Note

Meiotic Alterations in CAG Repeat Tracts

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ABSTRACT

We have investigated meiotic changes in CAG repeat tracts embedded in a yeast chromosome. Repeat tracts undergo either conversion events between homologs or expansion and contraction events that appear to be confined to a single chromatid. We did not find evidence for conversion of tract interruptions or excess exchange of flanking markers.

EXPANSIONS of CAG trinucleotide repeat tracts cause several human neurodegenerative diseases, including Huntington's disease and several spinocerebellar ataxias (Paulson and Fischbeck 1996). To understand the mechanisms by which CAG tracts undergo changes in length, we investigated CAG tract instability in the yeast *Saccharomyces cerevisiae*. During mitotic division the majority of changes observed are tract contractions, and the frequency of contractions is higher when CTG serves as the lagging strand template than when CAG serves as the lagging strand template (Maurer *et al.* 1996; Freudenreich *et al.* 1997). Rare tract expansions occur more frequently in the opposite orientation, *i.e.*, when CAG serves as the lagging strand template (Miret *et al.* 1998). While many yeast replication mutations exacerbate the frequency of tract contractions, two mutations—those in the flap endonuclease (Rad27p/Rth1p) and in DNA ligase I (Cdc9p)—elevate the frequency of CAG repeat tract expansions (Freudenreich *et al.* 1998; Schweitzer and Livingston 1998, 1999; Spiro *et al.* 1999; Ireland *et al.* 2000). After extensive characterization of CAG tract instability during yeast mitosis, here we turn our attention to examining their behavior during yeast meiosis.

To investigate tract instability during meiosis, we analyzed ~250 tetrads from each of four diploid repeat tract configurations: C78/Cii28, C78/no tract, D71/Dii28, and D71/no tract (Figure 1). We scored both the tract length in the spores and the configuration of the flanking markers. In spores where tract length changes had occurred we also scored for the presence of the *Sfa*NI sites (GCATC) to learn whether CAT interruptions were rearranged or transferred between chromosomes. We initially assigned the patterns of tract length changes for each tetrad to one of three classes: no change, a mitotic change, or a meiotic change. An example of the PCR products for each class is shown in Figure 2. The first class in which no tract length change has occurred comprises the largest number of tetrads (Table 1). An example of this class is shown in Figure 2A in which two copies of each parental tract length are present. The second class is composed of tract length changes that most likely occur during the mitotic divisions before cells enter meiosis. In this class two spores contain the same nonparental tract length and two spores maintain the same parental tract length (Figure 2B). These events resemble the mitotic events we have previously analyzed in haploid cells in three ways (Maurer *et al.* 1996; Schweitzer and Livingston 1999). First, as in mitosis where longer, uninterrupted tracts are more unstable than shorter, interrupted tracts, only the longer of the two tracts underwent changes in the tetrads of this class. Second, most of the mitotic events recorded in tetrads are contractions (94 out of 99 total changes), similar to the preponderance of tract contractions we have previously recorded during mitotic growth. Third, the events in this class of tetrads occur more frequently in the tract of the D orientation than in the tract of the C orientation (83 changes out of 479 tetrads for the D71 tract compared to 16 changes out of 506 tetrads for the C78 tract), again mimicking the orientation dependence found in mitotic division.

The third class comprises the smallest number of events (22 out of 985). In this class either one spore contains a repeat tract length not present in the parental cell (Figure 2C) or one spore has converted the tract from the homolog (Figure 2D). The details of each event of this class are given in Table 2. In all but one

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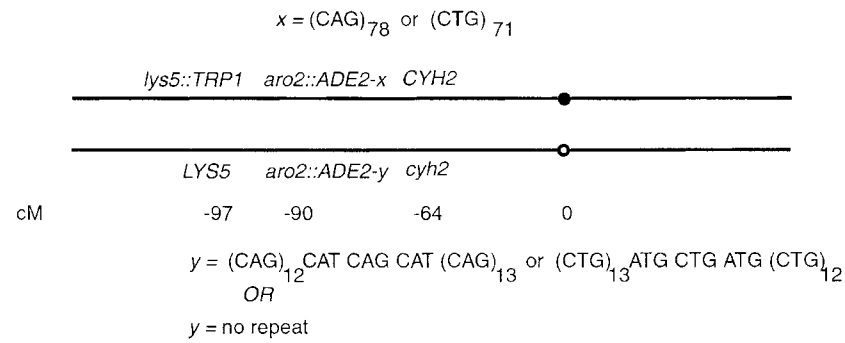

FIGURE 1.—Chromosome VII markers. Diploids were constructed by mating a haploid bearing a long CAG tract (*x* shown on the top homologue) and a haploid bearing a short, interrupted repeat tract or no repeat tract (*y* shown on the bottom homologue). We have described previously the placement of a CAG tract at the *ARO2* locus on yeast chromosome VII (MAURER *et al.* 1996). An autonomously replicating sequence located 5′ to the repeat tract directs replication through the tract. We denote tract C as the relatively stable orientation (CAG in the lagging strand template) and tract D as the relatively unstable orientation (CTG in the lagging strand template). The long tracts used in this study are C78 and D71. The short tracts of 28 repeat units (denoted Cii28 and Dii28) contain two CAT interruptions near the middle of the tract, creating cleavage sites for the restriction enzyme *Sfa*NI (MAURER *et al.* 1998). The CAT interruptions provide a marker that could potentially be transferred from one homologue to the other during recombination (CHUNG *et al.* 1993). Flanking markers on chromosome VII, *lys5::TRP1* and *cyh2*, were used to assess exchange frequencies in intervals flanking *ARO2*. The map distances from the centromere in centimorgans are noted below each locus.

example from this class, the flanking markers segregated 2:2 (Table 2). As described below, while some events in this class could arise during the final round of DNA replication preceding meiosis, we suspect that these events originate during meiosis.

Seven of the nine tetrads scored as a conversion within the third class occurred with the exact transfer of an entire tract (or by the transfer of the absence of a tract) from one homolog to the other. In the other two events, a chromosome with the short tract (event 1, Table 2) or a chromosome without a tract (event 21, Table 2) acquired a long tract. While not exact conversions, each of these two events likely results from a transfer of sequence information between homologs. In the first case (event 1, Table 2), the tract of new length is devoid of CAT interruptions, making it unlikely to have occurred by intramolecular expansion of the short tract that contains two CAT interruptions. In the second case (event 21, Table 2), the acquisition of a tract by a chromosome lacking a tract obligatorily occurs by transfer of sequence information from one homolog to the other. Both events are novel in that the new tracts are longer than the long parental tract that serves as a donor and are suggestive of a mechanism involving reiterative copying of the donor tract (RICHARD *et al.* 2000).

We also note that eight of nine conversion events occur in the diploids with the C tract. While this might signify that this orientation is subject to double-strand breakage in meiosis, the results show that conversions occur almost equally in both directions from long tract to short tract/no tract and from short tract/no tract to long tract. If the long C tract were especially susceptible to double-strand breaks in meiosis, we would have expected it to act as the recipient in most events.

That the majority of conversion events included an exact transfer of a tract (or the absence of a tract) suggests that the conversion events are not likely to initiate by invasion of a portion of a repeat tract into the tract located on the homolog. An exact transfer of tract length by a mechanism in which repeat units from one homolog invade a homologous repeat tract demands an exact positional match of repeat units. Inexact alignments lead to tract lengths different from the parental lengths. Tract lengths different from the parent are not frequent. Also, in the case of conversions of the short interrupted tract into the long uninterrupted tract, we might have expected retention of one or both of the CAT interruptions in some events if portions of the short tract invaded the longer tract during recombination. We never observed retention of CAT interruptions in these events.

The expansions and contractions that occur in the remainder of the events (13 out of 22) in the third class might arise by a number of mechanisms. One possibility is that these events initiate during the round of replication immediately preceding meiosis and are completed during meiotic replication. If a loop of repeat units on the template or on the newly synthesized strand is established during the round of replication preceding meiosis, and if the loop persists until the next round of replication during meiotic S, then its resolution would yield a 3:1 segregation pattern. The nature of the events makes this mechanism unlikely. First, we note that the events occur almost equally on tracts of both orientations, while mitotic events occur more frequently in tract D than in tract C. Second, tract expansions occur approximately as frequently as tract contractions among this group of events, while contractions occur far more frequently than expansions during mitosis. The one resemblance of these meiotic events to the mitotic events is that all but one occurs in the longer of the tracts. We favor a second mechanism in which these events are initiated during or after meiotic S. Double-strand breaks that occur within tracts during or after meiotic replication could give rise to expansions and contractions either by a single-strand annealing reaction involving one DNA duplex or by sister chromatid conversion involving two DNA duplexes (PAQUES and HABER 1999). (While

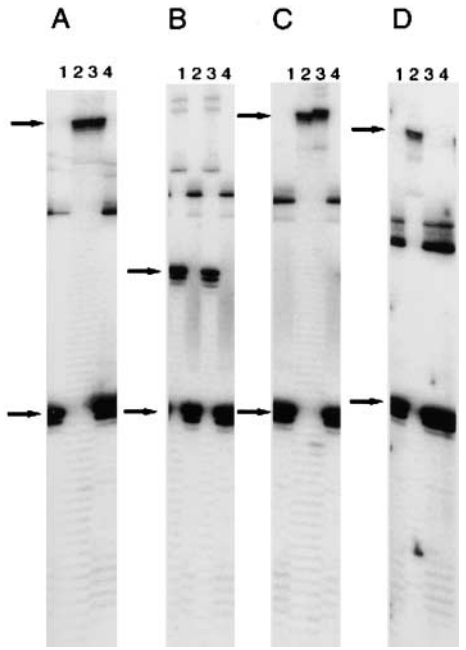

FIGURE 2.—PCR analysis of tetrads bearing CAG repeat tracts. For these studies, diploid strains were grown into colonies that were transferred by replica plating to potassium acetate plates. After dissection of tetrads a portion of each spore colony was tested for nutritional markers. The remainder of each colony was used to isolate DNA for PCR determination of repeat tract length, as described previously (MAURER *et al.* 1996; SCHWEITZER and LIVINGSTON 1999). The four tetrads illustrate the types of tetrads recorded in this analysis. All four came from the C_{78}/C_{ii28} cross (Tables 1 and 2). In A–D, the bottom arrow points to the C_{ii28} repeat tract band and the top arrow points to the long repeat tract band C_{78} or a new length. (A) This tetrad is an example of a tetrad that has not undergone a change in CAG tract length. Lanes 1 and 4 are C_{ii28} spores and lanes 2 and 3 are C_{78} spores. (B) This tetrad has undergone a mitotic change in tract length, a contraction of 33 repeat units in the longer tract. There are two spores with a parental tract length (C_{ii28}, lanes 2 and 4) and two spores with the same, new tract length (C_{45}, lanes 1 and 3). (C) This tetrad has undergone a meiotic change in tract length, an expansion of one repeat unit in the longer tract. Three spores maintain a parental tract length (C_{ii28}, lanes 1 and 4; C_{78}, lane 2) and one spore has a new tract length (C_{79}, lane 3). (D) This tetrad has undergone a conversion event ($C_{78} \rightarrow C_{ii28}$) with a 3:1 segregation of parental repeat tract lengths. Three spores have a C_{ii28} tract (lanes 1, 3, and 4) and one spore has a C_{78} tract. In this tetrad and the other three that are shown, the flanking markers segregated 2:2.

conversion between homologs could account for some of these events, half of these events take place when the homologous chromosome lacks a repeat tract and are precluded from this mechanism.) We note that if the sister chromatid is used to repair the double-strand break, crossing over could not be permitted because unequal sister chromatid exchange would likely lead to two nonparental tract lengths. No tetrads were found with two nonparental tract lengths.

While the events comprising the two meiotic patterns we observed, the conversions and the expansions and

TABLE 1

Mitotic and meiotic changes in repeat tract lengths

Tract configuration	No. of tetrads	Mitotic changes (all in long tract)		Meiotic changes in long tract			Meiotic changes in short tract/no tract		
		Expansions	Contractions	Expansions	Contractions	Conversions	Expansions	Contractions	Conversions
C_{78}/C_{ii28}	250	1	10	2	1	2[a]	0	1	2[c]
C_{78}/no tract	256	1	4	1	2	2[b]			2[d]
D_{71}/D_{ii28}	223	2	46	0	3	0	0	0	0
D_{71}/no tract	256	1	34	2	1	0			1[e]

[a] In these events C78 was replaced by Cii28 (events 3 and 6, Table 2).
[b] In these events C78 was eliminated (events 13 and 14, Table 2).
[c] In one of these events, Cii28 was replaced by C78, and in the other event Cii28 was replaced by an uninterrupted tract longer than C78 (events 7 and 1, respectively, Table 2).
[d] In these events a chromosome lacking a repeat tract acquired tract C78 (events 11 and 12, Table 2).
[e] In this event a chromosome lacking a repeat tract acquired an uninterrupted tract longer than D71 (event 21, Table 2).

TABLE 2

Meiotic events

Event no.	Tract configuration	Change in length	Type of event	Crossover
1	C_{78}/C_{ii28}	(+62)	Tract conversion, $C_{ii28} \rightarrow C_{90}$	None
2	C_{78}/C_{ii28}	+1	C_{78} expansion	*ARO2* to *CYH2*
3	C_{78}/C_{ii28}	(−50)	Tract conversion, $C_{78} \rightarrow C_{ii28}$	None
4	C_{78}/C_{ii28}	−35	C_{78} contraction	None
5	C_{78}/C_{ii28}	−10	Tract contraction, $C_{ii28} \rightarrow C_{18}$	None
6	C_{78}/C_{ii28}	(−50)	Tract conversion, $C_{78} \rightarrow C_{ii28}$	*LYS5* to *CYH2*[a]
7	C_{78}/C_{ii28}	(+50)	Tract conversion, $C_{ii28} \rightarrow C_{78}$	*LYS5* to *CYH2*[a]
8	C_{78}/C_{ii28}	+1	C_{78} expansion	*ARO2* to *CYH2*
9	C_{78}/no tract	−2	C_{78} contraction	None
10	C_{78}/no tract	−16	C_{78} contraction	None; gene conversion at *LYS5*
11	C_{78}/no tract	(+78)	Tract conversion, no tract $\rightarrow C_{78}$	*LYS5* to *CYH2*[a]
12	C_{78}/no tract	(+78)	Tract conversion, no tract $\rightarrow C_{78}$	None
13	C_{78}/no tract	(−78)	Tract conversion, $C_{78} \rightarrow$ no tract	*LYS5* to *CYH2*[a]
14	C_{78}/no tract	(−78)	Tract conversion, $C_{78} \rightarrow$ no tract	*LYS5* to *CYH2*[a]
15	C_{78}/no tract	+1	C_{78} expansion	None
16	D_{71}/D_{ii28}	−24	D_{71} contraction	None
17	D_{71}/D_{ii28}	−17	D_{71} contraction	None
18	D_{71}/D_{ii28}	−21	D_{71} contraction	None
19	D_{71}/no tract	−51	D_{71} contraction	None
20	D_{71}/no tract	+25	D_{71} expansion	None
21	D_{71}/no tract	(>100)	Tract conversion, no tract $\rightarrow$ long tract	None
22	D_{71}/no tract	+4	D_{71} expansion	*ARO2* to *CYH2*

[a]A crossover occurred between *LYS5* and *CYH2*, but the interval could not be determined.

contractions, may both be initiated by double-strand breaks, they do not appear to share other mechanistic similarities. The conversions are biased to diploids with the long C tract, while the expansions and contractions take place nearly equally in both orientations. The conversions take place on chromosomes bearing long tracts, short tracts, or no tracts, whereas the expansions and contractions take place almost exclusively in the long tract. The outcomes suggest that breaks within tracts lead to intramolecular recombination (or possibly sister chromatid conversion without exchange), giving rise to expansions and contractions, and that breaks with no remnant of repeat units are invasive into the homolog and lead to conversion.

We also compared the frequencies of flanking marker exchange in our diploid strains to an isogenic diploid lacking repeat tracts. A compilation of the results (Table 3) shows that the flanking intervals are neither appreciably expanded nor contracted by the presence of repeat tracts. The coincidence of exchange events accompanying the meiotic events (Table 2) shows that exchange can accompany both types of meiotic events but is not significantly different between the two classes. (Five of 9 conversion events and 3 of 13 expansion/contraction events are accompanied by crossing over.)

Comparison of our studies with other studies on CAG repeat tracts during meiosis shows similarities and differences. Like the meiotic studies of Cohen *et al.* (1999), we too find that the high ratio of tract contraction to tract expansion events among mitotic events is reduced in meiosis to bring the ratio closer to unity. In addition, they noted that the orientation bias present during mitosis disappears during meiosis. Our results are interesting in that while the meiotic events comprising tract contractions and expansions occur almost equally between the two tract orientations, the orientation bias in the conversion events is tilted toward the tract that is more stable during mitotic division. While Cohen *et al.* (1999) noted that repeat tracts appear to undergo more meiotic events when no repeat tract is present on the homologous chromosome, we do not see this bias. Another similarity between the two studies is that the frequencies of meiotic events in our diploids are comparable to those in the yeast artificial chromosomes studied by Cohen *et al.* (1999). This is noteworthy in that some meiotic studies have indicated that CAG repeat tracts may be hyperrecombinational (Jankowski *et al.* 2000). Taking all meiotic events together, the frequency of meiotic tract length changes we recorded (~2% of tracts) and the map distances to flanking markers we measured indicate that at this chromosomal locus, CAG repeat tracts do not appear to be hyperrecombinational.

Other studies on CAG repeat tracts have reported tetrads with postmeiotic segregation events (Moore *et al.* 1999). CAG repeats might yield postmeiotic segregants either by the failure to resolve heteroduplex structures that arise in recombination or by loops of repeat units that arise during meiotic S that are not resolved

TABLE 3

Exchange frequencies of markers flanking the repeat tracts

Tract configuration	No. of tetrads	Map distance (cM) *LYS5::TRP1-ARO2*	Map distance (cM) *ARO2-CYH2*
C_{78}/C_{ii28}	250	5.6	25.4
C_{78}/no tract	256	5.7	24.8
D_{71}/D_{ii28}	223	3.1	28.7
D_{71}/no tract	256	5.5	24.4
aro2::ADE2/ARO2	221[a]	4.1	25.1

[a] Two additional tetrads contained conversions from *ARO2* to *aro2::ADE2*.

until spore germination. While we looked for postmeiotic segregants, we did not observe any. We believe that we could have observed postmeiotic segregation events by our PCR assay in which a long tract formed heteroduplex with a short tract or with an allele lacking a tract as might have occurred in the conversion events (Table 2).

What makes our study unique is that we designed our chromosomes to mimic the configuration that would be present in humans with spinocerebellar ataxia type 1 where a long, uninterrupted CAG tract is opposed by a short, interrupted tract (Chung *et al.* 1993). In humans, no transfers of CAT interruptions have been observed in passage of disease alleles from parent to child (Chung *et al.* 1993). We too have found no transfer of CAT interruptions in yeast. By using marked repeats we have also been able to show that the meiotic events in yeast comprise two patterns, one that includes gene conversion between homologs and one that appears confined to one chromatid.

LITERATURE CITED

Chung, M. Y., L. P. Ranum, L. A. Duvick, A. Servadio, H. Y. Zoghbi *et al.*, 1993 Evidence for a mechanism predisposing to intergenerational CAG repeat instability in spinocerebellar ataxia type I. Nat. Genet. **5:** 254–258.

Cohen, H., D. D. Sears, D. Zenvirth, P. Hieter and G. Simchen, 1999 Increased instability of human CTG repeat tracts on artificial yeast chromosomes during gametogenesis. Mol. Cell. Biol. **19:** 4153–4158.

Freudenreich, C. H., J. B. Stavenhagen and V. A. Zakian, 1997 Stability of a CTG/CAG trinucleotide repeat in yeast is dependent on its orientation in the genome. Mol. Cell. Biol. **17:** 2090–2098.

Freudenreich, C. H., S. M. Kantrow and V. A. Zakian, 1998 Expansion and length-dependent fragility of CTG repeats in yeast. Science **279:** 853–856.

Ireland, M. J., S. S. Reinke and D. M. Livingston, 2000 The impact of lagging strand replication mutations on the stability of CAG repeat tracts in yeast. Genetics **155:** 1657–1665.

Jankowski, C., F. Nasar and D. K. Nag, 2000 Meiotic instability of CAG repeat tracts occurs by double-strand break repair in yeast. Proc. Natl. Acad. Sci. USA **97:** 2134–2139.

Maurer, D. J., B. L. O'Callaghan and D. M. Livingston, 1996 Orientation dependence of trinucleotide CAG repeat instability in yeast. Mol. Cell. Biol. **16:** 6617–6622.

Maurer, D. J., B. L. O'Callaghan and D. M. Livingston, 1998 Mapping the polarity of changes that occur in interrupted CAG repeat tracts in yeast. Mol. Cell. Biol. **18:** 4597–4604.

Miret, J. J., L. P. Brandao and R. S. Lahue, 1998 Orientation-dependent and sequence-specific expansions of CTG/CAG trinucleotide repeats in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **95:** 12438–12443.

Moore, H., P. W. Greenwell, C.-P. Liu, N. Arnheim and T. D. Petes, 1999 Triplet repeats form secondary structures that escape DNA repair in yeast. Proc. Natl. Acad. Sci. USA **96:** 1504–1509.

Paques, F., and J. E. Haber, 1999 Multiple pathways of recombination induced double-strand breaks in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **63:** 349–404.

Paulson, H. L., and K. H. Fischbeck, 1996 Trinucleotide repeats in neurogenetic disorders. Annu. Rev. Neurosci. **19:** 79–107.

Richard, G. F., G. M. Goellner, C. T. McMurray and J. E. Haber, 2000 Recombination-induced CAG trinucleotide repeat expansions in yeast involve the MRE11–RAD50–XRS2 complex. EMBO J. **19:** 2381–2390.

Schweitzer, J. K., and D. M. Livingston, 1998 Expansions of CAG repeat tracts are frequent in a yeast mutant defective in Okazaki fragment maturation. Hum. Mol. Genet. **7:** 69–74.

Schweitzer, J. K., and D. M. Livingston, 1999 The effect of DNA replication mutations on CAG tract instability in yeast. Genetics **152:** 953–963.

Spiro, C., R. Pelletier, M. L. Rolfsmeier, M. J. Dixon, R. S. Lahue *et al.*, 1999 Inhibition of FEN-1 processing by DNA secondary structure at trinucleotide repeats. Mol. Cell **4:** 1079–1085.

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