP1-CDC24* in the bisection and *ADE1-PAU7::LEU2::PHO11* in the deletion, were relatively high and did not vary significantly from the controls, YAL049*::LEU2-CDC24* and *ADE1-PAU7::LEU2*, respectively, which were not adjacent to telomeres. The control for the bisection used a different selectable marker (*TRP1 vs. LEU2*) in otherwise isogenic strains. However, previous studies showed that heterozygous marker identity had no effect on reciprocal recombination in the adjacent homologous regions (Su *et al.* 2000). The rate of recombination in the 6.8-kb *ADE1-PAU7::LEU2::PHO11* interval in deletion Δ3 equaled that found in the slightly smaller and well-investigated *ADE1-CDC15* interval as well as the entire 47.0-kb *ADE1-pHIS3* interval on normal copies of chromosome *I* (Mortimer and Schild 1980; Mortimer and Schild 1985; Kaback *et al.* 1989a). Therefore, the translocated telomeres did not lower recombination rates.

These results are in contrast to an ~2.5-fold decrease in recombination observed adjacent to a translocated telomere in a different chromosome *I* bisection (Kaback *et al.* 1999). The cause of this apparent paradox is likely to be that the bisection that affected recombination rates removed a prominent DSB site normally adjacent to the interval (*iARG4-fun30::LEU2*) that had been examined (Klein *et al.* 1996).

In total, these results show that telomere insertion cannot be correlated with any predictable repression of meiotic reciprocal recombination. Therefore, we conclude that the low recombination rates observed near both ends of chromosome *I* must be due mostly to the composition of the subtelomeric DNA sequences. Any repression due to telomere proximity must be less than twofold and is therefore incapable on its own of producing the observed low recombination rates throughout these 20- to 30-kb subtelomeric regions. It is still possible that a localized TPE prevents recombination in the outermost 2–4 kb of the chromosome end because this region was not examined. A telomere-dependent suppression of mitotic recombination has been observed (Stavenhagen and Zakian 1998). However, this effect appears to be specific to $C_{1\text{-}3}A$ telomeric repeats, not to subtelomeric DNA. Furthermore, there was no apparent effect on recombination within the telomere-proximal *S. carlsbergensis* sequences. The absence of any telomere effect on meiotic recombination is further supported by the high level of ectopic recombination and recombination-induced marker loss observed near telomeres of chromosome fragments that are missing subtelomeric sequences (Arbel *et al.* 1999) and by the observation that low levels of recombination at the end of chromosome *I* were independent of *SIR2* and *SIR3*, both of which are required to produce transcriptional TPEs (Su *et al.* 2000). The results presented here also are inconsistent with the possibility that recombination is prevented by physical constraints due to the association of telomere DNA with the nuclear periphery during meiotic prophase unless subtelomeric DNA itself is involved in the perinuclear localization of chromosomes.

Reciprocal recombination induces crossover interference. To determine whether crossovers in the *iLEU2-4-pURA3* subtelomeric region exhibited interference on the adjacent *pURA3-PYK1* interval, previously described

TABLE 1

Meiotic reciprocal recombination rates in subtelomeric regions

Chromosome *I* construct	Strain[a] (reference)	Interval	No. of asci					Recombination rate (cM/kb ±SE)
			kb	PD[b]	NPD[b]	TT[b]	cM[c]	
Normal	YPS175 (Su *et al.* 2000)	*iLEU2-4-iHIS3*	4.7	573	0	2	0.2	0.04 ± 0.03
		iHIS3-iTRP1-23	14.1	560	0	18	1.6	0.11 ± 0.03
		iTRP1-23-pURA3	9.7	532	0	47	4.1	0.41 ± 0.06
		pURA3-PYK1	40.1	217	10	335	35.6	0.89 ± 0.06
S. carlsbergensis, INSERTION 1	YPSY31	*iLEU2-iHIS3*	19.5	198	2	51	12.5	0.63 ± 0.12
		iHIS3-iTRP1-23	14.1	238	0	15	3.0	0.21 ± 0.05
		iTRP1-23-pURA3	9.7	242	0	11	2.2	0.23 ± 0.06
		pURA3-PYK1	40.1	95	5	124	35.2	0.88 ± 0.09
S. carlsbergensis, INSERTION 1A	YPSY31-kan	*iLEU2-kanmx*	9.8	194	0	23	5.3	0.54 ± 0.14
		kanmx-iHIS3	9.8	201	0	16	3.7	0.38 ± 0.09
		iHIS3-iTRP1-23	14.1	202	0	16	3.7	0.25 ± 0.06
		iTRP1-23-pURA3	9.7	199	0	17	3.9	0.40 ± 0.09
		pURA3-PYK1	40.1	101	1	116	28.0	0.70 ± 0.05
Normal	YPS159 (Su *et al.* 2000)	*iLEU2-4-iHIS3*	4.7	132	0	0	0	0
		iHIS3-iTRP1-16	7.5	130	0	1	0.4	0.05 ± 0.05
		iTRP1-16-pURA3	16.3	119	0	10	3.9	0.24 ± 0.07
		pURA3-PYK1	40.1	53	4	72	38.8	0.97 ± 0.16
S. carlsbergensis, INSERTION 2	YPSY41	*iLEU2-iHIS3*	19.5	168	2	61	16.0	0.82 ± 0.11
		iHIS3-iTRP1-16	7.5	228	0	4	0.9	0.11 ± 0.07
		iTRP1-16-pURA3	16.3	216	0	16	3.4	0.21 ± 0.05
		pURA3-PYK1	40.1	94	5	124	35.3	0.88 ± 0.09
S. carlsbergensis, INSERTION 3	YPSW15A	*iLEU2-4-iHIS3*	4.7	299	0	4	0.7	0.15 ± 0.07
		iHIS3-iTRP1-16	7.5	297	0	8	1.3	0.18 ± 0.07
		iTRP1-16-pURA3	16.3	278	1	26	5.4	0.34 ± 0.09
		pURA3-PYK1	40.1	130	5	168	33.1	0.83 ± 0.07
Normal	CAB38	*ADE1-pLYS2*	47.0	27	1	72	39.1	0.83 ± 0.08
		pLYS2-PHO11::ADE2	16.0	95	0	7	3.4	0.21 ± 0.08
Chromosome *I–II* translocation II	CAB36	*ADE1-pLYS2*	47.0	111	0	92	22.7	0.48 ± 0.04
		pLYS2-PHO11::ADE2	16.0	199	0	7	1.7	0.11 ± 0.04
Normal	JL52 (Kaback *et al.* 1999)	*ADE1-CDC24*	105.7	208	73	663	67.7	0.64 ± 0.05
		YAL049::TRP1-CDC24	12.0	840	0	112	5.9	0.49 ± 0.04
Bisection III	JL94	*ADE1-CDC24*	105.7	62	17	111	74.4	0.70 ± 0.17
		YAL049::LEU2-CDC24	12.0	166	0	14	3.9	0.33 ± 0.08
Normal	DK388 (Rothstein 1983)	*ADE1-pHIS3*	47.0	103	0	156	30.1	0.64 ± 0.03
		pHIS3-PHO11::LEU2	16.0	262	0	10	1.8	0.11 ± 0.04
Normal	DK411	*ADE1-PAU7::LEU2*	6.8	166	0	17	4.6	0.65 ± 0.05
		CEN1-ADE1	19.0	171		20	5.4	0.28 ± 0.05
Deletion Δ3	DK408	*ADE1-PAU7::LEU2::PHO11*	6.8	223	1	18	5.2	0.73 ± 0.28
		CEN1-ADE1	19.0	224		23	4.6	0.26 ± 0.05

Methods: Genetic analysis was carried out using standard protocols as previously described (Sambrook *et al.* 1989; Burke *et al.* 2000). Recombinant DNA techniques were carried out by standard protocols (Sambrook *et al.* 1989) and all constructs were verified by blot hybridization (Southern 1975). Strains that were compared were either isogenic or congenic, composed of spores from the same inbred diploid. Deletion Δ3 was also compared with previously published data from normal chromosomes. Markers were introduced by standard techniques (Sambrook *et al.* 1989; Burke *et al.* 2000). The *pLYS2* marker is identical to the previously described *pHIS3* marker (Steensma *et al.* 1989) except that it contains the *S. cerevisiae LYS2* gene on a 4.9-kb *Hin*dIII fragment instead of the *HIS3* gene. This marker was introduced into the parents of CAB36 and CAB38 (Kaback *et al.* 1999) following selection of spontaneous *lys2* mutants on α-aminoadipic-acid-containing medium (Burke *et al.* 2000). YAL049*::TRP1* in JL52 was previously described as *iTRP1* (Kaback *et al.* 1999). JL94 is derived from spores obtained from JL51 (Kaback *et al.* 1992), the isogenic parent of JL52. It contains YAL049*::LEU2*, a 2.1-kb *Hpa*I fragment containing *LEU2* inserted at the identical position as YAL049*::TRP1* on chromosome bisection III fragment *IB*-180 (Kaback *et al.* 1992) by one-step gene replacement (Rothstein 1983). Strains YPSY31 and YPSY41 were constructed by inserting 15.5 kb of *S. carlsbergensis* chromosome *III* DNA

tetrad data (Su *et al.* 2000) was further analyzed. Reciprocal recombination between *pURA3* and *PYK1* was significantly ($P < 0.001$) reduced from 38.0 ± 1.6 cM [447 parental ditypes (PDs), 28 nonparental ditypes (NPDs), and 724 tetratypes (TTs)] in tetrads that did not have a crossover in the subtelomeric region to 27.2 ± 4.0 cM (74 PDs, 2 NPDs, and 62 TTs) in tetrads that contained a crossover in the subtelomeric region. The same data were analyzed by ranking tetrads within the multiply marked subtelomeric intervals from *iLEU2-pURA3* and by calculating interference with the Tetrads program (Mortimer *et al.* 1989). Crossovers within this subtelomeric region again exhibited crossover interference ($k = 0.2 \pm 0.1$; based on 1303 no-crossover tetrads, 161 single-crossover tetrads, 2 double-crossover tetrads, and 0 >2-crossover tetrads, where k is equivalent to the coefficient of coincidence). In sum, these data indicate that a crossover in subtelomeric DNA induces crossover interference elsewhere on chromosome *I*.

Subtelomeric regions in yeast have been compared to telomeric heterochromatin of other eukaryotes and some noncoding structural role has been suggested (Bussey *et al.* 1995). As chiasmata that occur near the ends of chromosomes appear to be much less efficient at promoting segregation (Ross *et al.* 1996) but cause crossover interference, their occurrence is likely to lower the probability that a functional crossover occurs elsewhere on the chromosome. Indeed, it has been shown that interference can affect >200 kb, the approximate size of the two smallest yeast chromosomes (Kaback *et al.* 1999). The absence of subtelomeres would presumably lead to a normal rate of recombination within the endmost 30 kb of a chromosome. While the effect of losing a single subtelomeric sequence is expected to be relatively modest, certainly requiring the analysis of thousands of asci to quantitate, the loss of both subtelomeric regions is predicted to produce >1% meiotic nondisjunction for each of the small chromosomes (Su *et al.* 2000). Since this effect is expected to be additive, it is likely that a successful organism would not tolerate these high levels of nondisjunction. Therefore, we propose that subtelomeric regions function to prevent crossovers from occurring where they cannot promote segregation. The absence of subtelomeres would lead to high levels of crossing over near the ends of chromosomes and meiotic chromosome nondisjunction, especially on the smaller chromosomes.

It should be emphasized that these results were obtained with the smallest yeast chromosome. Since this chromosome has the fewest crossovers, it is likely to be most affected by the possible deleterious effects of high rates of recombination near telomeres. Thus, it is possible that the subtelomeric sequences on this chromosome are unique. Nevertheless, the apparent low level of DSB sites near the ends of all yeast chromosomes suggests that all subtelomeric DNA sequences may indeed play an active role in preventing nonfunctional meiotic recombination on all chromosomes.

The mechanism for lowering rates of recombination in these regions is not known but is clearly dependent on the subtelomeric sequences. Why subtelomeric DNA sequences are inherently nonrecombinogenic during meiosis may reflect the paucity of functional transcription promoter elements or *cis*-acting inhibitors capable of preventing recombination over a distance. It appears to be unrelated to ARS proximity as the *S. carlsbergensis* DNA inserted near the left telomere has two ARS elements that appear to be active in *S. cerevisiae* (Yang *et al.* 1999). Finally, some cases of human trisomy 21 (Down syndrome) that arose due to improper meiosis I chromosome segregation have been attributed to crossovers that occurred near the ends of chromosome 21, rather than to an absence of crossing over (Lamb *et al.* 1997). These cases suggest that humans might also have a

TABLE 1

(Continued)

(the ligation product of fragments 1b3a and 15Ba5; Yang *et al.* 1999) by one-step gene replacement (Rothstein 1983) using the chromosome *I* insert from plasmid pLF263 (Su *et al.* 2000) to target the host DNA of the parents that were used to construct the control strains YPS175 and YPS159 (Su *et al.* 2000). The *S. carlsbergensis* DNA was marked with a 2.5-kb *Xho*I fragment with *lox*P*LEU2lox*P (*iLEU2*) located ~1 kb from its left end as shown in Figure 1B. Where necessary, the *LEU2* gene was excised in the haploid transformants using an inducible *Cre* recombinase as previously described (Sauer 1996). The construction of the *PAU7::LEU2::PHO11* marker present in strain DK408 was described previously (Barton *et al.* 1997), where it was referred to simply as deletion Δ3. An identical deletion construct using a 1.7-kb *Bam*HI fragment containing the *S. cerevisiae HIS3* (Struhl and Davis 1980) gene as the selectable marker was introduced on the homologous chromosome. Thus the genotype of this strain is *PAU7::LEU2::PHO11/PAU7::HIS3::PHO11*. Similarly, the identical *LEU2* and *HIS3* fragments were inserted as controls adjacent to *PAU7* (position 176,627 bp) in the two parent haploids of strain DK411 that contain normal copies of chromosome *I*. All other heterozygous insertion markers have been described previously (Kaback *et al.* 1989b, 1992, 1999; Su *et al.* 2000).

[a] Noted strains were homozygous for the chromosome *I* construct and heterozygous for the markers shown.

[b] Ascus types: PD, parental ditype; NPD, nonparental ditype; TT, tetratype. *TRP1* was used as the centromere (*CEN1*) marker in strains DK408 and DK411. Asci showing first-division segregation of *ADE1* and *TRP1* are shown in the PD column and those showing second-division segregation are shown in the TT column.

[c] Centimorgans (cM) and standard errors were calculated using the Tetrads program (courtesy of J. Kans; Mortimer *et al.* 1989). Data from control strains previously described are referenced.

mechanism for preventing crossovers from occurring near telomeres.

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