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Genetics

Clustered tRNA genes in *Schizosaccharomyces pombe* centromeric DNA sequence repeats

(fission yeast/tRNA^{Ile}/tRNA^{Ala}/tRNA^{Val}/tRNA^{Glu})

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ABSTRACT **The centromere-associated B′ and B DNA sequence repeats of *Schizosaccharomyces pombe* chromosomes I and II have been found to contain clusters of tRNA genes. The centromere II region (*cen2*) includes at least 22 tRNA genes distributed among five copies of the B sequence repeat containing genes specifying tRNA^{Ile}, tRNA^{Ala}, and tRNA^{Val}. Individual B repeats are variously associated with other tRNA genes, including those specifying tRNA^{Lys}, tRNA^{Arg}, and tRNA$_2^{Glu}$. The centromere I region (*cen1*) contains at least six tRNA genes in two copies of the B′ repeated element, including genes specifying tRNA^{Ile}, tRNA^{Ala}, and tRNA$_3^{Glu}$. Multiple tandemly arranged clusters of tRNA genes are presumably conserved due to restricted recombination frequencies in the centromere regions.**

The centromeres of eukaryotic organisms play an important role in the faithful execution of cell division. Proper chromosome segregation and chromosomal copy-number control in mitotic and meiotic divisions depend upon an intact centromere. In the budding yeast *Saccharomyces cerevisiae*, essentially all of the cis-acting sequences required for full centromere function can be accounted for within ≈125 base pairs (bp) of DNA (reviewed in refs. 1–3). There are no repeated sequences in *Sa. cerevisiae* centromeres, and transcribed genes are found immediately adjacent (4). Certain single base-pair substitutions within a highly conserved 25-bp consensus sequence (CDEIII) are sufficient to inactivate the budding-yeast centromere (5, 6).

By contrast, the centromere regions of the fission yeast *Schizosaccharomyces pombe* are characterized by the presence of several classes of repeated DNA sequences somewhat reminiscent of the heterochromatic repeated sequences in the centromere regions of higher eukaryotic chromosomes (1, 2). No polyadenylylated messenger RNA is transcribed from the several tens of kilobases (kb) of repeated DNA sequences that comprise most of the centromere regions of *S. pombe* chromosomes I (*cen1*; ≈40 kb) and II (*cen2*; ≈80 kb) (7). Structurally, *cen1* and *cen2* consist of a central core of 5–7 kb of unique DNA flanked by a large (≈30 kb) inverted repeat (Fig. 1*a*). Although the arrangements vary, most of the DNA sequence repeats within the large inverted repeat are found on all three chromosomes of *S. pombe* and are confined to the centromere regions (7, 8). A large inverted repeat structure has also been reported for the centromere region of *S. pombe* chromosome III (*cen3*; ref. 8).

The *S. pombe cen2* region is organized into an asymmetric arrangement that includes four copies of a 14-kb K–L–B repeat unit (Fig. 1*a*; ref. 9). Two of these units comprise most of the large inverted repeat that flanks the unique *cen2* central core (*cc2*; Fig. 1*a*). The *cen1* inverted repeat consists of several classes of repeated sequences that share homology

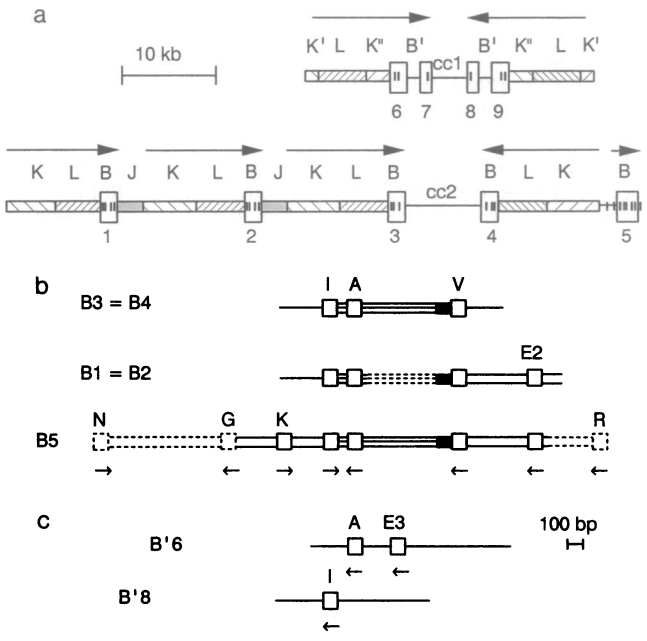

FIG. 1. (*a*) Map of *S. pombe* centromeres I and II showing locations of DNA sequence repeats K, K′, K″, L, B1–B5, and B′6–B′9. B- and B′-region sequences are indicated by boxes, long repeats by arrows, and tRNA genes by short vertical lines. Size of box indicates extent of nucleotide sequence determination, as described in the text; cc1 and cc2 indicate centromere central cores. B′7 and B′9 were not sequenced. (*b*) *cen2* B repeat regions, sequence determination (indicated by solid lines), and tRNA genes (boxes). The 964-bp tRNA cluster is found in all five B repeat regions (triple lines). B1 and B2 share identity with B3 and B4 on the left side of the cluster (single lines) and with B5 on the right side (double lines). Other sequence elements are as follows: yn1 (13) (filled box); proposed direction of tRNA transcription (arrows); DNA not sequenced in this study (broken lines). Fragments are arranged in the same orientation as in *a* except B4 is reversed to show identity with B3. Uppercase letters, tRNA genes encoding isoacceptors for asparagine (N), glycine (G), lysine (K), isoleucine (I), alanine (A), valine (V), glutamic acid (E2, ref. 14), and arginine (R). (*c*) Regions of sequence determination of *cen1* B′ regions (lines). There is no homology between *cen1* and *cen2* other than the tRNA genes marked I and A. Single-letter designations for the tRNA genes are described in *b*, with the addition of E3 (a new tRNA$_3^{Glu}$).

with the K, L, and B repeats of *cen2*. However, in *cen1* the L repeat splits the K repeat into two parts, K′ and K″, and K″ is inverted relative to K′, as compared to their arrangement in *cen2* (Fig. 1*a*). This structural motif has also been noted by Chikashige *et al.* (8), who have used the designations dhI and dgI for (K′ + L) and K″, respectively. The B repeats in *cen2* share only limited homology with the B′ repeats in *cen1*. The

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central core plus the inverted repeats of *cen1* and *cen2* each encompass about 38 kb of DNA.

The functional significance of the *S. pombe* centromeric repeated DNA sequences has not been fully determined. However, studies utilizing a functional minichromosome assay system have identified certain centromeric elements necessary for proper chromosome segregation (9–11). Artificial circular and linear minichromosomes containing DNA segments covering the entire *cen1* (≈40 kb) and *cen2* (≈80 kb) regions are fully functional mitotically in *S. pombe* and segregate properly as a fourth genetic linkage group through both meiotic divisions, demonstrating that functional centromere sequences are contained within the DNA segments that include the centromere-specific repeats (10). Additional functional studies with artificial chromosomes carrying portions of *cen1* or *cen2* have shown that the centromere-specific sequence repeats play a role in maintenance of sister chromatid attachment in meiosis I (9, 11). No relatively short DNA sequence taken from the *S. pombe* centromere region has been found to possess centromere function.

Our interest in the *cen1* and *cen2* B′ and B repeats was prompted by their conserved placement as inverted copies flanking their respective centromeric central cores; by the presence of at least one copy of B or B′ in all functional *S. pombe* centromere constructs studied thus far (9–11); and by the apparent absence of nucleosomal packaging of this region within the cell (12). In this study, we have determined the nucleotide sequences† of various fragments containing the five dispersed copies of the B repeat in *cen2* and the two core-associated B′ repeats of *cen1*. Our analysis shows that each B repeat region of *cen2* consists of a cluster of tRNA genes, oriented on both strands of the DNA. Variable numbers of tRNA genes (from three to eight) occur within or adjacent to the different B repeats in *cen2*. Two of the tRNA genes of the *cen2* B repeat cluster are also found in the *cen1* B′ repeat, along with at least one additional tRNA gene.

MATERIALS AND METHODS

Strains, Enzymes, and Molecular Cloning. The cloning of the various *S. pombe cen2* B repeats (7, 9, 15) and *cen1* B′ repeats (11) has been described. All subclones were derived originally from *S. pombe* strain Sp223, were constructed in pBluescript vectors (Stratagene), and were used to transform *Escherichia coli* strain DH5α (Bethesda Research Laboratories) by standard procedures (16). The B repeats of *cen2* were originally obtained in *E. coli* plasmid vectors by using a plasmid integration/excision strategy (9). For this study, clone opp-Sph, carrying repeat B3, was obtained by integration/excision using a 2.5-kb *Nco* I–*Eco*RI fragment from pSp3-Sph (Fig. 2*b*; ref. 9). All other subclones were obtained from plasmids previously described, as detailed below.

Repeats B3 and B4 were subcloned into pBluescript KS(+) or KS(−) as 1.5-kb *Eco*RI–*Hin*dIII fragments from plasmids opp-Sph and pSp15, respectively (Fig. 2*b*; ref. 7). B5 was subcloned as two *Hin*dIII–*Bam*HI fragments (1.9 and 2.8 kb) from pSp3Sal-dBstE (9). B′6 was subcloned as 0.7- and 1.3-kb *Spe* I fragments from plasmid 2E, and B′7 and B′8 as 1.7-kb *Eco*RI–*Cla* I and 1.5-kb *Eco*RI fragments, respectively, from pSp-9Sph (Fig. 2*a*; ref. 11). The B1 and B2 regions were subcloned from pSp1-S and pSp2-S, respectively (9); B1 as a 1.4-kb *Eco*RI fragment, B2 as 1.4-kb *Eco*RI and 3.5-kb *Eco*RI–*Kpn* I fragments. Southern analysis was performed as described (17).

Deletions, Sequencing, and Data Bank Searches. Plasmid deletions were constructed using exonuclease III and mung bean nuclease (18). Nucleotide sequence determination was performed on both strands, except where noted, by using double-stranded plasmid templates (19), the dideoxy sequencing method (20), and dITP where required (21). The nucleotide sequences of repeat regions B3 (1489 bp), B4 (1489 bp), B′6 (1959 bp), and B′8 (1397 bp) subclones were determined. The 1.9-kb fragment of the B5 region was sequenced in both directions and 0.4 kb of the adjacent 2.8-kb *Bam*HI–*Hin*dIII fragment was sequenced in one direction from the *Bam*HI site, resulting in 2303 bp of sequence from the B5 region. These sequences have been submitted to GenBank.† The 1.4-kb fragments containing repeats B1 and B2 were sequenced on both ends and the 3.5-kb *Eco*RI–*Kpn* I B2 fragment on the *Eco*RI end in one direction for 300 bases. Analysis of sequences and GenBank searches were performed with software from the University of Wisconsin Genetics Computer Group (22).

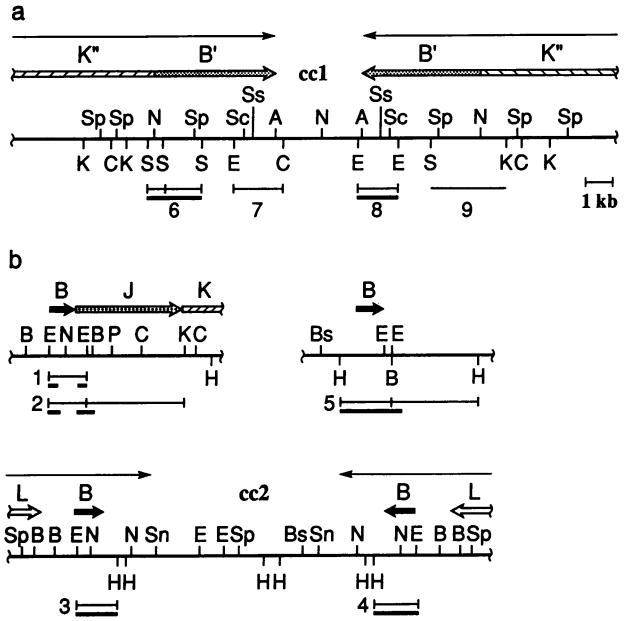

FIG. 2. Physical map of centromeric DNA of *S. pombe*: *cen1* (*a*) and *cen2* (*b*). In the B and B′ regions, the thin lines with vertical bars below the restriction map indicate various DNA fragments, subcloned as discussed in the text. The heavy lines represent regions whose sequence was determined. Thin arrows indicate extent and orientation of arms of the inverted repeat; wide arrows indicate B, B′, K, K″, J, and L repeats; cc1 and cc2, central cores. Restriction sites: A, *Aha* II; B, *Bam*HI; Bs, *Bst*EII; C, *Cla* I; E, *Eco*RI; H, *Hin*dIII; K, *Kpn* I; N, *Nco* I; P, *Pst* I; S, *Spe* I; Sc, *Sca* I; Sn, *Sna* BI; Ss, *Ssp* I; Sp, *Sph* I.

RESULTS

Organization of Centromeric B Repeats. The *S. pombe* centromeric B repeat was originally defined as any DNA sequence that hybridizes to a 2.2-kb *Bst*EII–*Bam*HI fragment from the B5 region of *cen2* (Fig. 2*b*; refs. 7, 9, and 15). There are five regions of B-hybridizing DNA in *cen2* and four in *cen1* (Fig. 1*a*). The numbering of the B repeats 1–5 in Fig. 1*a* follows the nomenclature of Clarke and Baum (9) and is continued for the *cen1* region (B′6–B′9) as shown in the figure. The B-hybridizing sequences of *cen1* (B′) are located in the inverted repeats adjacent to the central core (*cc1*) and are now known to contain only limited homology to the B repeats of *cen2*.

The structural organization of the B′ and B repeats within *cen1* and *cen2* was determined by a plasmid integration/excision strategy (9, 11). In the case of *cen2*, a 2.0-kb *Sau*3A1–*Bam*HI fragment from pSp25-OB (15) was used to

†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M57698–M57702).

target a pBR322-derived plasmid into *cen2* via homologous site-directed integration (23). This fragment carries B repeat DNA from either B3 or B4 (Fig. 1*a*). The symmetry of the central core region precludes determination of the exact origin of this fragment because pSp25-OB was obtained from a plasmid library. Southern analysis of restriction enzyme-digested genomic DNA of various integrant clones permitted identification and mapping of five classes of integrants, representing each of the five B repeats of *cen2* (9). Subsequent excision of integrated plasmid DNA with surrounding centromeric sequences from each of the five classes with a variety of restriction enzymes, followed by self-ligation and plasmid rescue in *E. coli*, yielded a set of plasmids, representative of the entire *cen2* region, from which the overall structure of the region was deduced and from which subclones of the B repeat regions were obtained for nucleotide sequencing. A similar approach was employed for the identification and cloning of the *cen1* B′ repeats (11).

Clustered tRNA Genes in the *S. pombe* B and B′ Repeats. Regions of interest within the various B repeat nucleotide sequences were identified by a variety of methods. A "moving-window" composition analysis (22) quantified regions of high G+C content, poly(A) or poly(T) tracts (A_5 and T_5), and alternating purine-pyrimidine [$(RY)_6$] residues (Fig. 3). A "window" of 100-bp width is moved in increments of 3 bp across the entire sequence and the number of times the given sequence appears within the window is recorded for each interval. The resulting plot reflects regions of the sequence with significant deviations from the average. This analysis is particularly suited to *S. pombe* DNA because of its high A+T content.

Regions of high G+C content (Fig. 3*d*) adjacent to poly(A) or poly(T) tracts (Fig. 3 *b* and *c*) suggested structural significance and ultimately identified both the location and direction of embedded tRNA genes. Poly(T) sequences, representing putative tRNA transcription terminators (24), are easily identified by this method, as are poly(A) tracts, signifying tRNA genes on the opposite strand. Not all such G+C-rich regions bounded by poly(A) or -(T), however, marked the location of tRNA genes. This method also identified a region of alternating purine and pyrimidine within each of the B and B′ repeats of the *cen* regions (Fig. 3*a*). Additional sequences of interest were found by comparison of B repeats between the two centromeres. The B regions of *cen2* were found to share homology with B′6 and B′8 of *cen1*

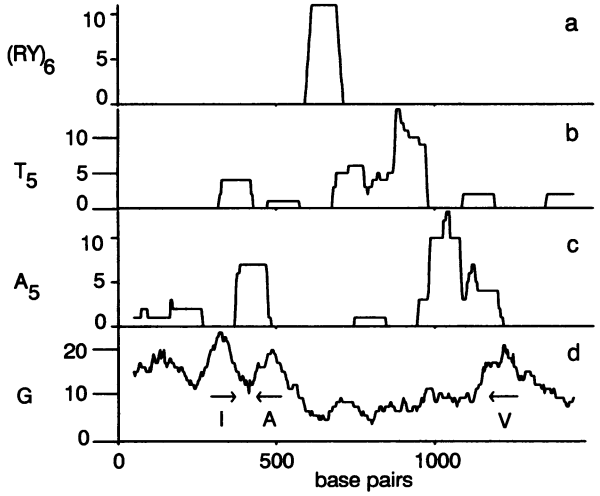

FIG. 3. "Moving window" plot of B3-region DNA sequence. Horizontal axis, nucleotide number, vertical axes, number of occurrences of (purine-pyrimidine)$_6$ (*a*), T_5 (*b*), A_5 (*c*), and guanine (*d*). Arrows, proposed direction of transcription of tRNAs. I, A, and V are tRNA genes as in Fig. 1*b*.

only to the extent of ≈100 bp (a single tRNA gene) in each case.

Sequence analysis reveals that in *cen2* all five B repeats contain a common 964-bp fragment bearing three tRNA genes ($tRNA^{Ile}$, $tRNA^{Ala}$, and $tRNA^{Val}$), identified by their anticodons. All five B repeat regions of *cen2* share perfect identity throughout the 964-bp B cluster (Fig. 1*b*). The three tRNA genes in the B5 region are flanked on both sides by a total of five additional tRNA genes. Three of these tRNA genes—$tRNA^{Lys}$, $tRNA_2^{Glu}$, and $tRNA^{Arg}$—have been cloned previously by hybridization of a plasmid library with radiolabeled tRNAs (14). Our sequence determination of the B5 region did not extend as far as the $tRNA^{Arg}$ gene previously reported, but because the exact arrangement of tRNAs in B5 is unique with respect to the other B repeats (Fig. 1*b*), and because our sequence is identical to that previously reported, it is clear we have cloned the same fragment. We have also identified two previously unreported tRNA genes in the sequence of the adjoining fragment reported by Chikashige *et al.* (8), tentatively identified as $tRNA^{Asn}$ and $tRNA^{Gly}$. The sequences of the 1.4-kb *Eco*RI fragments containing B1 and B2 were determined only on each end for 300–500 bp. On their core-proximal ends they are identical to B5 through $tRNA_2^{Glu}$ but do not contain $tRNA^{Arg}$, and on their core-distal ends they match B3 and B4 (Fig. 1*b*).

A region of alternating purine/pyrimidine is found between $tRNA^{Ala}$ and $tRNA^{Val}$ in each B repeat sequence of *cen2* (Fig. 3*a*). Of 110 possible pairs of nucleotides, 89 are adjacent purine/pyrimidine (RY or YR), including 30 consecutive nucleotides, $(ACAT)_3ATGT(AT)_7$, seen graphically as a peak in Fig. 3*a*. Each B repeat also contains two imperfect inverted repeats of 75 and 150 bp, rich in poly(A) and -(T), including three regions of 10 or more of the same base. Seen graphically in Fig. 3 *b* and *c* at 450 and 1000 bp, the inverted repeats are located between the $tRNA^{Ile}$ and $tRNA^{Ala}$ genes and between the RY repeat (Fig. 3*a*) and the $tRNA^{Val}$ gene, respectively. Similar motifs are present in *cen1*, including less extensive RY repeats in B′6 and B′8 and an inverted repeat of poly(A) and poly(T) in B′6.

The sequence yn1, originally reported by Nakaseko *et al.* (13) as appearing three times in *cen2*, appears in the region adjacent to $tRNA^{Val}$ in all five B repeats of *cen2* (Fig. 1*b*). Part of this sequence comprises the putative terminator for $tRNA^{Val}$. The yn1 sequence is not found in B′ of *cen1*.

In *cen1*, sequence analysis of the B′ repeats reveals limited homology to the B cluster. Region B′6 contains the identical $tRNA^{Ala}$ gene that appears five times in *cen2*, representing the entire sequence homology between B′6 and *cen2* (Fig. 1*c*), as well as a $tRNA_3^{Glu}$. We infer that the two tRNA genes in B′6, $tRNA^{Ala}$ and $tRNA_3^{Glu}$, also occur in B′9, because the restriction map surrounding B′9 is identical to that of B′6 in an inverted orientation (Fig. 2*a*) and because B′9 and B′6 hybridize to the *cen2* B repeat region to a similar extent.

Our sequence of B′8 identifies another copy of the same $tRNA^{Ile}$ gene that appears in *cen2* five times. This is the only tRNA gene in the 1.4-kb *Eco*RI fragment (Figs. 1*c* and 2*a*), and is the only DNA within this fragment with sequence homology to *cen2*. B′6 and B′8 share no homology with each other. Restriction analysis of a 1.6-kb *Eco*RI–*Cla* I fragment from the core-associated repeat (B′7) shows that it shares internal restriction sites for ≈1.0 kb, in an inverted repeat arrangement, with the 1.4-kb *Eco*RI–*Eco*RI fragment from the other side of the central core, B′8 (Fig. 2*a*). Southern blot analysis shows that B′7 contains $tRNA^{Ile}$ in the expected location.

Properties of Centromeric tRNA Genes. All tRNA genes described here encode putative tRNAs that possess features common to most tRNAs (Fig. 4): a 7-bp acceptor stem, an internal promoter consisting of a conserved YGG sequence in the D loop and conserved size and consensus in the TΨC

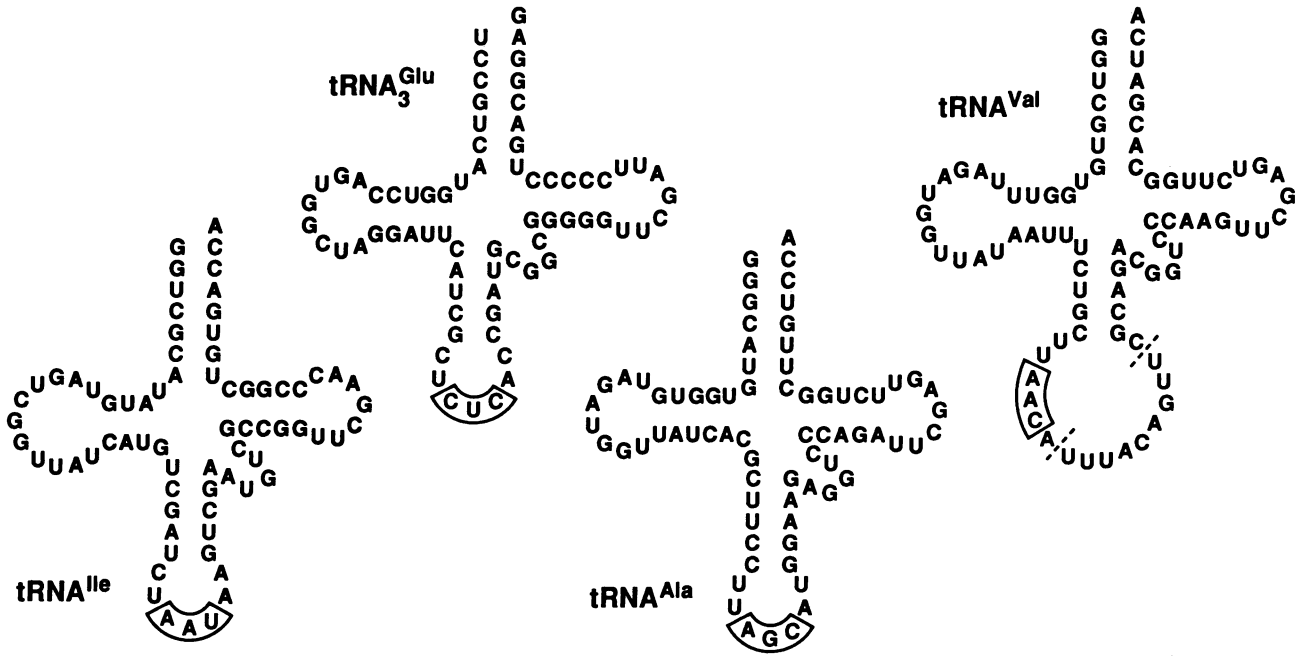

FIG. 4. Proposed secondary structure of tRNAs described in this study. Boxes indicate anticodons; dashed lines indicate a proposed intron in the anticodon loop.

loop, a 5-bp anticodon stem, and a 7-base anticodon loop containing the consensus YU-anticodon-R (24, 25). There is no CCA encoded at the 3′ end of the acceptor stem of the proposed tRNAs. Each tRNA gene is flanked on its 3′ end by a poly(T) putative transcription-terminator tract (24).

The tRNA^{Val} gene contains an intron of 9 bases in the anticodon loop (Fig. 4). The choice of the sequence AAC as the anticodon was made by noting conformity to the consensus sequence YU-anticodon-R and because the proposed intron lies at the typical location for an anticodon-loop intron, 1 base 3′ to the proposed anticodon (14, 26). Further, in most tRNAs containing introns, structures can be drawn involving base-pairing of the anticodon with other bases in the loop (26), and an AAC anticodon would be able to base-pair with a GUU found in the proposed intron.

The tRNA$_3^{Glu}$ genes of B′6 and B′9 differ from the two previously described tRNA^{Glu} genes at 13 and 15 nucleotide positions, including the anticodon (14). The tRNA^{Ala} gene has an unusual arrangement in the TΨC loop: The G·C base pair usually found at the stem–loop junction is located 1 bp from the end. Thus, the canonical GUUCRANNC becomes GAUUCRANNUC, though the size of both the stem and the loop remain unchanged.

The putative tRNAs of the centromere region have several similarities to each other and to other *S. pombe* tRNAs. tRNA^{Val} and tRNA^{Ala} have the same TΨC loop and very similar TΨC stems (Fig. 4). Each of the four tRNAs depicted in Fig. 4 has an extra arm of 4 or 5 bases between the anticodon stem and the TΨC stem. tRNA$_3^{Glu}$ has considerable homology to the *S. pombe* tRNA^{His} gene (14), including perfect matches to the anticodon stem and TΨC loop and to most of the D stem and loop.

DISCUSSION

We have detected an unusual clustering of tRNA genes embedded within the *S. pombe* centromere-specific DNA sequence repeats. The total number of tRNA genes in *Sa. cerevisiae* and *S. pombe* has been reported to be 360 and 200–300, respectively (14, 26). Assuming random distribution, these numbers would predict a density of about one tRNA gene per 40–50 kb of genomic DNA. A pairwise co-occurrence of serine- and methionine-specific tRNA genes in several *S. pombe* genomic locations has been reported (25), but no cluster has been identified as extensive as those seen in the centromere-associated repeated sequences. We have identified 22 individual tRNA genes within the five B repeats of *cen2*, and an additional 6 tRNA genes within the two B′ regions of *cen1*. It is quite likely that additional centromeric tRNA genes remain to be uncovered, since our nucleotide sequencing was confined to the B and B′ regions of *cen1* and *cen2*, and B-hybridizing sequences are known to exist in *cen3* as well (15). Thus, the fission-yeast centromeres have a tRNA gene density at least an order of magnitude higher than a random distribution would predict.

The question of why this unusual clustering of tRNA genes occurs within the centromeric DNA sequence repeats can be addressed by considering two complementary possibilities. The tRNA genes may in some way be involved in centromere function, or conversely, they could utilize some existing property of the centromere. With regard to the former possibility, tRNAs could perhaps function as special primers for controlled replication of centromere DNA, or the tRNA genes could adopt a conformation used as recognition sites for centromere proteins. The B and B′ regions with their associated tRNA gene clusters occur in the inverted repeat sequences immediately flanking the centromeric central core (Fig. 1*a*). Studies of centromeric chromatin have shown that the B and B′ regions appear to be a structural transition zone between the central core, which lacks normal nucleosomes, and K and L in the arms of the inverted repeats, which are packaged into standard nucleosomal arrays (12). Thus, the disrupted chromatin structure in the B repeat regions could be a direct consequence of the presence of actively transcribed tRNA genes. In addition, it is possible that alternating purine/pyrimidine motifs such as those observed in the B and B′ repeat regions (Fig. 3*a*), and which could possibly form Z-DNA in regions of high negative superhelical density *in vivo* (27), could play a role in nucleosomal disruption.

The centromere regions of most eukaryotes, including *S. pombe*, undergo a reduced level of meiotic recombination, signified by a low ratio of genetic to physical distance (13, 15). This reduced recombination would facilitate maintenance of multiple copies of tandemly repeated DNA sequences con-

taining tRNA genes. Thus, the high concentration of tRNA genes within the centromeric DNA sequence repeats could have evolved as a mechanism to maintain multiple gene copies of important tRNAs. The three tRNA genes contained in all five B clusters ($tRNA^{Ile}$, $tRNA^{Ala}$, and $tRNA^{Val}$) each encode the isoacceptor predicted to be the most abundant by using a codon-frequency table derived from the 4300 codons of six *S. pombe* genes specifying acid phosphatase, actin, adenylyl cyclase, H^+-ATPase, calmodulin, and cdc10 (22).

At this point, several lines of evidence indicate that the centromeric tRNA genes are transcribed. A previous report (7) has indicated that no RNA transcripts can be detected from the *S. pombe* centromeric DNA sequences, although small tRNAs might have escaped detection in that study. The B repeat sequences hybridize only to the three large genomic *Sal* I restriction fragments that contain the *S. pombe* centromeres (15), indicating the absence of B-repeat tRNAs at other genomic locations. Hybridization studies indicate that the centromeric $tRNA^{Lys}$, $tRNA^{Ile}$, $tRNA^{Ala}$, and $tRNA^{Val}$ isoacceptor genes are not found elsewhere in the genome (R.M.K., unpublished data). A plasmid clone of the $tRNA^{Lys}$ gene was originally identified by hybridization to labeled tRNAs, indicating that this centromeric B5 tRNA gene is expressed (14). In addition, the several repeated copies of the centromeric tRNA genes are absolutely conserved in sequence, indicating a functional role. The $tRNA^{Ala}$ and $tRNA^{Ile}$ genes in the *cen1* B′ region are identical in sequence to the same genes as they occur in *cen2*, although they are embedded in regions that are otherwise highly divergent. Finally, the centromere-region tRNAs possess most of the conserved structural features known to be common to all tRNAs, including the standard cloverleaf conformation, stem lengths, and loop sizes, again suggesting a functional role for these tRNAs (Fig. 4).

It is remarkable that the five copies of the B repeat in *cen2* are identical in sequence over a 1-kb region, even in the long noncoding region separating the $tRNA^{Ala}$ and $tRNA^{Val}$ genes. It is unlikely that the expression of the tRNA genes would require the conservation of this long intergenic region. On the contrary, it is known that the *S. pombe* $tRNA^{Ser}$ genes require no sequence homology in the flanking DNA to be expressed (28, 29). This virtual identity among the many copies of B suggests a centromere-associated functional role for the B repeat. These multiple copies must be maintained in perfect identity by a sequence-homogenization mechanism, such as intra- and interchromosomal gene conversion. tRNA genes on different chromosomes are known to gene-convert readily, with a frequency dependent upon the size of the homology tract (30, 31).

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