The Chromatin Structure of Centromeres from Fission Yeast: Differentiation of the Central Core that Correlates with Function

Catherine Polizzi and Louise Clarke

Department of Biological Sciences, University of California, Santa Barbara, California 93106

Abstract. We have examined the chromatin structure of centromere regions from the fission yeast *Schizosaccharomyces pombe.* The large and complex centromere regions of the *S. pombe* chromosomes encompass many kilobase pairs of DNA and contain several classes of tandemly repeated DNA sequences. The repeated sequences are further organized into a large inverted repeat flanking a central core, a conserved structural feature among all three centromeres in *S. pombe.* The nucleosomal configuration of the centromere regions is nonuniform and highly varied. Most of the centromere-specific repeated DNA sequences are packaged into nucleosomes typical of bulk chromatin. However, the central core and core-associated repeated sequences from the centromere regions of chromosomes I (*cen1*) and II (*cen2*), when present in *S. pombe*, show an altered chromatin structure, with little or no evidence of regular nucleosomal packaging. The atypical chromatin organization of the *cen2* central core is not due to transcription, as no transcripts from this region were detected. These same DNA sequences, however, are packaged into nucleosomes typical of bulk chromatin when present in a nonfunctional environment on a minichromosome in the budding yeast *Saccharomyces cerevisiae.* Because the *cen2* central core sequences themselves do not preclude regular nucleosomal packaging, we speculate that in *S. pombe* they constitute a specialized site of kinetochore protein assembly. The atypical nucleosomal pattern of the *cen2* central core remains constant during the cell cycle, with only minor differences observed for some sequences. We propose that the unusual chromatin organization of the core region forms the basis of a higher order structural differentiation that distinguishes the centromere from the chromosome arms and specifies the essential structure for centromere function.

THE centromere of a eukaryotic chromosome is a highly specialized, multifunctional region containing the kinetochore, a DNA-protein complex that mediates chromosome attachment to the spindle and participates in the movement of replicated chromatids along the spindle during mitotic and meiotic cell divisions. The centromere also holds sister chromatids together in mitotic metaphase and throughout the first meiotic division, thus ensuring proper segregation of chromosomes to daughter cells. Such a specialized chromosome function implies the existence of a characteristic chromatin structure that confers specificity for carrying out the precise, timed events of mitotic and meiotic cell divisions. Because the centromere functions in the context of a higher order nucleoprotein assembly, understanding the molecular mechanisms of centromere function and the basis of its differentiation from chromosome arms will depend in large part on the elucidation of the chromatin architecture of the centromere region.

The chromatin structure of the relatively simple centromeres from the budding yeast *Saccharomyces cerevisiae* has been well-characterized (Bloom and Carbon, 1982). The functional *S. cerevisiae* centromeres are specified by ~125 bp of DNA (Cottarel et al., 1989) and lack repeated sequences (reviewed in Clarke and Carbon, 1985; Fitzgerald-Hayes, 1987). The chromatin organization of *S. cerevisiae* centromeres is distinct, with a small nuclease-resistant core of ~200 bp flanked by DNase I hypersensitive sites and an array of positioned nucleosomes (Bloom and Carbon, 1982). Because of their small size and lack of untranscribed heterochromatic repeated DNA sequences, however, *S. cerevisiae* centromeres bear no obvious relationship to the much larger and more complex centromeres of higher eukaryotes, whose pericentric repetitive DNA sequences may span many megabases of DNA. Moreover, in contrast to many higher eukaryotic organisms, *S. cerevisiae* chromosomes fail to condense significantly during mitosis, and the mitotic spindle persists during most of the cell cycle (Pringle and Hartwell, 1981). The relatively simple *S. cerevisiae* centromere regions may represent a more primitive form of the mitotic apparatus, reflecting a permanent attachment of the microtubule spindle to the chromosome throughout cell division (Bloom et al., 1989).

The recent isolation and functional delineation of centromeres from the fission yeast *Schizosaccharomyces pombe* has provided an excellent opportunity to study their chromatin structure (Hahnenberger et al., 1989, 1991; Clarke and Baum, 1990). *S. pombe* centromeres are considerably larger and more complex than those from *S. cerevisiae*, encompass-

ing many kilobases of DNA and containing several classes of repeated DNA sequences that are not transcribed into polyadenylated RNAs (Clarke et al., 1986; Nakaseko et al., 1986; Fishel et al., 1988). The repetitive elements are further organized into a large inverted repeat structure flanking a central core, a conserved structural feature of the centromeres of all three chromosomes in *S. pombe* (Chikashige et al., 1989; Clarke and Baum, 1990; Hahnenberger et al., 1991). Structure/function studies of the centromere region of chromosome II (*cen2*) have shown that the central core or one arm of the inverted repeat alone is not sufficient for function and that at least most of the inverted repeat sequences along with the central core are needed for centromere-mediated maintenance of sister chromatid attachment in the first meiotic division, indicating that in *S. pombe* the centromere-specific repeat sequences play a role in centromere function (Clarke and Baum, 1990). These findings have been confirmed and extended in similar functional dissections of the centromere from chromosome I (*cen1*; Hahnenberger et al., 1991). In contrast to *S. cerevisiae*, *S. pombe* exhibits a more typically eukaryotic cell cycle, with distinct G1, S, G2, and M phases and division by fission (Nurse, 1985). Additionally, the *S. pombe* chromosomes condense during mitosis (Robinow, 1977; Toda et al., 1981). Thus, the chromatin structure of *S. pombe* centromeres may share characteristics of the chromatin structure of centromeres from higher eukaryotes, providing a valuable model system for detailed study.

The nucleosome is the fundamental structural subunit of chromatin (reviewed by Kornberg, 1977; van Holde, 1989). In addition to the well-understood compaction function of nucleosome packaging, nucleosomes must also accommodate other DNA-binding proteins in order to allow chromosomal processes such as transcription or replication to occur (reviewed by Gross and Gerrard, 1988; Lorch et al., 1987; Piña et al., 1990; Simpson, 1990). A recent report has shown that a portion of *S. pombe* centromeric repeat K (dgIIa) is packaged into nucleosomes and may contain nuclease hypersensitive sites (Chikashige et al., 1989). However, the fragment tested comprises a small part of the centromere region and represents only one repeat class of DNA. A complete study of nucleosomal structure of *S. pombe* centromeres must include all known repeat classes as well as the central core in order to provide a comprehensive view of the chromatin structure of the region.

In this study, we have examined the chromatin structure of the centromere regions of *S. pombe* using partial micrococcal nuclease digestion to determine nucleosome configuration. The results presented here provide evidence that the central core and the core-proximal repeats are complexed into a structure differentiated from the flanking centromeric repeat arms, and that this distinct chromatin configuration may play a role in specifying centromere function.

Materials and Methods

Strains and Media

The *S. pombe* haploid strain Sp233 (*h⁻ leu1.32 ura4.294 ade6.216*) was a gift from D. Beach (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Temperature-sensitive *S. pombe* cell division cycle (*cdc*) strains *cdc10.129(h⁻leu1.32)*, *cdc25.22(h⁺leu1.32)*, and *cdc13.117(h⁺leu1.32)* were gifts from P. Russell (Research Institute of Scripps Clinic, La Jolla, CA). *S. cerevisiae* strain AB1380/pSp(cen2)52-L that contains the entire *S. pombe cen2* region cloned in a yeast artificial chromosome vector has been described (Hahnenberger et al., 1989; Clarke and Baum, 1990). *S. pombe* cells were grown in yeast extract/glucose (YEA; Gutz et al., 1974) and *S. cerevisiae* cells in YPD (Sherman et al., 1986).

Preparation and Digestion of Nuclei

Cells were grown to logarithmic phase (~1 $\times 10^7$ cells/ml), harvested, and washed in H_2O. For conversion to spheroplasts, cells were resuspended in SH buffer (20 mM Hepes, pH 7.4, 1.2 M sorbitol, 0.5 mM PMSF) and digested with novozyme (0.5 mg/ml) and zymolase 100T (1 mg/g cells). Spheroplasts were washed in SP buffer (20 mM Pipes, pH 6.8, 1.2 M sorbitol, 1 mM PMSF) and lysed by resuspension (15 ml/gram cells) in lysis buffer (20 mM Pipes, pH 6.6, 18% Ficoll 400, 0.5 mM $MgCl_2$, 1 mM PMSF). After stirring on ice for 15 min, the mixture was vortexed and layered on a glycerol/Ficoll cushion (20 mM Pipes, pH 6.6, 20% glycerol, 8% Ficoll 400, 0.5 mM $MgCl_2$, 1 mM PMSF). Nuclei were harvested by spinning at 25,000 *g* for 40 min at 4°C.

For nuclease digestion, pelleted nuclei were resuspended in PC buffer (20 mM Pipes, pH 6.4, 0.1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 1 mM PMSF; 1 ml/g cells). After prewarming at 32°C for 3 min, micrococcal nuclease (Boehringer Mannheim Biochemicals, Indianapolis, IN) was added to 500 U/ml. Aliquots were withdrawn at 10', 20', and 40'. Digestion was stopped by adjusting samples to 2% SDS and 20 mM EDTA.

DNA Purification, Gel Electrophoresis, and Southern Transfer

DNA was purified from nuclei by extraction with phenol/chloroform/isoamyl alcohol (24:1). After ethanol precipitation, samples were resuspended in STE (10 mM Tris-HCl, pH 8, 100 mM NaCl, 1 mM EDTA) and digested with RNase A (50 μg/ml; 30 min at 37°C) and proteinase K (100 μg/ml; 15 min at 37°C). After another phenol extraction, DNA was precipitated and dissolved in STE. DNA (~5 μg per lane) was analyzed on 1.4% agarose gels (200 V, 2 3/4 h) in 1 × TBE (0.09 M Tris-borate, pH 8.3). Resolved DNA was transferred to a nylon membrane (Zeta Probe; BioRad Laboratories, Richmond, CA) following manufacturer's instructions. Blots were stripped according to manufacturer's instructions if reprobing was necessary.

DNA Probes and Hybridizations

S. pombe centromeric DNA fragments were obtained from various plasmids whose isolation has been described (Clarke and Baum, 1990; Hahnenberger et al., 1991). A 1.8-kb Hind III fragment containing the *S. pombe ura4* gene, a 0.8-kb Eco RI fragment containing a portion of *S. pombe ade6* gene, and a 0.9-kb Kpn I/Eco RI fragment containing a portion of the *S. cerevisiae LEU2* gene (Ratzkin and Carbon, 1977) were isolated from plasmids constructed in this laboratory (L. Clarke and K. Hahnenberger, unpublished observations). Isolated restriction fragments were radiolabeled with ^{32}P-dCTP (800 Ci/mMole; Amersham Corp., Arlington Heights, IL) by nick translation to a specific activity of at least 1 × 10^8 cpm/μg. Labeled DNA was hybridized to blots for 16–24 h at 65°C as described (Reed, 1982) except that the hybridization buffer contained 7% SDS, and all wash solutions contained 1% SDS.

RNA Isolation and Northern Analysis

RNA was isolated as described (Sherman et al., 1986). Poly A+ RNA was purified using a prepacked oligo(dT)-cellulose spun column (Pharmacia Fine Chemicals, Piscataway, NJ) according to manufacturer's instructions. Formaldehyde/agarose gel electrophoresis was as described (Davis et al., 1986). Resolved RNA fragments were transferred to ZetaProbe according to manufacturer's instructions.

Results

Most of the Centromeric DNA Sequence Repeats in S. Pombe Are Organized in a Typical Chromatin Structure

The structural organization of the centromere regions from *S. pombe* chromosome 1 (*cen1*) and II (*cen2*) as deduced

from a set of excision plasmids derived by site-directed homologous integration techniques is shown in Fig. 1 (Clarke and Baum, 1990; Hahnenberger et al., 1991). The 38-kb *cenI* region contains two 17-kb *cenI* repeat units composed of repeated elements, K′, K″, L, and B′, portions of which occur at all three centromeres in *S. pombe* (Fig. 1, *cenI*; Fishel et al., 1988; Hahnenberger et al., 1991). These *cenI* repeat units are further organized into an inverted repeat structure flanking a 4–5-kb central core (Fig. 1, *ccI*; Chikashige et al., 1989; Hahnenberger et al., 1991). The innermost portion of the inverted repeat structure includes a 4-kb *cenI* core-associated repeat B′ (Fig. 1, *stippled box*) that directly flanks the *cenI* central core. With the exception of two transfer RNA genes that are also present within the *cen2* B repeat (see below; Kuhn et al., 1991) the sequences comprising the *cenI* B′ repeat appear to be unique to that centromeric location in the *S. pombe* genome (Hahnenberger et al., 1991).

The *S. pombe cen2* region is over 80 kb in length and is organized into four 14-kb tandemly repeated units containing centromeric repeats K, L, and B (Fig. 1, *cen2*; Clarke and Baum, 1990). Repeat J, a portion of which is also found in the *cen3* region, is located between the three leftmost *cen2* repeat units (Fig. 1; Clarke and Baum, 1990). As in *cenI*, two of the 14-kb *cen2* repeat units are organized into a large inverted repeat that flanks the unique 7-kb *cen2* central core (Fig. 1, *cc2*). The 1.5-kb *cen2* core-associated repeats, unique to that centromere, are designated by black boxes (Fig. 1, *cen2*). The inverted repeat/central core structural motif is present at all three *S. pombe* centromeres, although the composition and organization of the repeat units appears to be different for each chromosome (Chikashige et al., 1989; Clarke and Baum, 1990; Hahnenberger et al., 1991).

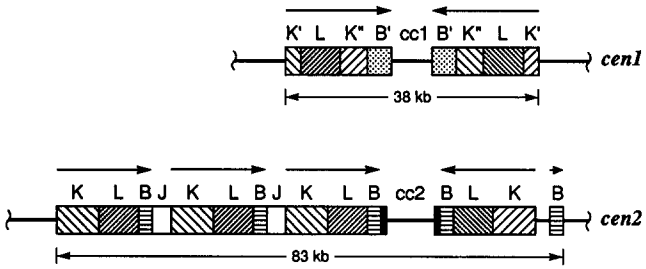

Figure 1. Schematic representation of *S. pombe* centromere regions from chromosome I (*cenI*) and II (*cen2*). The two large *cenI* repeat units, indicated by the arrows, are composed of centromeric repeats K′, K″, L, and B′. The *cenI* repeat units are further organized into an inverted repeat flanking a 4–5-kb central core (*ccI*). The stippled boxes directly flanking the central core denote the core-associated repeat B′, the major portion of which is specific to *cenI*. Derivation of the *cenI* structure is described elsewhere (Hahnenberger et al., 1991). In *cen2*, four tandemly arranged 14-kb repeat units, indicated by the arrows, are composed of centromeric repeats K, L, and B. The three leftmost repeat units are separated by repeat J. Two of the large *cen2* repeat units are further organized into an inverted repeat flanking a 7-kb central core (*cc2*). The black boxes directly flanking the central core denote the core-associated repeats (Clarke and Baum, 1990) specific to *cen2*. K′ in *cenI* is a 2.2-kb portion of the K repeat. K′ and K″ together comprise a complete K repeat (Clarke et al., 1986), except that K″ in *cenI* occurs in reverse orientation relative to K′. K′ plus the L repeat in *cenI* have also been designated dhl and K″ has been designated dgl by Chikashige et al., (1989). The only sequence homologies between repeats B (*cen2*) and B′ (*cenI*) are two transfer RNA genes (Kuhn et al., 1991).

The nucleosomal configuration of centromeric repeated elements was examined by treatment of *S. pombe* nuclei with micrococcal nuclease and electrophoretic separation of the purified DNA fragments in 1.4% agarose. Ethidium bromide staining shows a typical nucleosome ladder representative of bulk chromatin, with the repeat length between nucleosome units estimated at 155 bp (Fig. 2 *b*, *EtBr*). When the fragments were transferred to a nylon membrane and probed with a ^{32}P-labeled 1.8-kb Hind III fragment containing the *S. pombe ura4* gene (encoding orotidine-5′-phosphate decarboxylase; Gutz et al., 1974), a nuclease cleavage pattern identical to that of the ethidium bromide–stained gel was obtained, indicating that sequences from this region are packaged into nucleosomes characteristic of bulk chromatin (Fig. 2 *b*, *ura4*). Sequences from a portion of the *S. pombe ade6* gene (encoding 5-aminoimidazole ribonucleotide carboxylase; Gutz et al., 1974) used as probe gave the same result, further confirming the nucleosomal integrity of the preparations (not shown).

Genomic DNA sequences representative of the various classes of repeated DNAs found within *S. pombe* centromere regions were tested for nucleosomal configuration by hybridizing the homologous ^{32}P-labeled fragments to Southern blots of nucleosome ladders (Fig. 2 *b*). A restriction map of a portion of the *cen2* region containing a *cen2* repeat unit, with probe fragments designated as numbered bars under the figure, is shown in Fig. 2 *a*. Most repeated sequences are common to all three centromeres. Fragments 2r and 4r (Fig. 2 *a*), representative of the K and L repeats, respectively, revealed a nucleosomal pattern indistinguishable from that of the *ura4* gene (Fig. 2 *b*, *2r* and *4r*). The variation of intensity of nucleosomal bands hybridizing to fragment 2r was presumably due to a nonuniform accessibility of internucleosomal cutting sites. When the entire 6.4-kb K repeat (*3r*; Fig. 2 *a*) was used as a probe, however, this variation of nucleosomal band intensity disappeared (not shown). Although sequences hybridizing to repeats K and L are found at all three centromeres (Fishel et al., 1988), genomic sequences containing these repeats behave as a single class with respect to their chromatin structure, indicating that these repeats are packaged into nucleosomes typical of bulk chromatin in all their locations in the genome. Fragment 5r, another portion of the L repeat (Fig. 2 *a*), and fragment 6r, a *cen2*-specific repeat (Fig. 2 *a*), also displayed a typical nucleosome pattern (not shown).

A probe representing centromeric repeat J, which is present at *cen2* and *cen3* but not at *cenI* (*1r*, Fig. 2 *a*; Clarke and Baum, 1990), showed a slightly higher background hybridization to DNA between the nucleosomal bands, indicating that at least some sequences within the J repeat lack typical nucleosomal structure (Fig. 2 *b*, *1r*). The relative sharpness of one of the nucleosome bands (indicated by an asterisk in Fig. 2) reflects a more discrete size class of protected fragments than in bulk chromatin, perhaps due to more site-specific cleavage by the enzyme.

Genomic DNA sequences hybridizing to a 600-bp fragment containing the *cen2* centromeric B repeat displayed an unusual nuclease cleavage pattern (*7r*; Fig. 2, *a* and *b*). A broad band representing a class of fragments ranging in size from 300–400 bp was detected, while the basic nucleosomal

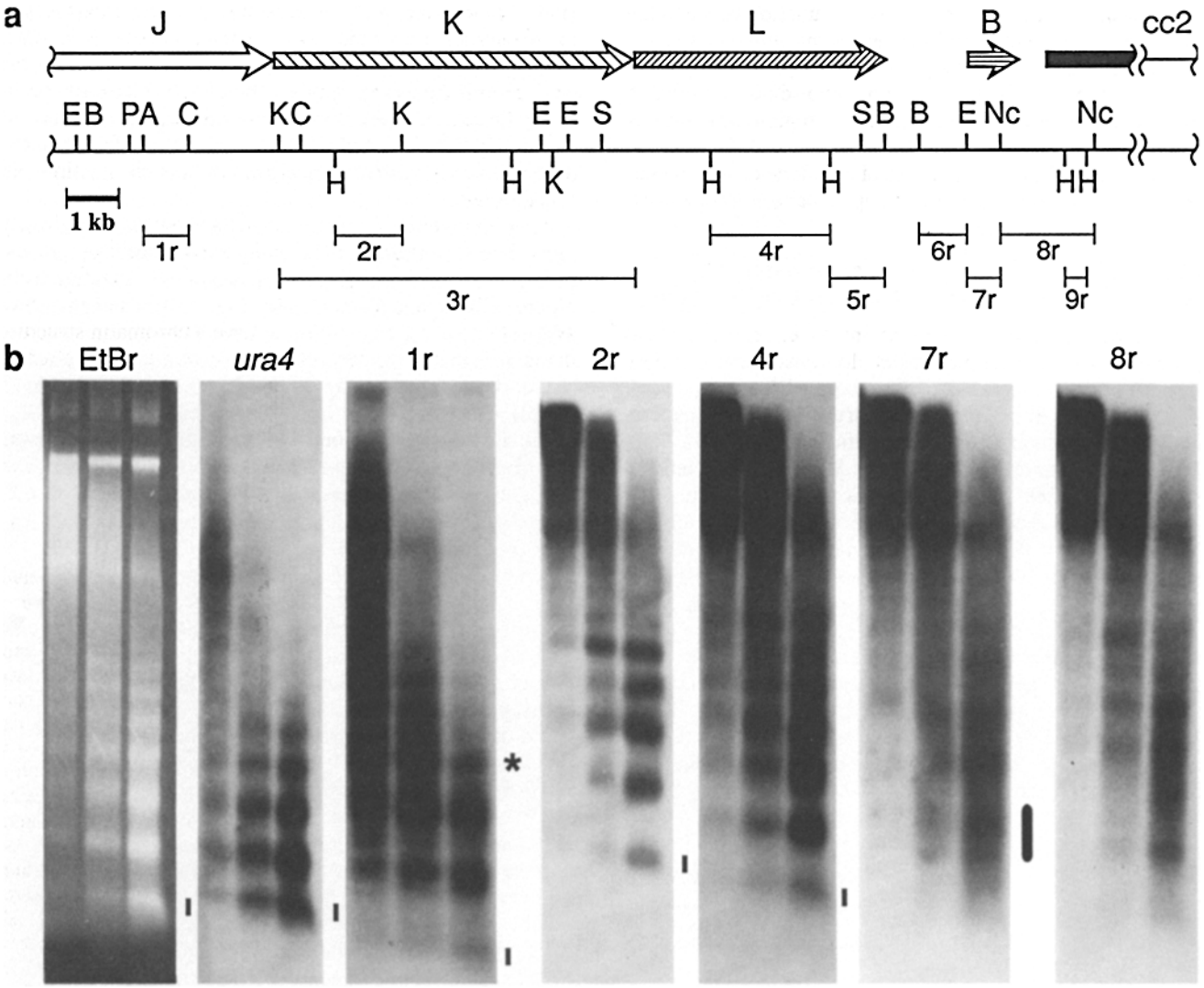

Figure 2. Chromatin structure of *S. pombe* centromeric repeated sequences. Isolated *S. pombe* nuclei were treated with micrococcal nuclease, and the deproteinized DNA fragments electrophoresed, blotted to a nylon membrane, and probed with ^{32}P-labeled fragments representing the various centromeric repeat classes. (*a*) Restriction map of a *cen2* repeat unit with the spacer J repeat. DNA fragments used as probes are designated by the numbered bars under the figure. Unless otherwise noted, sequences within probe fragments occur at all three centromeres: *1r*, 0.7 kb Ava I/Cla I, J repeat, present on *cen2* and *cen3*; *2r*, 1.1 kb Hind III/Kpn I, K repeat; *3r*, 6.4 kb Cla I (Clarke et al., 1986), K repeat; *4r*, 2.1 kb Hind III, L repeat; *5r*, 0.9 kb Hind III/Bam HI, L repeat; *6r*, 0.9 kb Bam HI/Eco RI, *cen2*-specific repeat; *7r*, 0.6 kb Eco RI/Nco I, B repeat; *8r*, 1.5 kb Nco I, the core-distal portion of which is present in *cen3*; *9r*, 0.3 kb Hind III, *cen2*-specific repeat and part of the core-associated repeat. Restriction sites are as follows: *A*, Ava I; *B*, Bam HI; *C*, Cla I; *E*, Eco RI; *H*, Hind III; *K*, Kpn I; *Nc*, Nco I; *P*, Pvu II; *S*, Sph I. (*b*) Southern blots of *S. pombe* nucleosome ladders probed with various centromeric repeated sequences. The panel designated EtBr is an ethidium bromide–stained gel of a nucleosome ladder showing the micrococcal nuclease cleavage pattern of bulk chromatin. Subsequent lanes within each panel represent longer times of micrococcal nuclease digestion. A 1.8-kb Hind III fragment containing the *ura4* gene was used as a control probe for chromatin preparation integrity (*ura4*). Numbers above each blot denote the fragment used as probe; repeats *j* (*1r*), *K* (*2r*), *L* (*4r*), and *B* (*7r*) are shown. Fragment 8r is on the core-proximal side of repeat B and spans the boundary between the distal repeat arms and the core-associated repeats. The asterisk next to blot 1r indicates a discrete size class of protected fragments. The rounded bar next to blot 7r denotes the nonnucleosomal class of protected fragments generated by nuclease digestion and displayed by this probe. Small bars mark monomer positions in the various blots, where applicable.

subunit, the monosome, was not (Fig. 2 *b*, *7r*). This class of presumably protected fragments is indicated as a bar next to the autoradiograph. Additionally, there was considerable background hybridization, making the bands difficult to distinguish. When the same blot was stripped and reprobed with *ura4* sequences, a noncorrespondence was established between the bands observed for the 600-bp probe and those of *ura4*, although the repeat distance, in base pairs, between protected fragments was the same for both probes (not shown). This singular digestion pattern was not present when deproteinized DNA was cut by micrococcal nuclease (not shown), confirming that the observed pattern of nuclease cutting is chromatin specific. The results indicate that most of the sequences hybridizing to this B-containing fragment are not packaged into a typical nucleosomal configuration but may be associated with another class of protein.

Fragment 8r, which maps adjacent to the core-proximal B

repeat sequence in *cen2* and spans the junction between the core-associated repeats and the remaining sequences in the inverted repeat, displayed a partially disrupted nucleosomal configuration, with high background hybridization. When a small (300 bp) subfragment from this region was used as probe (*9r*; Fig. 2 *a*), a nucleosomal pattern was found, indicating that small localized groups of positioned nucleosomes could account for the partially disrupted pattern (not shown).

The cen2 and cen1 Central Cores and Associated Repeats Lack a Regular Nucleosomal Configuration

Genomic DNA sequences comprising the entire *cen2* central core region were examined for nucleosomal structure (Fig. 3). Sequences from the nonhomologous 7-kb *cen2* central core showed an altered chromatin structure, with the micrococcal nuclease cleavage pattern often yielding a uniform smear rather than the characteristic ladder pattern, indicating complete lack of a regular periodic nucleosomal array (Fig. 3 *b*, *4c2* and *7c2*). In some nuclei preparations a faint or grossly smeared ladder was detectable (Fig. 3 *b*, *9c2*). However, in no instance did any of the sequences from the *cen2* central core show regular, complete nucleosome packaging typical of bulk chromatin. Subsequent probing of stripped blots with *ura4* sequences confirmed the presence of the nucleosomal structure typical of bulk chromatin in all preparations.

Approximately 1.5 kb of repeated sequences that directly flank the *cen2* central core are unique to that centromere and centromeric location within the limits of hybridization techniques (Clarke and Baum, 1990; Fig. 3 *a*). The *cen2* core-associated repeats were found to have a chromatin structure distinct from the other repetitive centromeric elements, resembling instead the structure of the central core (Fig. 3 *b*, *1c2*).

The disrupted nucleosomal pattern of the central core was also observed in *cen1* (Fig. 4). Fragments representing most of the *cen1* central core and the core-associated repeat B′

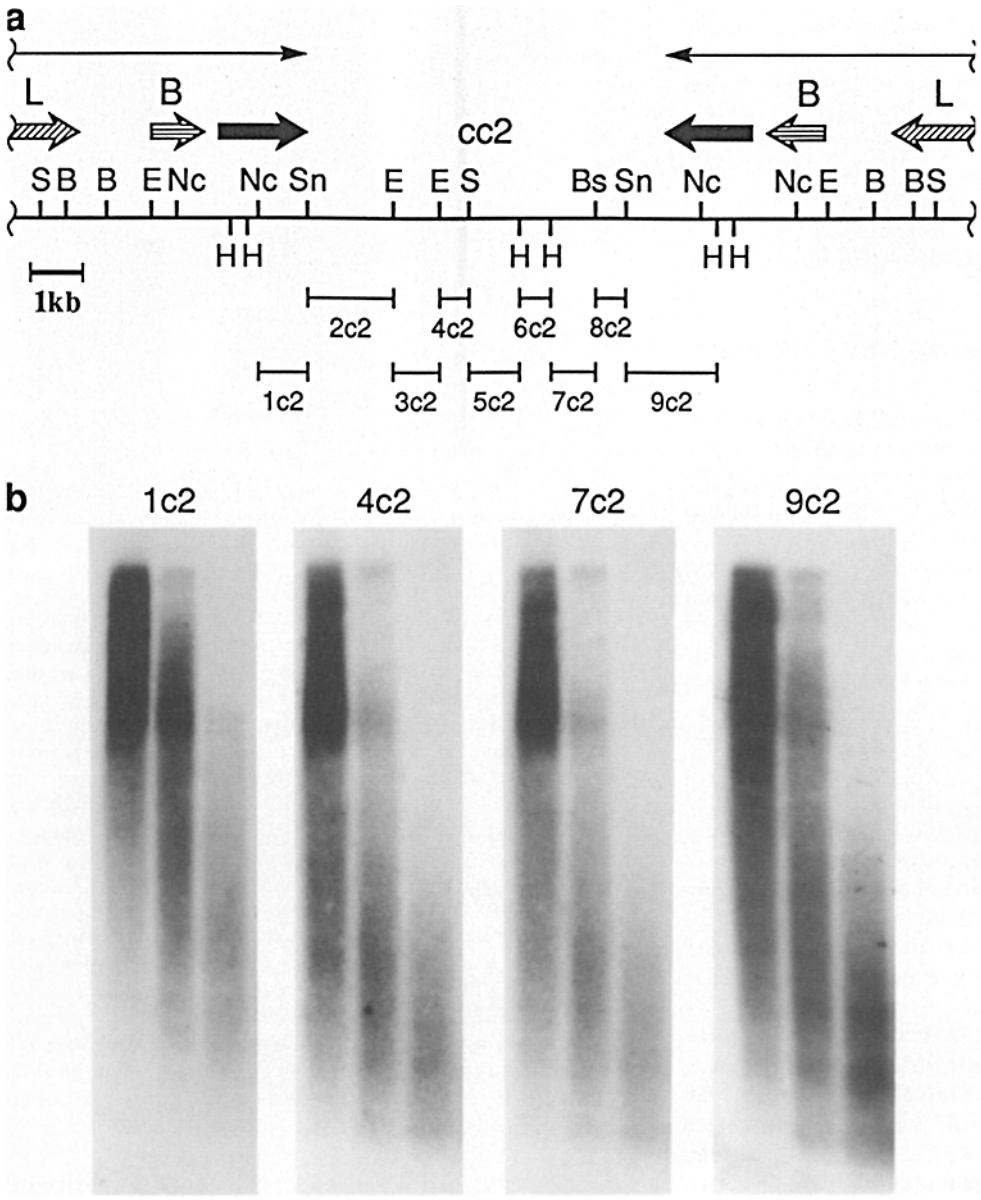

Figure 3. Chromatin structure of the *cen2* central core and associated repeats. (*a*) Restriction map of the *cen2* central core and core-associated repeats. The *cen2* central core and its associated repeats encompass ~10 kb, with 7 kb of nonhomology, as determined by EM and blot hybridization analysis (Clarke and Baum, 1990). Fragments used as probes are shown as numbered bars under the figure. Unless otherwise noted, all sequences tested are unique to the central core regional of *cen2*: *1c2*, 0.8 kb Nco I/Sna BI, core-associated repeat; *2c2*, 1.7 kb Sna BI/Eco RI; *3c2*, 0.9 kb Eco RI, *4c2*, 0.6 kb Sph I/Eco RI; *5c2*, 0.9 kb Sph I/Hind III; *6c2*, 0.5 kb Hind III; *7c2*, 0.8 kb Hind III/Bst EII; *8c2*, 0.6 kb Bst EII/Sna BI; *9c2*, 1.7 kb Sna BI/Hind III, the core-distal portion of which contains part of the core-associated repeat. Restriction sites are: *Bs*, Bst EII; *Sn*, Sna BI; remaining designations are as in Fig. 2. (*b*) Micrococcal nuclease cleavage patterns of *cen2* central core sequences. *S. pombe* nuclei were isolated and digested with micrococcal nuclease as described in Materials and Methods, and the purified DNA fragments separated and probed with nick-translated central core restriction fragments. Numbers above the blots denote the probes utilized.

were used as probes on nucleosome ladders as described above (Fig. 4, *a* and *b*). The *cen1* central core exhibited the same uniform nuclease cleavage pattern as *cen2*, with only the two smallest nucleosome subunits, the monosome and the disome, barely detectable (Fig. 4 *b*, *3c1* and *5c1*). None of the *cen1* central core or core-associated repeat B′ probes tested showed a nucleosome ladder typical of bulk chromatin or of the distal repeated sequences. Thus, the unusual chromatin configuration of the central core regions is conserved among at least two of the three *S. pombe* centromeres. We conclude that the *cen1* and *cen2* central cores and their associated repeats are complexed in a chromatin structure distinct from the flanking repeated centromeric sequences.

The Atypical Chromatin Structure of the cen2 Central Core Region Is Not Due to Transcription

Examination of certain transcribed regions of chromosomes using nucleases has shown increased sensitivity and even loss of nucleosome structure as chromatin becomes transcriptionally active (Wu et al., 1979; Bellard et al., 1982; van Holde, 1989). We determined whether the *cen2* central core sequences are transcribed by probing *S. pombe* total and poly A+ RNA with two fragments representing the left and right halves of the central core (Fig. 5, lanes *1–4*). No transcripts from any part of the central core region were detected. The presence of the 1.2-kb *ura4* transcript confirmed the integrity of both preparations (Fig. 5, lanes *5* and *6*). The results indicate that the unusual chromatin structure of the *cen2* central core region is not related to transcriptional activation.

The cen2 Central Core Chromatin Structure Remains Unchanged during the Cell Cycle

In testing the *cen2* central core sequences for structural variability by probing more than one nuclei preparation with a given fragment, it was observed that in some instances the degree of disruption varied somewhat between preparations, ranging from complete absence of to a barely detectable nucleosome ladder. Because nuclei were isolated from asynchronous cultures, the faint nucleosome ladder seen with some nuclei preparations as well as the variability of the degree of disruption for a given sequence from one nuclei preparation to another might be explained by the presence of a small population of cells with *cen* central cores organized in a typical nucleosomal configuration. The possibility that this small population could represent cells in a certain point in the cell cycle led us to ask whether the chromatin structure of the central core remains constant during the cell cycle. Moreover, the proportion of cells in mitosis in a given asynchronous population of *S. pombe* cells is low (Toda et al., 1981), making it difficult to assess whether any significant differences exist in chromatin structure while the chromosomes are condensed and engaged in mitotic spindle attachment.

We examined the chromatin structure of the *cen2* central core region during the cell cycle by using temperature-sensitive cell division cycle (*cdc*) mutants to arrest cells at G1 (*cdc10.129*; Nurse et al., 1976), G2 (*cdc25.22*; Fantes, 1979), and M (*cdc13.117*; Nurse et al., 1976). Nuclei were isolated as described, digested with micrococcal nuclease,

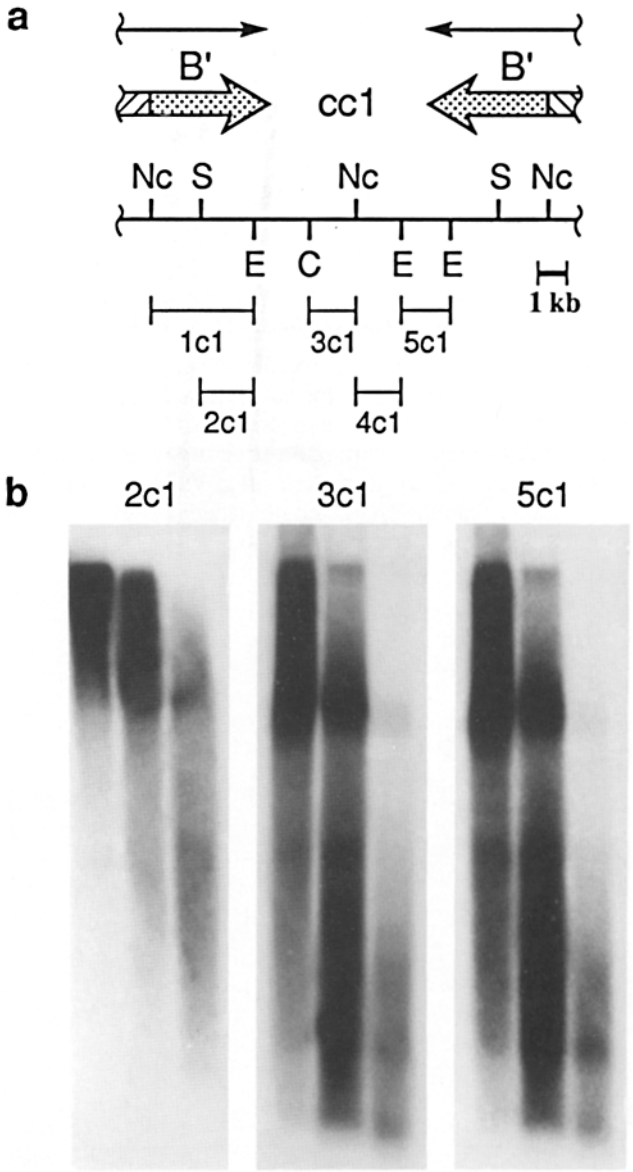

Figure 4. Chromatin structure of the *cen1* central core and associated repeats. (*a*) Restriction map of the central core and associated repeats from *cen1*, with fragments used as probes delineated by the numbered bars under the figure. The *cen1* central core and its associated repeats encompass ~12 kb, including a 4–5-kb central core. The detailed structural analysis of the *cen1* region is described elsewhere (Hahnenberger et al., 1991). Unless otherwise noted, fragments tested are unique to the *cen1* central core region: *1c1*, 3 kb Nco I/Eco RI; *2c1*, 1.7 kb Sph I/Eco RI; *3c1*, 1.5 kb Cla I/Nco I; *4c1*, 1.5 kb Nco I/Eco RI; *5c1* 1.5 kb Eco RI. Fragments 2c1 and 3c1 hybridize to *cen3*; fragments 1c1 and 5c1 share with the *cen2* B repeat an identical $tRNA^{Ala}$ gene and $tRNA^{Ile}$ gene, respectively (Kuhn et al., 1991). The boundaries of the nonhomologous central core with the B′ repeat sequences have not been mapped precisely, but occur within the 1.5-kb Eco RI fragment (*5c1*) and the 1.6-kb Eco RI/Cla I fragment. Restriction site designations are as in Fig. 2. (*b*) Micrococcal nuclease cleavage patterns of *cen1* central core region sequences. Numbers above the autoradiographs indicate the fragment tested.

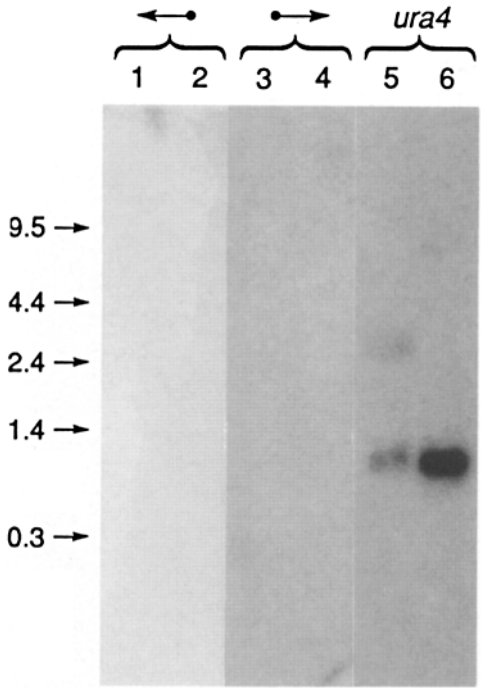

Figure 5. Northern analysis showing lack of transcription from the *cen2* central core. 10 μg of total RNA and 2 μg of poly A+ RNA isolated from *S. pombe* were fractionated by 1% agarose/formaldehyde gel electrophoresis, blotted to a nylon membrane, and probed with fragments representing the left half (←) and the right half (→) of the *cen2* central core region. Lanes *1* and *2*, total and poly A+ RNA probed with a 4-kb Nco I/Sph I fragment containing the left half of the central core; lanes *3* and *4*, total and poly A+ RNA probed with a 7.5-kb Sph I/Bam IH fragment containing the right half of the central core as well as core-associated repeats, the B repeat, and part of the L repeat; lanes *5* and *6*, total and poly A+ RNA probed with the 1.8-kb Hind III fragment containing the *S. pombe ura4* gene. Position of size markers (in kb) are indicated.

and the Southern blots probed with DNA fragments from the *cen2* central core region. All sequences from the *cen2* central core region were tested. Probing a blot of a nucleosome ladder with the *ura4* fragment confirmed the nucleosomal integrity of the preparations (not shown). Genomic DNA sequences hybridizing to the *cen2* fragment containing the B repeat (*7r*; Fig. 2*a*) showed no change during the cell cycle (Fig. 6 *a*); similarly, repeat K sequences (*3r*; Fig. 2 *a*) remained unchanged (not shown). The variability previously observed for central core sequences in asynchronous populations of cells was no longer observed, with multiple preparations of all three *cdc* strains giving consistent results. For all points in the cell cycle that were tested, the *cen2* central core showed no detectable periodic nucleosomal arrays (Fig. 6*b*). However, one sequence located near the center of the central core and contained in a 0.9-kb Eco RI fragment (*3c2*; Fig. 3 *a*) displayed a slightly different nuclease cleavage pattern at mitosis in the strain carrying the *cdc13.117* allele. Two darker band-like areas were present (Fig. 6 *c*, *arrows*) whose positions did not correspond to those of the bulk genomic nucleosomal bands. These bands observed with digestion of chromatin from arrested *cdc13.117* nuclei were not observed when digested chromatin isolated from wild type cells arrested at mitosis using the microtubule inhibitor thiabendazole was probed with fragment 3c2 (not shown). Because neither thiabendazole-arrested wild type cells nor temperature-arrested strains carrying the *cdc13.117* allele contain a mitotic spindle, the unusual cleavage pattern may be a consequence of the chromosomal hypercondensation characteristic of temperature-arrested strains carrying the *cdc13.117* allele (Nasmyth and Nurse, 1981). In conclusion, we observe no apparent change in the chromatin structure of centromeric repeats or *cen2* central core sequences at the points tested throughout the cell cycle.

The S. pombe cen2 Central Core Is Packaged into Nucleosomes When Present in S. cerevisiae

Studies have shown that certain DNA sequences preclude the formation of nucleosomes due to their inability to bend around the core particle (Soumpasis, 1985; Travers, 1987). The lack of periodic nucleosomal structure for the central core region could be due to a sequence constraint that prevents stable association of histones; alternatively, there may be recognition proteins in *S. pombe* that bind to the core sequences, such as those proteins specifically involved in kinetochore function, preventing a fully packaged nucleosomal structure. To understand the basis of the atypical chromatin structure of the central core region, we examined the chromatin conformation of these sequences when present on a minichromosome in the budding yeast *S. cerevisiae*. The entire *cen2* region has been isolated as a 100-kb Sal I fragment cloned into an artificial chromosome in *S. cerevisiae* (Hahnenberger et al., 1989; Clarke and Baum, 1990). In this system, the *cen2* sequences are nonfunctional and are also completely removed from their functional context and protein environment, providing an excellent opportunity to determine whether the observed atypical chromatin structure of the central core region in *S. pombe* is correlated with function.

Nuclei from *S. cerevisiae* strain AB1380/pSP(*cen2*)52-L containing an artificial chromosome carrying the entire *cen2* region (Hahnenberger et al., 1989; Clarke and Baum, 1990) were isolated and digested with micrococal nuclease as described. Two fragments representing the center of the *cen2* central core (*3c2*; Fig. 3 *a*) and one end of the *cen2* central core encompassing some core-associated repeats (*9c2*; Fig. 3 *a*) were used as probes to examine the genomic chromatin conformation. Both central core sequences were found to be packaged into nucleosomes typical of bulk chromatin. A comparison of *S. pombe* and *S. cerevisiae* nucleosome ladders probed with one of the fragments (3c2) is shown in Fig. 7. The nucleosomal pattern observed for both *cen2* central core fragments was indistinguishable from *S. cerevisiae* bulk chromatin as revealed by a portion of the *LEU2* gene (Fig. 7). We conclude that the DNA sequences of the central core region do not in themselves preclude regular nucleosome formation, and that the distinct chromatin conformation of this region in *S. pombe* most likely correlates with centromere function.

Discussion

As a first step toward an analysis of the relationship between chromatin organization and *S. pombe* centromere function, we have examined the centromere regions in terms of the

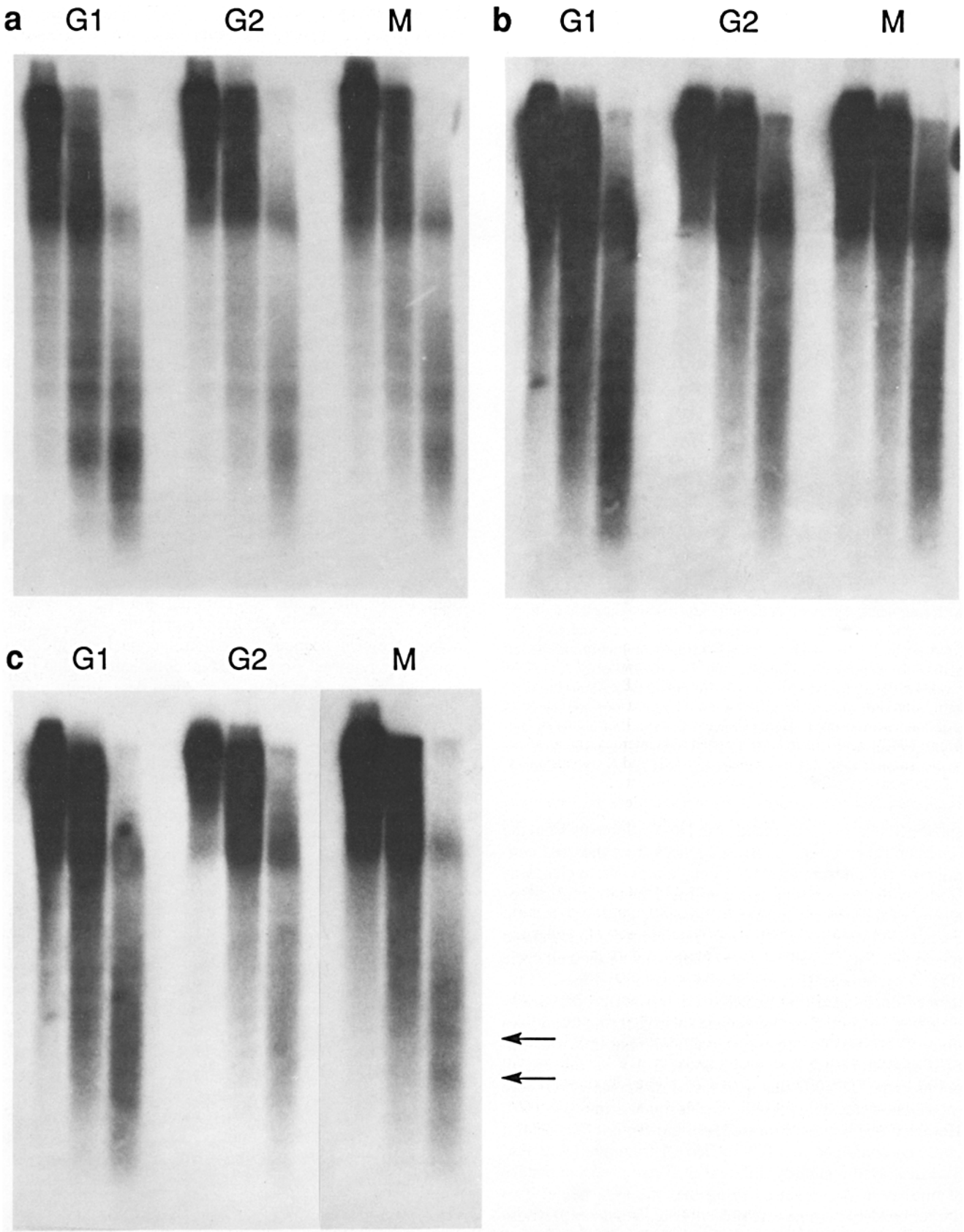

Figure 6. The *cen2* central core chromatin configuration at various points of the cell cycle. Cells from *S. pombe* strains *cdc10.129* (G1 arrest), *cdc25.22* (G2 arrest), and *cdc10.113* (M arrest) were grown at the permissive temperature (25°C) to early logarithmic phase and arrested by temperature shift to 35°C for 3–4 h. Cell synchrony was confirmed by microscopic examination. Nuclei were isolated as described, digested with micrococcal nuclease, and the resultant fractionated DNA fragments probed with representative fragments from

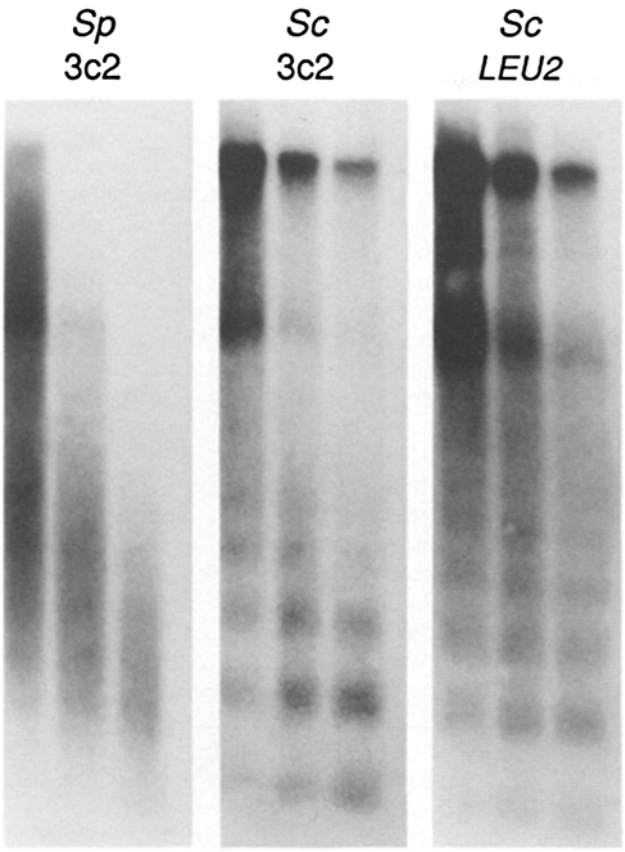

Figure 7. *S. pombe* centromeric central core sequences are packaged into nucleosomes when present in *S. cerevisiae*. Nuclei from *S. pombe* strain Sp223 and *S. cerevisiae* strain AB1380/pSp(*cen2*)-52-L, containing the entire *S. pombe cen2* region carried on a yeast artificial chromosome (Hahnenberger et al., 1989; Clarke and Baum, 1990), were isolated and digested with micrococcal nuclease. Nucleosome ladders from *S. pombe* (*Sp* 3c2) and *S. cerevisiae* (*Sc* 3c2) were probed with *cen2* central core fragment 3c2 (Fig. 3 *a*). As a control, a duplicate blot of an *S. cerevisiae* nucleosome ladder was probed with a 0.9-kb Eco RI/Kpn I *S. cerevisiae LEU2* fragment.

most fundamental structural unit of chromatin, the nucleosome. We find that the chromatin structure of the *S. pombe* centromeres is nonuniform, with the nucleosomal configuration of the central core and its associated repeats distinct from the flanking arms of the inverted repeat.

Most of the centromere-specific DNA sequence repeats are packaged into nucleosomes typical of bulk chromatin. Our observation that repeat K is organized into a nucleosome array agrees with a previous report by Chikashige et al. (1989). The centromere regions of higher eukaryotes are characterized by the presence of long runs of highly repetitive untranscribed sequences, termed pericentric heterochromatin (Singer, 1982; Miklos, 1985). The pericentric heterochromatic sequences examined thus far have shown a typical nucleosomal structure, although the role of these sequences in centromere function is unclear (Bostock et al., 1976; Lipchize and Axel, 1976; Brutlag, 1980; van Holde, 1989). In *S. pombe*, repeated sequences at the centromere have been implicated in contributing to an important meiotic centromere function, the maintenance of sister chromatid attachment during the first meiotic division (Clarke and Baum, 1990; Hahnenberger et al., 1991). It can be inferred that on the nucleosomal level of chromatin structure, this centromere function does not require a particular configuration that is significantly different from the rest of the genome.

A repeated DNA sequence found in five locations in the *cen2* region (*7r*; Fig. 2 *a*) was found to have a unique chromatin configuration, with a class of DNA fragments of ~300–400 bp constituting a protected region, rather than the canonical 146-bp monosome (Fig. 2 *b*, *7r*). As this digestion pattern is chromatin specific, we postulate that at least part of this centromeric repeat sequence is complexed into a nucleoprotein structure distinct from nucleosomes, although the presence of higher order nucleosomal bands implies this structure is flanked by nucleosomes. It is unclear whether the observed protection is conferred by histones or by other sequence-specific protein components. The atypical nuclease protection pattern may relate to the structural organization of two transfer RNA genes found within this sequence (Kuhn et al., 1991). No *cen1*-derived sequence analyzed shows this nuclease cleavage pattern, implying that the observed nonnucleosomal pattern may be *cen2* specific.

While most of the centromeric repeat sequences are packaged into nucleosomes typical of bulk chromatin, the central core and its associated DNA sequence repeats show little or no evidence of the nuclease cleavage pattern indicative of regularly spaced nucleosomes (Figs. 3 and 4). This lack of a distinct ladder pattern is conserved in both *cen1* and *cen2* central core regions, even though their sequences differ within the limits of detection by hybridization (Clarke and Baum, 1990; Hahnenberger et al., 1991). It has been reported that the chromatin structure of certain genes alters during active transcription, leading to a loss or smearing of the nucleosome ladder (Wu et al., 1979; Levy and Noll, 1981; Bellard et al., 1982). No transcripts were detected from the *cen2* central core region, however, indicating that a process other than transcription gives rise to the observed central core chromatin structure.

The differentiated chromatin structure of the central core and its associated repeats extends to the flanking B and B′ repeats, which appear to form the approximate boundary of the atypical chromatin structure of the central core region. The core-proximal fragments containing B′ sequences from *cen1* (*1cl*, *2cl*; Fig. 4) display a nucleosomal configuration similar to that of the central core itself (*3cl*, *4cl*, *5cl*; Fig.

the central core region. Fragments were used as probes on multiple chromatin preparations of each *cdc* strain to confirm reproducibility of results. Central core fragments used as probes were the same as described in the legend to Fig. 3*a*, with the following exception: fragment 2c2 was further subdivided into a 1-kb Sna BI/Nde I and a 0.7-kb Nde I/Eco RI fragment. Each panel represents nuclease cleavage patterns displayed by the following *cen2* fragments at specified points in the cell cycle (*G1*, *G2*, *M*): (*a*) 7r (Fig. 2 *a*), containing the *cen2* B repeat; (*b*) 5c2 (Fig. 3 *b*) from the *cen2* central core; (*c*) 3c2 (Fig. 3 *a*) from the *cen2* central core. Arrows point to bands that may represent specific nuclease cleavage sites in M.

4). In *cen2*, the B repeat, which is located on either side of the core and in three other locations dispersed throughout this centromere region, shows a highly smeared digestion pattern in addition to the unusual nonnucleosomal configuration already discussed (*7r*; Fig. 2 *b*); furthermore, the core-distal adjacent fragment from *cen2* (*6r*; Fig. 2 *a*) is packaged into regularly spaced nucleosomes (not shown). In *cen1* and *cen2*, B′ and B repeat sequences are in similar positions flanking the central core region, and all partially or fully functional *S. pombe* minichromosomes constructed and analyzed so far include at least one copy of either B or B′ (Clarke and Baum, 1990; Hahnenberger et al., 1991).

The uniform nuclease digestion pattern of the central core region might be explained by a completely random distribution of nucleosomes throughout the central core region. The appearance of faint ladders in some preparations argues against this possibility. Alternatively, the nucleosomes of the region could be very unstable and dissociate during isolation of the nuclei, rendering the DNA of the central core region uniformly accessible to the enzyme. Another explanation for the observed uniform nuclease cleavage pattern is that there are fewer nucleosomes associated with the region in order to allow binding of kinetochore proteins. It has been shown that nucleosomes can prevent formation of transcription initiation complexes as well as prevent replication initiation (Lorch et al., 1987; Workman and Roeder, 1987; Simpson, 1990). Conversely, histone depletion has been shown to activate some genes, presumably by nucleosome loss allowing certain proteins to bind regulatory sequences (Han and Grunstein, 1988). With an average nucleosomal repeat length of 155 bp, the linker length in *S. pombe* is on the order of 10 bp. Such an exceedingly small linker size may place constraints on sequence accessibility for DNA binding proteins, especially when a large complex assembly forms, as may be the case for the kinetochore. It seems likely that the central core and core-associated repeats of *S. pombe* centromeres comprise a kinetochore protein assembly site, although our experiments do not directly address this point.

The possibility that there may be transient nucleosome formation in the *cen2* central core was investigated by the cell cycle experiments. No marked changes were seen in the *cen2* central core chromatin structure when *S. pombe* cells were arrested at G1, G2, and M; furthermore, no nucleosome ladders were detected for central core sequences at these points. Similar studies in *S. cerevisiae* have shown that the distinct chromatin structure of the centromere region stays constant during the cell cycle, which may reflect a permanent attachment of the centromere to the spindle (Bloom et al., 1989). The mitotic spindle of *S. pombe* assembles and disassembles during the cell cycle (McCully and Robinow, 1971), and thus we do not take the constant chromatin structure we observed as indicative of permanent attachment of the kinetochore to the spindle, although the kinetochore structure per se may remain relatively constant during the cell cycle. Our results are consistent with the idea that the *S. pombe* centromere regions maintain specific chromatin configurations during the cell cycle; however, this does not rule out the possibility of other, more global morphological changes that would not be detected by extensive nuclease digestion.

On the surface, there appears to be little resemblance between the centromeric chromatin structures of *S. pombe* and *S. cerevisiae*. Both centromeres are similar in that centromeric sequences are complexed in a chromatin structure distinct from the surrounding sequences, although the basis of that distinction is quite different for each organism. In *S. cerevisiae*, the 200-bp nuclease-resistant core particle associated with functional centromere sequences is considered to represent the fundamental structural unit of the kinetochore (Bloom and Carbon, 1982). Given the dramatic differences between *S. pombe* and *S. cerevisiae* centromere regions in terms of their size and complexity, as well as the fundamental difference in the mode of cell division, it is not surprising that their centromeric chromatin structures should differ commensurately. This dissimilarity in terms of chromatin structure supports the idea that the budding and fission yeast centromeres represent two distinct classes of kinetochores (Carbon and Clarke, 1990; Clarke and Baum, 1990).

Is the irregular nucleosome packaging of the central core a functional configuration? The conservation of this unusual chromatin structure in both *cen1* and *cen2* supports this idea. In *S. cerevisiae*, a distinct chromatin structure is associated with centromere function; mutations abolishing centromere function also show loss of the characteristic protected core (Saunders et al., 1988). When the *S. pombe cen2* central core sequences are removed from their protein milieu and functional context by placing the entire *cen2* region on an artificial chromosome in *S. cerevisiae*, the sequences are organized into nucleosomes typical of bulk *S. cerevisiae* chromatin. This observation provides compelling evidence that the atypical central core chromatin structure in *S. pombe* is not a result of a generic sequence incompatibility with core histones, but is due to a specific interaction of *S. pombe* proteins with the central core sequences. The results conversely suggest that *S. cerevisiae* lacks those proteins that specify the distinct central core chromatin structure found in *S. pombe*. In fact, *S. pombe* centromeres do not appear to function in *S. cerevisiae*, as no dicentric behavior is observed when the artificial chromosomes carrying both *S. cerevisiae CEN4* and complete *S. pombe* centromeric sequences are propagated in *S. cerevisiae* (Hahnenberger et al., 1989). These results strongly suggest that the *S. pombe* central core and its associated repeats delineate a domain within the centromere whose functional specificity gives rise to the observed atypical chromatin structure.

An obvious role for the differentiated central core region would be to provide a site for spindle fiber attachment. Although the functional specificity of the central core has not been determined, the central core and its associated repeats are included in all functional minichromosomes examined so far (Clarke and Baum, 1990; Hahnenberger et al., 1991). This minimal functional requirement corresponds to the observed chromatin structure, which differentiates the central core and the associated repeats from the rest of the inverted repeat structure. In mammals, the kinetochores are structurally delineated from the flanking chromosome arms, embedded in domains of pericentric heterochromatin. The central core regions in *S. pombe* perhaps represent a smaller, simpler version of the higher eukaryotic kinetochore, providing the basis for the higher order structural differentiation that serves as a recognition site in condensed metaphase chromosomes.

The complex centromere regions of *S. pombe* probably do not contain a single small functional segment embedded in a mass of inert repeated DNA sequences but rather may be

large cooperative structures composed of several interacting functional domains with many protein components. Our initial chromatin studies represent a first step toward the dissection of this large multifunctional region.

We thank Paul Russell (Research Institute of Scripps Clinic, La Jolla, CA) for kindly providing us with *S. pombe cdc* strains, and Kerry Bloom (University of North Carolina, Chapel Hill, NC) and Bill Garrard (University of Texas Health Science Center, Dallas, TX) for helpful discussions. We also thank Karen Hahnenberger (Syva Corp., Palo Alto, CA) for providing data before publication and Mary Baum, John Carbon (University of California, Santa Barbara, CA), and Karen Hahnenberger for helpful discussions and critical reading of the manuscript.

This research was supported by a Public Health Service grant (GM-33783) from the National Institutes of Health.

Received for publication 6 June 1990 and in revised form 26 September 1990.

References

Bellard, M., G. Dretzen, F. Bellard, P. Oudet, and P. Chambon. 1982. Disruption of the typical chromatin structure in a 2500 base-pair region at the 5' end of the actively transcribed ovalbumin gene. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:223–230.

Bloom, K. S., and J. Carbon. 1982. Yeast centromere DNA is in a unique and highly ordered structure in chromosomes and small circular minichromosomes. *Cell.* 29:305–317.

Bloom, K., A. Hill, M. Kenna, and M. Saunders. 1989. The structure of a primitive kinetochore. *Trends Biochem. Sci.* 14:75–86.

Bostock, C. J., S. Christie, and F. T. Hatch. 1976. Accessibility of DNA in condensed chromatin to nuclease digestion. *Nature (Lond.).* 262:516–519.

Brutlag, D. L. 1980. Molecular arrangement and evolution of heterochromatic DNA. *Annu. Rev. Genet.* 14:121–144.

Carbon, J., and L. Clarke. 1990. Centromere structure and function in budding and fission yeasts. *The New Biologist.* 2:10–19.

Chikashige, Y., N. Kinoshita, Y. Nakaseko, T. Matsumoto, S. Murakami, O. Niwa, and M. Yanagida. 1989. Composite motifs and repeat symmetry in *S. pombe* centromeres: direct analysis by integration of NotI restriction sites. *Cell.* 57:739–751.

Clarke, L., and M. P. Baum. 1990. Functional analysis of a centromere from fission yeast: a role for centromere-specific repeated DNA sequences. *Mol. Cell. Biol.* 10:1863–1872.

Clarke, L., and J. Carbon. 1985. The structure and function of yeast centromeres. *Annu. Rev. Genet.* 19:29–56.

Clarke, L., H. Amstutz, B. Fishel, and J. Carbon. 1986. Analysis of centromeric DNA in the fission yeast *Schizosaccharomyces pombe*. *Proc. Natl. Acad. Sci. USA.* 83: 8253–8257.

Cottarel, G., J. A. Shero, P. Hieter, and J. H. Hegeman. 1989. A 125 base-pair *CEN6* DNA fragment is sufficient for complete meiotic and mitotic centromere functions in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 9:3342–3349.

Davis, L. G., M. Dibner, and J. F. Battey. 1986. Formaldehyde gel for electrophoretic separation of RNA and northern blot. *In* Basic Methods in Molecular Biology. Elsevier, New York. 143–146.

Fantes, P. 1979. Epistatic gene interactions in the control of division in fission yeast. *Nature (Lond.).* 279:428–430.

Fishel, B., H. Amstutz, M. P. Baum, J. Carbon, and L. Clarke. 1988. Structural organization and functional analysis of centromeric DNA in the fission yeast *Schizosaccharomyces pombe*. *Mol. Cell. Biol.* 8:754–763.

Fitzgerald-Hayes, M. 1987. Yeast centromeres. *Yeast.* 3:187–200.

Gross, D. S., and W. T. Gerrard. 1988. Nuclease hypersensitive sites in chromatin. *Annu. Rev. Biochem.* 57:159–197.

Gutz, H., H. Heslot, U. Leupold, and N. Loprieno. 1974. *Schizosaccharomyces pombe*. *In* Handbook of Genetics; R. D. King, editor. Plenum Publishing Corp, New York. 395–446.

Hahnenberger, K. M., M. P. Baum, C. M. Polizzi, J. Carbon, and L. Clarke. 1989. Construction of functional artificial minichromosomes in the fission yeast *Schizosaccharomyces pombe*. *Proc. Natl. Acad Sci. USA.* 86:577–581.

Hahnenberger, K. M., J. Carbon, and L. Clarke. 1991. Identification of DNA regions required for mitotic and meiotic functions within the centromere of *Schizosaccharomyces pombe* chromosome I. *Mol. Cell. Biol.* In press.

Han, M., and M. Grunstein. 1988. Nucleosome loss activates yeast downstream promoters in vivo. Cell. 55:1137–1145.

Kornberg, R. 1977. Structure of chromatin. *Annu. Rev. Biochem.* 46:931–954.

Kuhn, R. M., L. Clarke, and J. Carbon. 1991. Clustered tRNA genes in *Schizosaccharomyces pombe* centromeric DNA sequence repeats. *Proc. Natl. Acad. Sci. USA.* In press.

Levy, A., and M. Noll. 1981. Chromatin fine structure of active and repressed genes. *Nature (Lond.).* 289:198–203.

Lipchize, L., and R. Axel. 1976. Restriction endonuclease cleavage of satellite DNA in intact bovine nuclei. *Cell.* 9:355–364.

Lorch, Y., J. W. LaPointe, and R. D. Kornberg. 1987. Nucleosomes inhibit the initiation of transcription but allow chain elongation with the displacement of histones. *Cell.* 49:203–210.

McCully, E., and C. Robinow. 1971. Mitosis in the fission yeasts *Schizosaccharomyces pombe*: a comparative study with light and electron microscopy. *J. Cell Sci.* 9:475–507.

Miklos, G. L. G. 1985. Localized highly repetitive DNA sequences in vertebrate and invertebrate genomes. *In* Molecular Evolutionary Genetics. J. R. MacIntyre, editor. Plenum Publishing Corp., New York. 241–313.

Nakaseko, Y., Y. Adachi, S. Funahashi, O. Niwa, and M. Yanagida. 1986. Chromosome walking shows a highly repetitive sequence present in all centromere regions of fission yeast. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:1011–1021.

Nasmyth, K., and P. Nurse. 1981. Cell division cycle mutants altered in DNA replication and mitosis in the fission yeast *Schizosaccharomyces pombe*. *Mol. & Gen. Genet.* 182:119–124.

Nurse, P. 1985. Cell cycle control genes in yeast. *Trends Genet.* 3:51–55.

Nurse, P., P. Thuriaux, and K. Nasmyth. 1976. Genetic control of the cell division in the fission yeast *Schizosaccharomyces pombe*. *Mol. & Gen. Genet.* 146:167–178.

Piña, B., U. Brüggemeier, and M. Beato. 1990. Nucleosome positioning modulates accessibility of regulatory proteins to the mouse mammary tumor virus promoter. *Cell.* 60:719–731.

Pringle, J., and L. Hartwell. 1981. The *Saccharomyces cerevisiae* cell cycle. *In* The Molecular Biology of the Yeast Saccharomyces. J. Strather, E. Jones, and J. Broach, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 97–142.

Ratzkin, B., and J. Carbon. 1977. Functional expression of cloned yeast DNA in *E. coli*. *Proc. Natl. Acad. Sci. USA.* 74:487–491.

Reed, S. 1982. Preparation of product-specific antisera by gene fusion: antibodies specific for the product of the yeast cell-division-cycle gene *CDC28*. *Gene (Amst.).* 20:255–265.

Robinow, E. F. 1977. The number of chromosomes in *Schizosaccharomyces pombe*: light microscopy of stained preparations. *Genetics.* 87:491–497.

Saunders, M., M. Fitzgerald-Hayes, and K. Bloom. 1988. Chromatin structure of altered yeast centromeres. *Proc. Natl. Acad. Sci. USA.* 85:175–179.

Sherman, F., G. R. Fink, and J. B. Hicks. 1986. Total yeast RNA isolation. *In* Laboratory Course Manual for Methods in Yeast Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 143–144.

Simpson, R. T. 1990. Nucleosome positioning can affect the function of a cis-acting DNA element in vivo. *Nature (Lond.).* 343:387–389.

Singer, M. 1982. Highly repeated sequences in mammalian genomes. *Int. Rev. Cytol.* 76:67–112.

Soumpasis, D. M. 1985. Effects of DNA sequence and conformation on nucleosome formation. *J. Biomol. Struct. & Dyn.* 3:1–10.

Toda, T., M. Yamamoto, and M. Yanagida. 1981. Sequential alterations in the nuclear chromatin region during mitosis of the fission yeast *Schizosaccharomyces pombe*: video fluorescence microscopy of synchronously growing wild-type and cold-sensitive cdc mutants by using a DNA-binding fluorescent probe. *J. Cell Sci.* 52:271–287.

Travers, A. A. 1987. DNA bending and nucleosome positioning. *Trends Biochem. Sci.* 12:108–112.

van Holde, K. E. 1989. Chromatin. Springer-Verlag, New York. 497 pp.

Workman, S. L., and R. G. Roeder. 1987. Binding of transcription factor TFIID to the major late promoter during in vitro nucleosome assembly potentiates subsequent initiation by RNA polymerase II. *Cell.* 51:613–622.

Wu, C., Y.-C. Wong, and S. C. R. Elgin. 1979. The chromatin structure of specific genes. II. Disruption of chromatin structure during gene activity. *Cell.* 16:807–814.