# Chromosome Condensation and Sister Chromatid Pairing in Budding Yeast

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*Abstract.* We have developed a fluorescent in situ hybridization (FISH) method to examine the structure of both natural chromosomes and small artificial chromosomes during the mitotic cycle of budding yeast. Our results suggest that the pairing of sister chromatids: (*a*) occurs near the centromere and at multiple places along the chromosome arm as has been observed in other eukaryotic cells; (*b*) is maintained in the absence of catenation between sister DNA molecules; and (*c*) is independent of large blocks of repetitive DNA commonly associated with heterochromatin. Condensation of a unique region of chromosome XVI and the highly repetitive ribosomal DNA (*rDNA*) cluster from chromosome XII were also examined in budding yeast. Interphase chromosomes were condensed 80-fold relative to B form DNA, similar to what has been observed in other eukaryotes, suggesting that the structure of interphase chromosomes may be conserved among eukaryotes. While additional condensation of budding yeast chromosomes were observed during mitosis, the level of condensation was less than that observed for human mitotic chromosomes. At most stages of the cell cycle, both unique and repetitive sequences were either condensed or decondensed. However, in cells arrested in late mitosis (M) by a *cdc15* mutation, the unique DNA appeared decondensed while the repetitive *rDNA* region appeared condensed, suggesting that the condensation state of separate regions of the genome may be regulated differently. The ability to monitor the pairing and condensation of sister chromatids in budding yeast should facilitate the molecular analysis of these processes as well as provide two new landmarks for evaluating the function of important cell cycle regulators like $p^{34}$ kinases and cyclins. Finally our FISH method provides a new tool to analyze centromeres, telomeres, and gene expression in budding yeast.

REPLICATED chromosomes (sister chromatids) are paired and condensed prior to their segregation in mitosis. The pairing between sister chromatids is needed to establish a stable bipolar attachment of sister chromatids to microtubules emanating from opposite poles of the mitotic spindle (Ault and Nicklas, 1989). This bipolar attachment in turn helps ensure that sister chromatids segregate from each other during anaphase. In addition, the dissolution of pairing appears to be a key event in governing the onset of chromosome segregation, more commonly known as the "metaphase to anaphase transition." The condensation of chromosomes shortens their length which may serve to minimize the entanglement of chromosomes with one other while they are being moved by the mitotic apparatus. This shortening may also help ensure that the lagging ends of segregating chromosomes are moved far enough away from the plane of cell division so as not to be cleaved by cytokinesis. Hence, both pairing and condensation of sister chromatids appear to be essential for proper chromosome segregation.

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Our understanding of the molecular basis of these two chromosomal features is founded upon relatively few observations. Classical cytological and genetic analyses strongly support the idea that sister chromatids are paired when they are in close proximity during or immediately following DNA replication. This contrasts with homolog pairing during meiosis where widely separated chromosomes move together. Sister chromatid pairing is observed along the entire length of the chromosome although the most persistent pairing occurs within blocks of heterochromatin (Lica et al., 1986; Cooke et al., 1987; Sumner, 1991; Carmena et al., 1993). Two mechanisms have been proposed for generating the cohesion between sisters which results in their pairing. Sister chromatids may be bound together by proteins such as the INCENP and CLiP proteins which are localized between sister chromatids while they are paired (Cooke et al., 1987; Rattner and Lin, 1988). Pairing has also been postulated to involve the catenation of sister DNA molecules (Murray and Szostak, 1985) since subsequent segregation of chromatids requires the action of topoisomerase II (Uemura et al., 1987; Holm et al., 1985). Finally, recent evidence suggests that the dissolution of pairing involves in some way the ubiquitin-dependent proteolysis (Holloway et al., 1993).

The condensation of chromosomes appears to involve at least the core histones (H2A, H2B, H3, and H4) and histone H1 (Wolffe, 1991). Presumably many additional factors have yet to be identified. The correlation between phosphorylation of H1 by p34 kinase and chromosome condensation has implicated H1 as a possible component for regulating condensation. However, several observations over the past few years have made this a less attractive possibility (Roth and Allis, 1992; Oshumi et al., 1993). Clearly many aspects of the molecular basis for the pairing and condensation of sister chromatids remain unknown.

Molecular and genetic studies have provided at least three ways to manipulate chromosome structure in vivo in the budding yeast *Saccharomyces cerevisiae*. First, the DNA component of chromosomes has been analyzed intensively. For example, a physical map of contiguous DNA sequences comprising almost the entire genome has been constructed using cosmid and lambda-based libraries (Olson et al., 1986; Link and Olson, 1991; Riles et al., 1993). Furthermore, the DNA sequences that participate in the formation of functional centromeres, telomeres, and origins of replication have also been determined (Fitzgerald-Hayes, 1987; Szostak and Blackburn, 1982; Shampay et al., 1984; Fangman and Brewer, 1991). With this knowledge it has been possible to alter the DNA structure of endogenous chromosomes as well as to generate novel artificial chromosomes (Murray and Szostak, 1983; Surosky et al., 1986; Shero et al., 1991). Second, the characterization of chromosomal structural proteins has been initiated. For example, the genes encoding the core histone proteins have been identified (Wallis et al., 1980; Choe et al., 1982; Smith and Murray, 1983). The few copies of the histone genes in yeast relative to other eukaryotes have made them much more amenable to detailed genetic analysis (Smith and Stirling, 1988; Megee et al., 1990). Third, the cell cycle of this organism has been studied extensively. These studies have led to the isolation of cell division cycle (*cdc*)[1] mutants that arrest at discrete stages of the cell cycle when grown at their nonpermissive temperature (Hartwell et al., 1973; Pringle and Hartwell, 1981). The stage of arrest has been assessed by such parameters as the onset and/or completion of DNA replication, duplication of spindle pole bodies, formation of spindles, spindle elongation, and segregation of replicated genomes. The use of these mutants and cell cycle inhibitors to generate cultures in which all cells are synchronized to a defined stage of the cell cycle should greatly facilitate an analysis of cell cycle dependent changes in chromosome structure.

With the ability to manipulate chromosome structure in vivo, budding yeast would appear to be an ideal organism to pursue a molecular analysis of chromosome condensation and sister chromatid pairing. However, the fundamental drawback with using this organism for such studies has been the absence of a good assay for monitoring chromosome condensation and sister chromatid pairing in situ. Individual chromosomes of budding yeast are not visible with conventional cytological techniques such as phase microscopy or fluorescent dyes that bind DNA in a sequence independent manner. This could simply reflect the small size of chromosomes (250–2,000 kb) (Carle and Olson, 1985) or perhaps the absence of conventional chromosome structure observed in other eukaryotes. Alternative approaches including electron microscopy and sedimentation analysis have also been used as a means to address chromosome structure in this yeast (Wintersberger et al., 1975; Peterson and Ris, 1976; Gordon, 1977; Pinon, 1978, 1979). Unfortunately, the low resolution of these methods and the variable results make their usefulness rather limited.

Fluorescence in situ hybridization (FISH) involves the hybridization of DNA or RNA probes to chromosomal DNA, and the visualization of those probes with appropriate fluorochromes. Improvements in this technology has enabled investigators to visualize the position of repetitive and unique chromosomal DNA sequences within fixed cells of many species. This methodology has been used to study the timing of replication of specific chromosomal loci in differentiating mammalian cells (Selig et al., 1992), the behavior of telomeres and centromeres in mouse cells and the fission yeast, *Schizosaccharomyces pombe* (Funabiki et al., 1993; Vourc'h et al., 1993), and the pairing of homologs in meiotic chromosome spreads of budding yeast (Scherthan et al., 1992).

These successes encouraged us to develop a FISH method for budding yeast which would allow us to address fundamental questions about chromosome condensation and sister chromatid pairing during vegetative growth. In this study we show that our FISH method is capable of determining the position of both unique and repetitive chromosomal DNA sequences in the nucleus of *S. cerevisiae*. We report the use of this method to characterize sister chromatid pairing and condensation in dividing cells and cells arrested at different stages of the cell cycle. The results obtained from our studies not only provide valuable insights into these processes but also demonstrate the value of using budding yeast as a model system to examine these processes.

## Materials and Methods

### Reagents

Zymolyase T100 was obtained from ICN Biomedicals, Inc. (Costa Mesa, CA) or Seikagaki. Polylysine was obtained from Sigma Chemical Co. (St. Louis, Missouri) and prepared as a 3 mg/ml solution in distilled $H_2O$. Restriction enzymes and proteinase K were obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). RNAse was obtained from Sigma Chemical Co. and made DNase free as previously described (Maniatis et al., 1982). Nick translations were carried out using the BioNick™ Labelling System from GIBCO BRL (Gaithersburg, MD). The in situ hybridization kit (Signal Amplification Reagent Set) was obtained from ONCOR (Gaithersburg, MD). Slides for in situ hybridization were obtained from Roboz Surgical (Rockville, MD). Yeast strains are listed in Table I.

### Procedure for Making Probes for In Situ Hybridization

Probes were labeled with biotin by nick translation using the BioNick™ Labelling System with two modifications. First, large DNA templates were digested with restriction enzymes to generate a mixture of fragments shorter than 3 kb before nick translation. Second, the optimal amount of DNA in the nick translation was determined empirically for each probe by varying the amount of template from 30 to 300 ng. Digoxigenin probes were also made using BioNick™ Labelling System except that 5 μl 10× nick translation buffer (0.5 M Tris-HCl, pH 7.8, 50 mM MgCl, 100 mM mercaptoethanol, 100 μg/ml BSA) and 1 μl digoxigenin labeling mixture from Boehringer Mannheim Biochemicals were substituted for the 5 μl of 10× dNTP mix from the kit.

The source of DNA templates used to make probes and the chromosomal position of the corresponding homologous sequences were as follows. The

1. *Abbreviations used in this paper*: cdc, cell division cycle; FISH, fluorescence in situ hybridization; noco, nocodazole; rDNA, ribosomal DNA.

*Table I. Yeast Strains*

| | |
|---|---|
| DS50B-4C | *Mat a CUP1 ura3-52 trp1Δ1 leu2Δ1 lys2-801 his3Δ200 ade2-101* YAC12#1 |
| VG810-15-1 | *Mat α CUP1 ura3 his3Δ200* |
| DK4521-001 | *Mat α leu2 ade2 ade3 his7 can1 sap3 gal1* |
| DK4523-042 | *Mat a cdc4-1 leu2 ade2 ade3 his7 can1 sap3 gal1* |
| DK5203-3-4 | *Mat a cdc14-1 leu2 ade2 ade3 his7 can1 sap3* |
| DK4536-151 | *Mat α cdc15-1 leu2 ade2 ade3 his7 can1 sap3 ura1* |
| H20c1a5 | *Mat a cdc20-1 his7 hom3 can1 sap3 gal1* |
| DK4522-282 | *Mat a cdc28-1 leu2 ade2 ade3 his7 can1 sap3 ura1 gal1* |
| DS50B | *Mat a/Mat α CUP1/CUP1 leu2Δ1/leu2Δ1 his3Δ200/his3Δ200 ade2-101/ade2-101 lys2-801/lys2-801 ura3-52/ura3-52 trp1Δ1/trp1Δ1 Cen6ΔCEN11-LEU2/CEN6* YAC12#1/YAC12#2 |
| BP5050 | *Mat a/Mat α leu2/LEU2 ade2/ADE2 ade3/ADE3 his7/his7 can1/can1 sap3/sap3 gal1/gal1 HOM3/hom3 CYC2/cyc2* |
| BP5306 | *MAT a/MAT α cdc4-1/cdc4-1 leu2/leu2 ade2/ade2 ade3/ade3 his7/his7 can1/can1 sap3/sap3 gal1/gal1* |
| DK205 | *Mat a/Mat α cdc6-1/cdc6-1 leu2/LEU2 ade2/ADE2 ade3/ADE3 his7/his7 can1/can1 sap3/sap3 gal1/gal1 hom3/HOM3* |
| DK204 | *Mat a/Mat α cdc7-1/cdc7-1 leu2/LEU2 ade2/ADE2 ade3/ADE3 his7/his7 can1/can1 sap3/sap3 gal1/gal1 hom3/HOM3 ura1/URA1* |
| DK206 | *Mat a/Mat α cdc9-1/cdc9-1 leu2/Leu2 ade2/ADE2 ade3/ADE3 his7/his7 can1/can1 sap3/sap3 gal1/gal1 hom3/HOM3 ura1/URA1* |
| DK207 | *Mat a/Mat α cdc13-1/cdc13-1 leu2/LEU2 ade2/ADE2 ade3/ADE3 his7/his7 can1/can1 sap3/sap3 gal1/gal1 hom3/HOM3* |
| DK210 | *Mat a/Mat α cdc14-1/cdc14-1 leu2/LEU2 ade2/ADE2 ade3/ADE3 his7/his7 can1/can1 sap3/sap3 gal1/gal1 hom3/HOM3* |
| DK211 | *Mat a/Mat α cdc15-1/cdc15-1 leu2/LEU2 ade2/ADE2 ade3/ADE3 his7/his7 can1/can1 sap3/sap3 gal1/gal1 hom3/HOM3 ura1/URA1* |
| DK208 | *Mat a/Mat α cdc16-1/cdc16-1 leu2/LEU2 ade2/ADE2 ade3/ADE3 his7/his7 can1/can1 sap3/sap3 gal1/gal1 hom3/HOM3* |
| DK209 | *Mat a/Mat α cdc17-1/cdc17-1 leu2/LEU2 ade2/ADE2 ade3/ADE3 his7/his7 can1/can1 sap3/sap3 gal1/gal1 hom3/HOM3 ura1/URA1* |
| DK229 | *Mat a/Mat α cdc20-1/cdc20-1 LEU2/leu2 ade2/ADE2 ade3/ADE3 his7/his7 can1/CAN1 sap3/SAP3 gal1/gal1 hom3/HOM3 ura1/URA1* |
| DK201 | *Mat a/Mat α cdc28-1/cdc28-1 leu2/LEU2 ade2/ADE2 ade3/ADE3 his7/his7 can1/can1 sap3/sap3 gal1/gal1 hom3/HOM3* |
| BP5071 | *Mat a/Mat α cdc31/cdc31 leu2/leu2 trp1/trp1 his3 or 7/his3 or 7 ura3/ura3 gal1/gal1 hom3/HOM3* |

Strain DS50B was obtained from the Hieter lab. All other strains came from this laboratory.

probe from chromosome I was a 40-kb contiguous unique DNA sequence made from a mixture of chromosome I inserts from the following plasmids: 12 kb from YCp50-2C, 9.6 kb from pLF183, 8.8 kb from pLF184, and 8.6 kb from pLF185 (Wickner et al., 1987; Riles et al., 1993). The centromere proximal edge of this probe is 20 kb from *CEN1* (see Fig. 1). The probe from chromosome VIII was made from a 2.0-kb *KpnI* fragment containing the *CUP1* gene from plasmid pJW6 (Fogel and Welch, 1982). *CUP1* is 100 kb from *CEN8* (Riles et al., 1993) (see Fig. 1). The strains, DS50B and VG810-15-1, contained eight tandem copies of this sequence on chromosome VIII (data not shown); therefore this probe detected a total of 16 kb of chromosomal sequences in these strains. The probes from chromosome XVI were made by nick translating entire cosmids purchased from the American Tissue Culture Collection (Rockville, MD). Probe 1 was made from the cosmid 71042, that contains a 35.6-kb yeast DNA insert. Probe 2 was made from the cosmid 70,905, that contains a 36.8-kb yeast DNA insert. Probe 3 was made from the cosmid 70,912, that contains a 41.6-kb yeast DNA insert and probe 4 made from the cosmid 70,982, that contains a 31.8-kb yeast DNA insert. Probe 5 was made from the cosmid 71,015, that contains a 36.8-kb yeast DNA insert. The centromere proximal edge of probe 1 is located 11–28 kb from *CEN16*. Probes 1 and 2 are separated by 110 kb. Probes 2 and 3 are separated by 80 kb and probes 3 and 4 are separated by 48 kb. The centromere proximal edge of probe 5 is located 0–12 kb from *CEN16*. The positions of the chromosome XVI probes were determined using contig maps provided by ATCC. The ribosomal DNA (*rDNA*) probes from chromosome XII were made either from a 4.6-kb *BglII* fragment from plasmid p362, that contains the 5′ half of the *rDNA* repeat (18S, IVS, 5.8S IVS, and 5′ end of 25S) or a 4.4-kb *BglII* fragment from plasmid p363, that contains the 3′ half of the *rDNA* repeat (3′ end of 25S, IVS, and 5S) (Keil and Roeder, 1984). The probe for the pDK243 minichromosome was made from 4.3 kb of vector sequences (pBR322).

## *Procedure for Fluorescent In Situ Hybridization*

***Cell Preparation.*** Cells were grown in YEPD to midlog phase. For nocodazole arrest, cells were then incubated at 23°C for 3 h in 15 μg/ml nocodazole. Cell division cycle mutants were arrested by incubation at 37°C for 3 h. Cells were fixed in 3.6% formaldehyde for 2 h at 23°C, washed 3× with distilled water then resuspended in 1 M sorbitol, 20 mM $KPO_4$, pH 7.4 (cells can be stored overnight at 4°C). Cells were treated with mercaptoethanol (1/50 vol) and then converted to spheroplasts in 30 μg/ml zymolyase 100T for 1 h at 23°C. Spheroplasts were mixed with an equal volume of 1% Triton X-100 (either in a microfuge tube or directly on the slide), transferred to polylysine-coated slides and incubated 15 min at room temperature to allow them to adhere to the slides. Triton X-100 was removed from the wells and replaced with 0.5% SDS for 3 min at room temperature. The SDS was removed from the wells and replaced with fresh 0.5% SDS for an additional 10 minutes at room temperature. As a consequence of SDS treatment, spheroplasts that adhered to the slide appeared swollen and transparent. Slides were submerged in 3:1 methanol/acetic acid (coplin jar) for 5 min at room temperature and then allowed to air dry overnight at room temperature (slides can be stored for >3 mo at 4°C in vacuum desiccator).

***In Situ Hybridization.*** RNase A (100 μg/ml in 2× SSC) was added to each slide well containing cells, and the slides were incubated in a humid chamber for 1 h at 37°C. The slides were washed four times by submersion in coplin jars containing 2× SSC at room temperature (2 min/wash), dehydrated by submersion in a series of 2 min ethanol (−20°C) washes (70, 80, and then 95%), and allowed to air dry. To denature chromosomal DNA, the slides were incubated for 2 min in a coplin jar containing 70% formamide, 2× SSC at 70°C and then immediately dehydrated by a series of 1 min ethanol (−20°C) washes (70, 80, 90, and 100%). Proteinase K (200 μg/ml in 20 mM Tris, pH 7.8, 2 mM $CaCl_2$) was added to slide wells and

then slides were incubated in a humid chamber for 15 min at 37°C. Slides were dehydrated by a series of 1 min ethanol (−20°C) washes (70, 80, 90, and 100%). Probes were denatured in 50% formamide, 10% dextran sulfate, 400 μg/ml salmon sperm DNA, 2× SSCP (0.3 M NaCl, 0.03 M sodium citrate, 0.04 M $NaH_2PO_4$, pH 6.0) for 10 min at 70°C and then chilled in ice water. Typically, ~150 picograms of probe was added to 10 μl hybridization mix (the amount was determined empirically for each probe). 5 μl of denatured probe mix were added to each well. Hybridization proceeded overnight at 35–36°C in a humid chamber. To remove nonspecific hybridization, slides were subjected to the following serial washes in coplin jars: once in 60% formamide, 2× SSC (37°C, 20 min), twice in 2× SSC (37°C, 5 min/wash), and then once in 1× PBD (0.1 M $NaH_2PO_4$, 0.1 M $Na_2HPO_4$, 0.1% [wt/vol] NP-40) (room temperature, 2 min). Hybridized biotinylated probes were detected using an in situ hybridization kit from ONCOR. Hybridized digoxigenin probes were detected by serial incubations with 1:250 dilutions of mouse anti-digoxigenin antibodies, goat anti-mouse antibodies conjugated with FITC and swine anti-goat antibodies conjugated with FITC (antibodies were obtained from Boehringer Mannheim Biochemicals and Jackson ImmunoResearch (West Grove, PA). The times of incubations and washes were done as described in the ONCOR kit. Total chromosomal DNA was detected using propidium iodide. Images were visualized using a standard Zeiss universal epi-fluorescence microscope. Propidium and FITC images were recorded digitally using a Hamamatso CCD camera (2400) and the Image-1/AT processing system (Universal Imaging Corp., Media, PA). This system allowed us to superimpose images.

### *Measurement of Distance between Pairs of Chromosome XVI Probes*

We wanted to assess chromosome condensation by measuring the separation of two FISH signals generated from hybridization of two probes from chromosome XVI. To obtain the appropriate pairs of probes for this study, haploid *cdc14* cells were arrested and hybridized with pairs of probes from chromosome XVI. The *cdc14* strain was used because distinct FISH signals were maximally separated compared to other strains analyzed. Two signals were observed in 90% of the nuclei hybridized with probes that were 145 kb (Fig. 1, probes *1* and *2*) or 255 kb (Fig. 1, probes *1* and *3*) apart and the distance between them was sufficient to allow the detection of any shortening that may occur at other arrest points (see Fig. 9). Two signals were also observed in 58% of nuclei hybridized with probes 80-kb apart (Fig. 1, probes *3* and *4*). Similar resolution of FISH signals has been observed for hamster and human chromosomes (Trask et al., 1989; Brandriff et al., 1991). However, since we could not consistently resolve the two signals from the probes 80-kb apart we did not use this probe pair in our analysis. Images of the FISH signals were magnified 4,000-fold (2,000-fold optically and two fold digitally). Distances between the signals was measured using the morphometric programs of Image 1. The separation of signals was measured as the distance between the middle of each FISH signal. Accordingly, the number of base pairs between the signals was calculated as the number of base pairs between the midpoints of the probes.

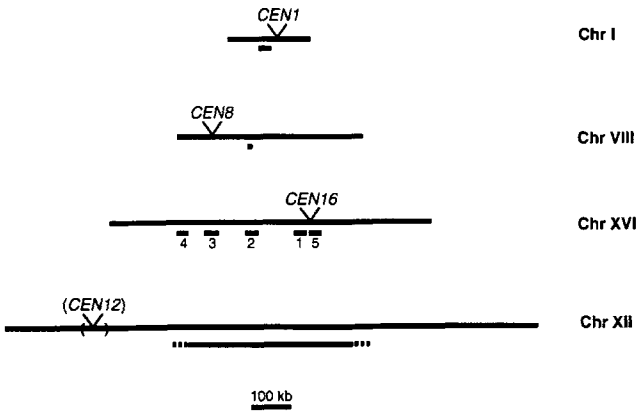



*Figure 1.* Schematic showing the position and size of the chromosomal targets for FISH. Probes were made using DNA from four different yeast chromosomes (see Materials and Methods). The length and position of the chromosomal sequences detected by the probes are drawn to scale. For chromosome XII, the parentheses around *CEN12* indicates its precise position is not known. The broken line at each end of the *rDNA* sequence indicates that the exact length of this sequence was not determined.

## *Results*

### *Establishing FISH As a Method to Follow the Position of Chromosomal Sequences in Budding Yeast*

No method for using FISH on vegetative cells of budding yeast had been reported prior to the initiation of our studies. To develop such a method, we modified a FISH method developed for mammalian cells. Yeast cells from asynchronous culture of haploid and diploid strains were fixed, stripped of cell wall, mounted on slides, and then treated to remove membranes, RNA, and protein (Materials and Methods).

Several conclusions could be drawn from the staining pattern of total chromosomal DNA in mounted cells after these treatments (Fig. 2). First, the diameter of the chromosomal DNA mass was about 2 to 3 μm for haploid cells and 3 to 4 μm for diploid cells. These diameters were about twice the diameters of the chromosomal mass in nuclei of intact cells suggesting that a small amount of swelling or flattening of nuclear contents may have occurred during the preparation of the cells for FISH. Second, the mounted cells could be placed into two categories based upon the staining pattern of total chromosomal DNA (Fig. 2). In the majority of cells the DNA stained as a spheroid (Fig. 2 *A*). From many other studies using conventional cytology, these spheroid chromosomal masses are derived either from cells that are in G1, S, G2, or early M (not yet undergoing spindle elongation) or from cells that are in late M (completed chromosome segregation and spindle elongation) (Pringle and Hartwell, 1981). In the remaining cells the chromosomal DNA appeared as a dumbbell-like shape (Fig. 2 *B*), a pattern diagnostic of cells undergoing anaphase.

The mounted cells were hybridized with a biotinylated DNA probe that was made with either centromere proximal (*CEN1*) or centromere distal (*CUP1*) sequences from chromosomes I or VIII, respectively (Fig. 1). A single hybridization signal was detected in most round nuclei from the haploid strain while two hybridization signals were detected in most round nuclei from the diploid strain (Figs. 2 *A* and 3). Less than 1% of the nuclei had signals in mock hybridizations in which the probes were omitted (data not shown) indicating that the signals were probe dependent. The extremely good correlation between the number of signals per nucleus and the ploidy of the strains indicated that our FISH method could reliably detect the presence of loci homologous to the probe. Furthermore, the significant spatial separation of the two signals produced in the diploid nuclei with various centromere distal probes (*CUP1*, Fig. 2 *A*; and data not shown) suggested that extensive somatic pairing of homologs does not occur in budding yeast as it does in some other organisms. This observation is consistent with the apparent lack of pairing between homologs in pre-meiotic cells (Scherthan et al., 1992). It also provides an explanation for the fact that homologous sequences are equally as likely to undergo mitotic recombination when they are at ectopic sites as when

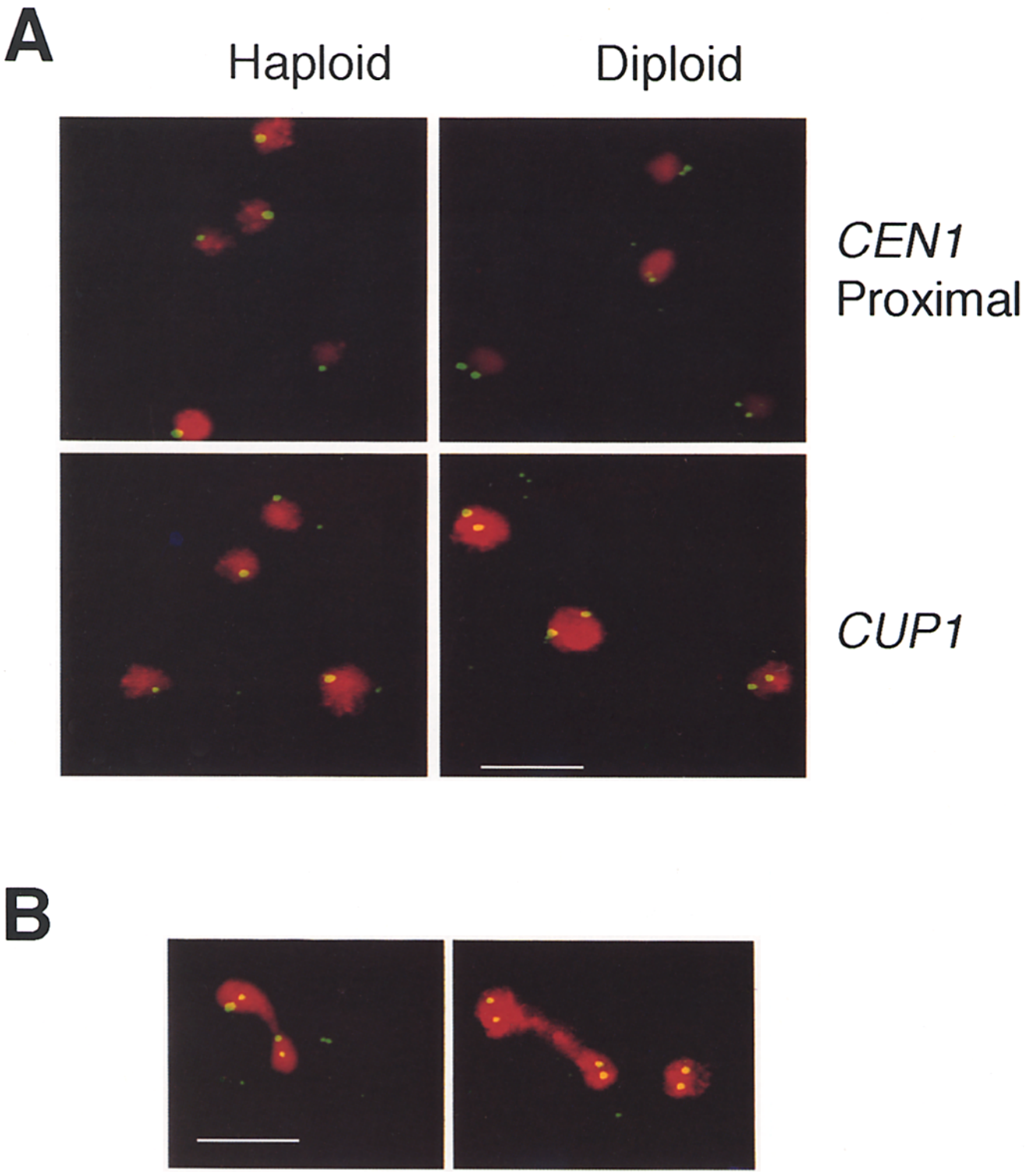



*Figure 2.* Micrographs from in situ hybridization of asynchronous yeast cells using probes from two different chromosomes. (*A*) Random cells from asynchronous cultures fixed and prepared for in situ hybridization. The ploidy of the cells and the probes used are as indicated. (*B*) Rare anaphase cells from an asynchronous diploid population hybridized with the centromere distal probe from chromosome VIII (*CUP1*). The chromosomal DNA was stained with propidium iodide and the hybridized biotinylated DNA probes were detected by FITC–avidin. Propidium and FITC images were pseudo-colored red and yellowish-green, respectively. Diploid strain DS50B and haploid strain VG810-15-1 were used for these hybridizations. Bar, 5 μm.

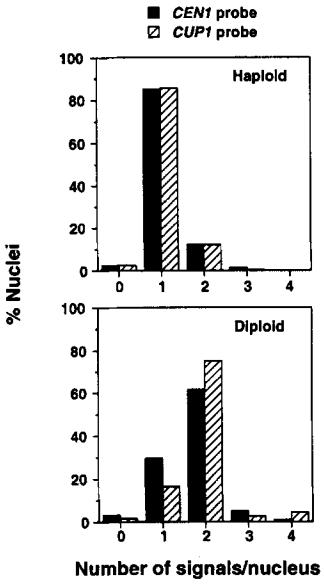



*Figure 3.* The number of spheroid nuclei in asynchronous cultures of haploid and diploid yeast that contained 0, 1, 2, 3, or 4 hybridization signals was determined and plotted as a percentage of total nuclei from each strain. Between 100 and 200 nuclei were scored for each probe used. Diploid strain DS50B and haploid strain VG810-15-1 were used for this analysis.

they are on homologs (Lichten et al., 1987). While the signals from *CEN1* proximal probes appeared more clustered than signals from the centromere distal probes (Fig. 2 *A*), subsequent analysis showed that clustering also occurred between nonhomologous centromeres (data not shown) as has been observed by FISH in other eukaryotic cells (Funabiki et al., 1993; Vourc'h et al., 1993). Therefore the proximity of the *CEN1* signals appears to be a centromere specific phenomenon rather than a consequence of localized somatic pairing.

In more than 80% of anaphase nuclei from diploid cultures we observed a total of four hybridization signals in which two signals were located in each separating DNA mass (Fig. 2 *B*). The number and distribution of these signals presumably arose from one sister chromatid of each homolog having segregated to opposite poles. In agreement with this interpretation, anaphase nuclei from haploid cells yielded two hybridization signals, one signal in each separating DNA mass (data not shown). These results demonstrated that our in situ hybridization method could also identify homologous sequences on unpaired sister chromatids as well as distinguish each homolog within a diploid nucleus.

### *Extent and Timing of Sister Chromatid Pairing*

In a typical asynchronous yeast culture, a significant fraction (30–60%) of cells with round nuclei have completed the replication of chromosomes but not yet begun anaphase; that is, they are somewhere in the window of the cell cycle from late S to early M phase. In haploid cells, the late S to early M nuclei should have two targets for hybridization, one from each sister chromatid, while in diploids these nuclei should have four targets. Our failure to observe 30–60% of haploid nuclei with two signals or diploid nuclei with four signals (Figs. 2 and 3) suggested that sister chromatids might be tightly paired after replication but prior to anaphase, rendering it difficult or impossible to resolve each chromatid as a separate signal.

To examine more carefully the pairing of yeast sister chromatids, we took advantage of a subset of well-characterized mutations in cell division cycle genes and inhibitors of the cell cycle. These reagents allowed us to generate yeast cultures in which >90% of the cells had a homogeneous DNA content and hence a known number of targets for our in situ probes. To obtain diploid nuclei containing a 2C DNA content (two sets of unreplicated chromosomes), diploid cells were arrested in the G1 phase using the *cdc4* mutation. To obtain nuclei containing a 4C DNA content (i.e., two sets of replicated chromosomes or four sets of sister chromatids), diploid cells were arrested in late S phase using the *cdc9* mutation, or after S but prior to anaphase using either the *cdc20* mutation or the microtubule inhibitor, nocodazole. Arrested cells were mounted and hybridized with different probes as described in Materials and Methods.

The number of signals per nucleus was determined in *cdc4*-, *cdc9*-, *cdc20*-, and nocodazole-arrested diploid cells hybridized with the *CEN1* proximal probe (Fig. 1). Most nuclei gave two hybridization signals while very few gave three or four signals regardless of whether the nuclei had two sets of unreplicated chromosomes (*cdc4* arrest) or four sets of sister chromatids (*cdc9*-, *cdc20*-, or nocodazole-induced arrest) (Fig. 4). Similar results were obtained with a chromosome VIII probe and four different chromosome XVI probes (data not shown). The failure to detect four signals in cells with four sets of sister chromatids did not reflect a limitation in our ability to resolve more than two signals in a nucleus. In fact we observed three to four signals in G1 diploid cells hybridized with a mixture of two probes from two different chromosomes (data not shown) and in haploid cells hybridized with a mixture of four probes to chromosome XVI (see below). Therefore, the fact that we observed only two signals in diploid cells containing replicated chromosomes indicated that sister chromatids must be tightly paired at all sites homologous to the probes, rendering it difficult or impossible to resolve each chromatid as a separate FISH signal. These conclusions were corroborated by analyses of haploid cells (Guacci et al., 1994).

### *Pairing of Sister Minichromatids*

Artificial circular minichromosomes have been shown to mimic reasonably well authentic yeast chromosomes, replicating once during S phase and segregating properly in >99% of cell divisions (Fangman et al., 1983; Hieter et al., 1985; Koshland et al., 1985). We wanted to determine whether replicated minichromosomes (sister minichromatids) also pair like authentic sister chromatids. For this purpose, we made an exponential culture of a haploid strain

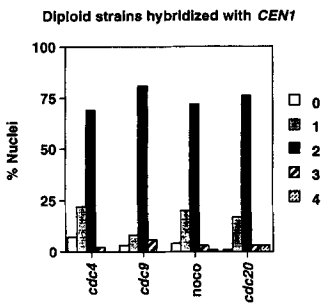



*Figure 4.* Proximity of sister chromatids during the cell cycle. The number of nuclei with 0, 1, 2, 3, or 4 hybridization signals determined for diploid cultures arrested by *cdc* mutations or nocodazole (see Materials and Methods) and plotted as a percentage of total nuclei for each strain. At least 100 nuclei were scored for each strain. The strains used for this analysis were: BP5306 (*cdc4*), DK206 (*cdc9*), BP5050 treated with nocodazole (noco), and DK229 (*cdc20*).

(DK4521-001) harboring the pDK243 minichromosome, which is a 14-kb pBR322-based plasmid that contains a functional centromere, origin of replication, and yeast-selectable markers (Koshland, et al., 1985). Cells from this culture were arrested after DNA replication but prior to mitosis by treatment with nocodazole and subjected to FISH. The number of FISH signals was determined in 100 nuclei hybridized to either a chromosome XVI probe (digoxigenin-labeled probe *1*, Fig. 1) or a minichromosome specific probe (digoxigenin-labeled pBR322). When cells were hybridized with the chromosome XVI probe, 77% had one FISH signal while only 13% had two signals and 9% had 0 signals. These results corroborated our previous conclusion that sister chromatids were paired in most nocodazole-arrested cells. When cells were hybridized with the minichromosome specific probe, 76% had one FISH signal while only 6% gave two signals and 18% gave zero signals. The fact that the plasmid and chromosome XVI probes gave only one signal in a similar fraction of nuclei suggested that sister minichromatids also were paired in most nocodazole-arrested cells. We had shown previously that at least 90% of pDK243 sister minichromatids were no longer catenated in nocodazole-arrested cells (Koshland and Hartwell, 1987). This result coupled with the results from the FISH analysis suggested that catenation of sister DNA molecules must not be required to maintain chromatid pairing. Furthermore as this minichromosome contained no repetitive DNA, repetitive DNA was not required for establishing or maintaining chromatid pairing.

### *Chromosome Condensation at the rDNA Locus*

In budding yeast, the *rDNA* locus encompasses a region on the right arm of chromosome XII (Petes, 1979*a*, *b*) that is as large or larger than many of the other yeast chromosomes (Carle and Olson, 1985) (Fig. 1). This locus is composed of approximately 50–70 copies of a 9-kb *rDNA* sequence arranged as tandem repeats (Petes, 1979*b*). Hence, hybridization of nuclei with a probe corresponding to one half of an *rDNA* repeat produced a signal that corresponded to the entire *rDNA* locus and allowed us to monitor chromosome structure over a large chromosomal segment (~500 kb).

Initially we used our FISH method to examine the *rDNA* signal in asynchronous cultures of diploid strains. In 85% of nuclei, the *rDNA* signal was a single large amorphous cap on the edge of the chromosomal mass (Fig. 5, *A*). This pattern of staining is similar to the staining pattern observed by indirect immunofluorescence when cells are probed with antibodies against nucleolar proteins (Granot and Snyder, 1991). The remaining nuclei exhibited intense discrete line-like signals (Fig. 5, *B–D*). This second class of nuclei could be subdivided into two categories based on whether the *rDNA* staining existed as loops (Fig. 5 *B*) or bars (Fig. 5, *C* and *D*). In the rare cells with the elongated nuclei diagnostic of anaphase (Fig. 5 *C*), the *rDNA* always appeared as bars. Two of these bars were observed in each separating chromosomal mass presumably reflecting the segregation of the sister chromatids of the two chromosome XII homologs. Similar ratios of amorphous and discrete *rDNA* signals were observed in cells from asynchronous haploid populations except anaphase cells had only one line-like signal in each segregating chromosomal mass as expected from the reduced ploidy (data not shown). The amorphous and discrete line-like *rDNA* signals were reminiscent of decondensed and condensed chromosomes, respectively, that have been observed in many other eukaryotes. Furthermore, the enrichment of *rDNA* bars structures in anaphase nuclei suggested that this apparent condensation might be regulated during the cell cycle.

To test whether a particular *rDNA* structure correlates with a particular stage of the cell cycle, *rDNA* structure was characterized in diploid strains that were arrested at various stages of the cell cycle using the well characterized *cdc* mutations or cell cycle inhibitors (Pringle and Hartwell, 1981). For each diploid strain the percent of nuclei with a particular *rDNA* structure was determined. These data are presented as micrographs from a subset of these strains (Fig. 6), and as a histogram (Fig. 7).

From an examination of these data a simple pattern emerges. In most nuclei from cells arrested in G1 (*cdc28, cdc4, cdc7*) or S (*cdc6, cdc17, cdc9*) phase, the *rDNA* signal was amorphous. In the nuclei of two G2-M–arrested populations (*cdc20*, wild-type cells treated with nocodazole), the *rDNA* pattern changed to two discrete loops or bars in almost every nucleus. The presence of only two loops or bars in these nuclei, even though they contained four sister chromatids as targets for our *rDNA* probe, corroborated further our conclusion that sister chromatids are paired extensively in the G2 and early M window of the cell cycle (see above). Finally among the late M-arrested cells the signal was either juxtaposed bars (*cdc15*) or amorphous (*cdc14*). Therefore, the enrichment of line-like *rDNA* signals among all of the different arrested diploids was limited to a subset of those diploids blocked in G2-M. These results coupled with the results from asynchronous cells strongly suggested that *rDNA* locus underwent cell cycle dependent rounds of condensation in G2 early M followed by decondensation in late M.

In diploids cells arrested by *cdc13*, *cdc16*, or *cdc31* mutations, the *rDNA* signal was variable within each population. For example, the *rDNA* signals in many nuclei from *cdc13*- and *cdc16*- arrested cells were more line-like in character than they were in nuclei from G1 or S arrested cells (Fig. 6). However it was more extended and did not resolve into two distinct signals as was observed in nocodazole- or *cdc20*-arrested cells. Likewise, in *cdc31*-arrested cells the *rDNA* signals in half the nuclei appeared decondensed like those in G1 and S mutants while the other half appeared condensed like those in *cdc20* (data not shown). While we can not explain all these morphological differences, it is possible that *cdc13*, *cdc16*, and *cdc31* cells arrest in a window of the cell cycle that spans the time when *rDNA* locus and perhaps other chromosomal sequences begins to condense.

Finally we noted that the FISH signal from the *rDNA* probe was usually at the periphery of the chromosomal mass in either dividing or arrested cells (Figs. 5 and 6). In fact, the *rDNA* locus in *S. pombe* exhibited very similar localization and morphology (Uzawa and Yanagida, 1992), and nonrandom positioning of *rDNA* bearing chromosomes has also been observed in other eukaryotic cells (Hernandez-Verdun, 1991). It is possible that the position of condensed *rDNA* in budding yeast and these other eukaryotic cells is a consequence of its association with the nucleolus which is peripheral in interphase. The two *rDNA* loci often were aligned

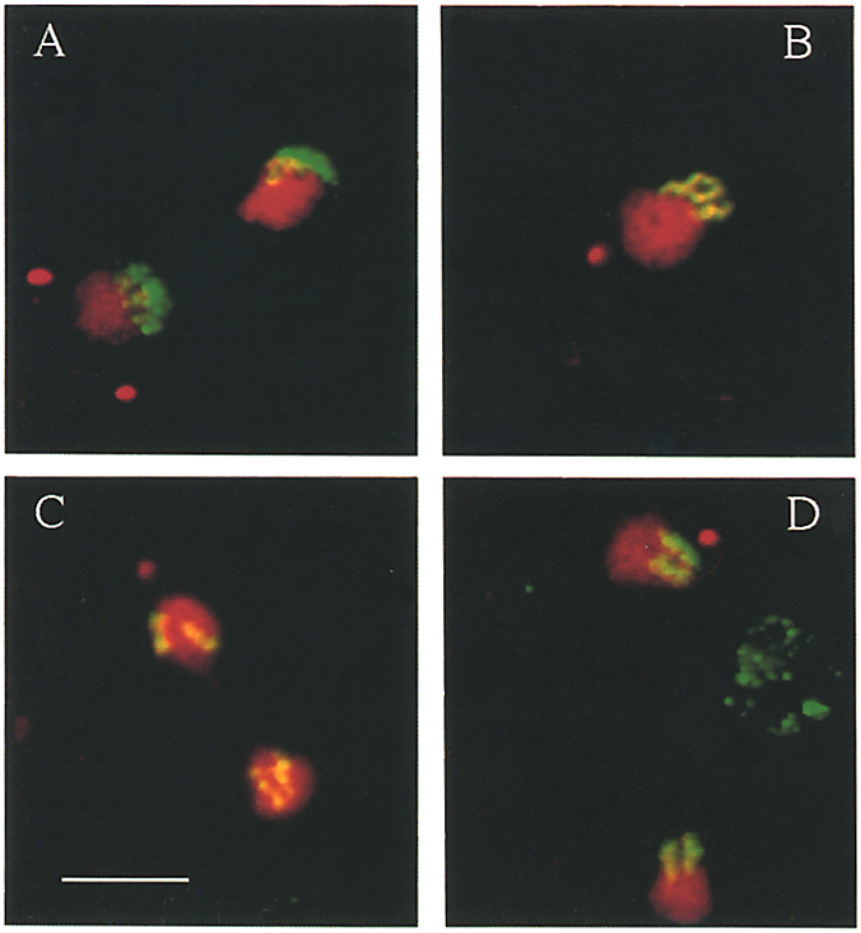



*Figure 5.* FISH staining of *rDNA* loci in cells from an asynchronous culture of diploid yeast. Mid-log cultures of diploid strain DS50B were analyzed by in situ hybridization using an *rDNA* probe as described in Materials and Methods. The chromosomal DNA was stained with propidium iodide and appeared red. The hybridized biotinylated DNA probes were detected by FITC–avidin and gave green or yellow signals. Nuclei exhibiting an amorphous *rDNA* signal (*A*). Nuclei exhibiting discrete line-like signals (*B–D*). In spheroid nuclei these discrete signals were at the periphery as either loops (*B*) or lines (*D*). In anaphase nuclei the signals often appeared as two lines within each separating DNA mass (*C*). Bar, 5 μm.

closely with each other in diploid cells arrested in late M by the *cdc15* mutation. The proximity of the two *rDNA* loci may have reflected the early stages of reformation of a single nucleolus since in this strain background both the *rDNA* (this study) and the nucleolus (Granot and Snyder, 1991) were located as a single peripheral mass in interphase cells.

### *Condensation of Chromosome XVI*

Given that *rDNA* locus has unusual structure (highly repetitive) and function, it was possible that its cell cycle condensation was not reflective of a general property of all yeast chromosomes. To assess condensation of a non-*rDNA* chromosomal region, four probes from chromosome XVI (Fig. 1) were mixed and hybridized to haploid cells arrested at different stages of the cell cycle. Haploid cells were used so that all of the hybridization signals could be assigned to a single chromosome. Three or four separate signals were observed in most nuclei from *cdc28* and *cdc15* haploid cells (Fig. 8). Similar results were obtained with *cdc4*- and *cdc14*-arrested cells (data not shown). In contrast, in nocodazole-arrested cells the hybridization signals were very close together, often as a contiguous line making it difficult to determine the number of hybridization signals (Fig. 8). A similar result was obtained with *cdc20*-arrested cells though the number of nuclei with distinct signals was greater than observed in nocodazole arrested cells. As the diameter of the chromosomal mass was indistinguishable in all of these strains (data not shown), the differences in the clustering of FISH signals in these strains was not a consequence of the cell preparation for in situ hybridization. The fact that the FISH signals were in closer proximity to each other in nocodazole- and *cdc20*-arrested haploid cells than in *cdc28*-, *cdc4*-, *cdc14*-, or *cdc15*-arrested haploid cells suggested that this region of chromosome XVI was more condensed in *cdc20* and nocodazole arrested cells than in the other four *cdc*-arrested cells analyzed.

To quantify the apparent difference in the separation of FISH signals, these arrested haploid cells were hybridized with pairs of probes from chromosome XVI, either probes 1 and 2 (145-kb apart) or probes 1 and 3 (255-kb apart) (Fig. 1). The distance between the two hybridization signals was measured in each nucleus and plotted in histogram format in order to compare the various arrested populations (Fig. 9 *A*). From this analysis, the arrested cells fell into two classes. In the first class (*cdc28*-, *cdc4*-, *cdc15*-, and *cdc14*-arrested cells) the distances between the two signals in each nucleus were evenly distributed amongst the first three intervals while in the second class (*cdc20* and nocodazole-arrested *CDC+* cells) the distances between the signals fell mostly into the shortest interval. The distinction between the two classes was validated when our data were used to calculate a mean separation of the signals (Fig. 9 *B*). The mean separation between probes 1 and 2 was statistically indistinguishable for all cells in the first class. However, mean separation for class 1 was significantly greater than the mean separation for cells in the second class. When cells were hybridized with probes 1 and 3, the mean distance between the FISH signals was greater in all strains than the mean dis-

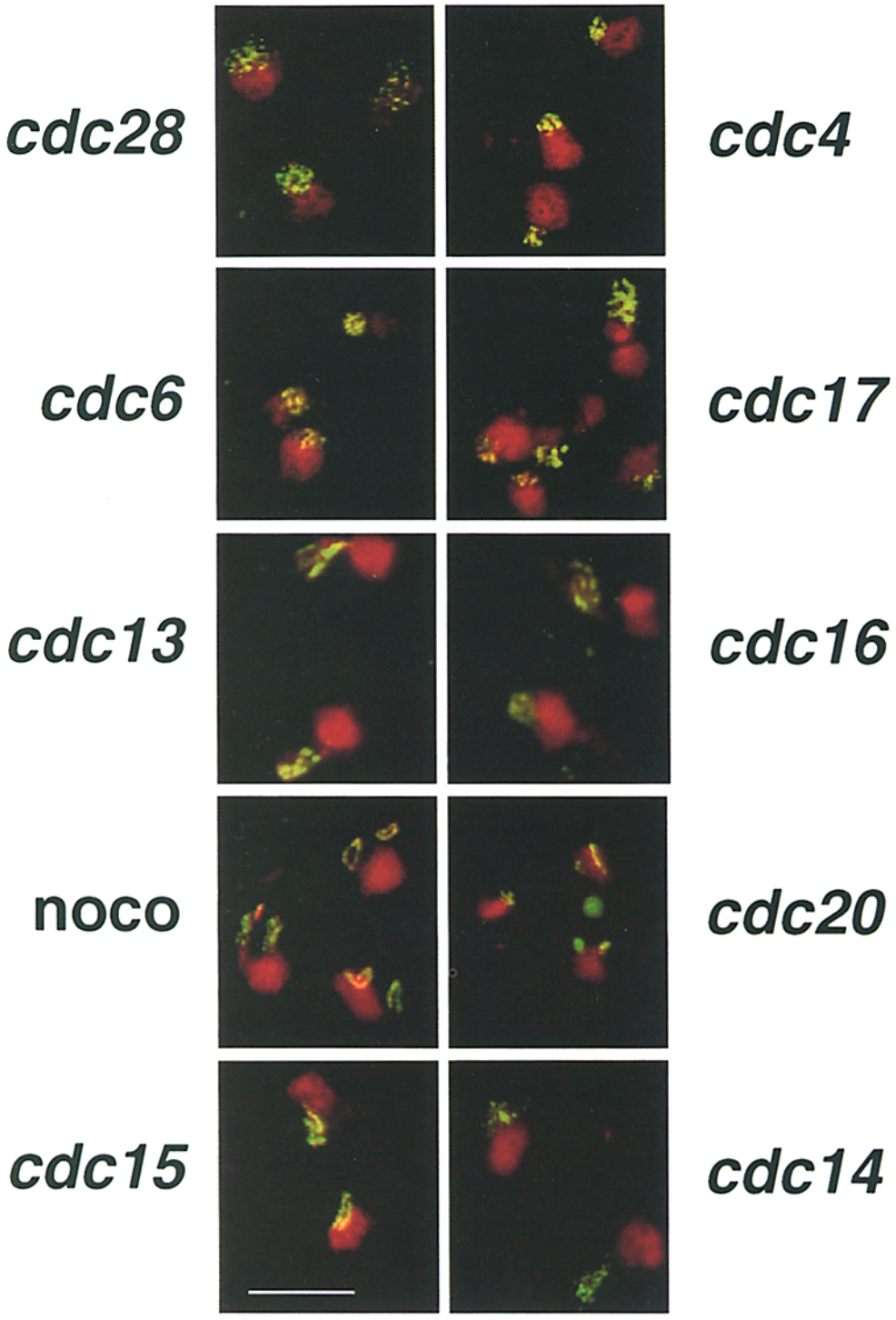



*Figure 6.* Visualization of *rDNA* morphology in diploid cells arrested at different stages of the cell cycle. Diploid yeast cells were arrested with *cdc* mutations or nocodazole (see Materials and Methods). Arrested cells were fixed and subjected to FISH using an *rDNA* probe (Fig. 1). The chromosomal DNA was stained with propidium iodide and appeared red. The hybridized biotinylated DNA probes were detected by FITC–avidin and gave green or yellow signals. The strains used for this analysis were: DK201 (*cdc28*), BP5306 (*cdc4*), DK205 (*cdc6*), DK209 (*cdcl7*), DK207 (*cdcl3*), DK208 (*cdcl6*), BP5050 treated with nocodazole (noco), DK229 (*cdc20*), DK211 (*cdcl5*), and DK210 (*cdcl4*). Bar, 5 μm.

tances observed with probes 1 and 2. This result was expected because it had been shown previously in both human and hamster cells that the distance between FISH signals increased as the genomic length between the probes increased (Lawrence et al., 1988; Trask, et al., 1989). However, the same classification of strains could be made with probes 1 and 3 as was made with probes 1 and 2, that is the mean distance between FISH signals was significantly greater in *cdc28*-, *cdc4*-, *cdcl4*-, and *cdcl5*-arrested cells than in *cdc20* or nocodazole-arrested cells (Fig. 9 *B*). These results strengthened our conclusion that this region of chromosome XVI was more condensed in *cdc20* and nocodazole-arrested cells than in *cdc28*-, *cdc4*-, *cdcl5*-, and *cdcl4*-arrested cells. Therefore, both unique sequences on chromosome XVI and repetitive sequences (*rDNA* locus) on chromosome XII appeared to be condensed or decondensed at similar times in the cell cycle. The one exception to this correlation occurred in *cdcl5*-arrested cells where the *rDNA* appeared condensed (see above) while chromosome XVI appeared decondensed.

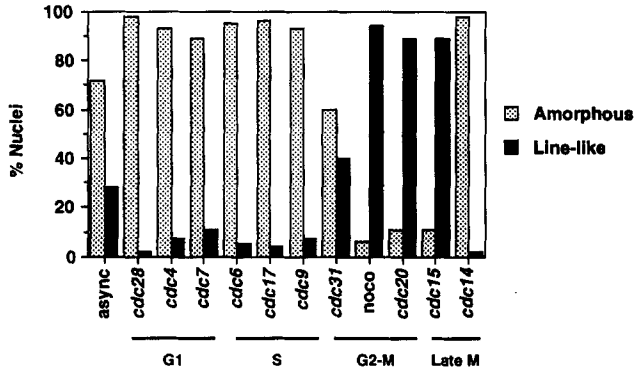



*Figure 7.* Quantitation of *rDNA* morphology in nuclei of cells arrested at different stages of the cell cycle. The *rDNA* signal in each nucleus from cells arrested at different stages of the cell cycle (Fig. 6) was scored as being either amorphous (punctate with little or no line-like character) or line-like (two distinct bars or loops). By these criteria, the *rDNA* morphology in *cdcl3*- and *cdcl6*-arrested cells did not fit clearly into either of these two categories and therefore the quantitative analysis of their *rDNA* structure was omitted from this figure. The strains used for this analysis were: BP5050 (async), DK201 (*cdc28*), BP5306 (*cdc4*), DK204 (*cdc7*), DK205 (*cdc6*), DK209 (*cdcl7*), DK206 (*cdc9*), BP5071 (*cdc31*), BP5050 treated with nocodazole (noco), DK229 (*cdc20*), DK211 (*cdcl5*), and DK210 (*cdcl4*).

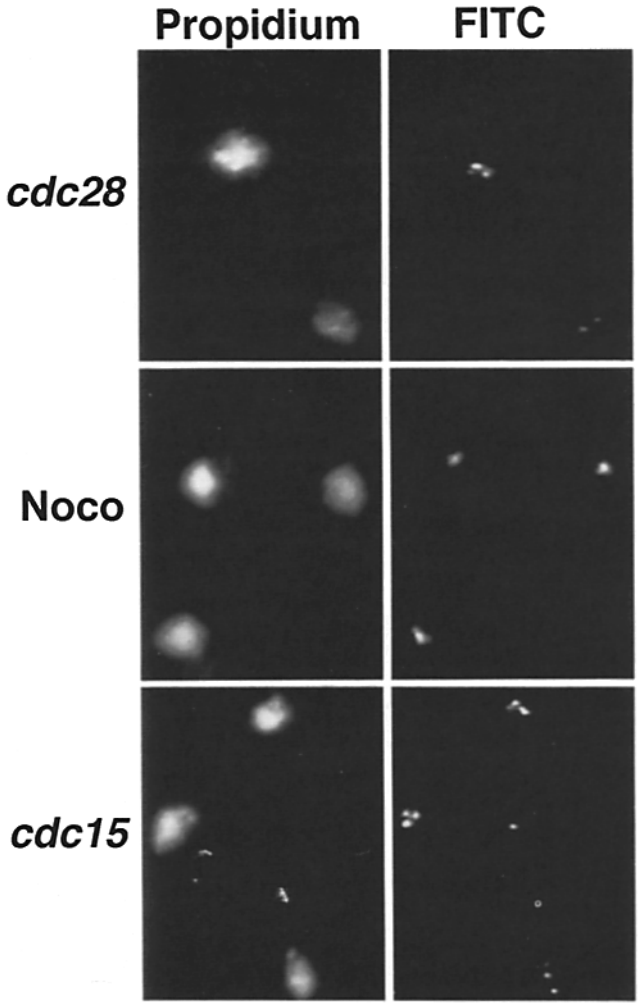



*Figure 8.* Visualization of chromosome XVI condensation in cells arrested at different stages of the cell cycle. Haploid cells were arrested at various stages of the cell cycle and analyzed by in situ hybridization using a mixture of four probes to chromosome XVI (Fig. 1). The chromosomal DNA was detected with propidium iodide and the hybridized biotinylated DNA probes were detected by FITC-avidin (*FITC*). The strains used for this analysis were: DK4522-282 (*cdc28*), DK4521-001 treated with nocodazole (noco), and DK4536-151 (*cdcl5*). Bar, 5 μm.

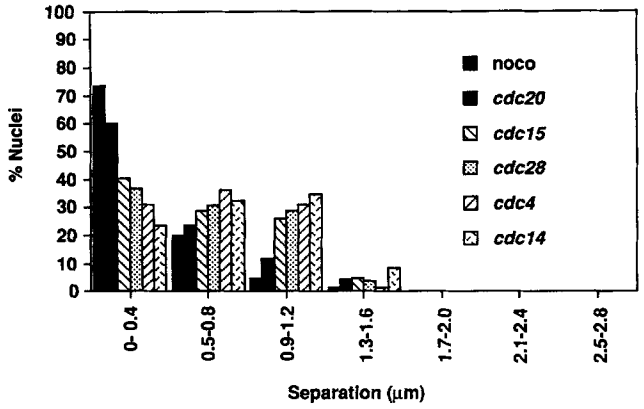
## Discussion

### Sister Chromatid Pairing

In this study we used fluorescent in situ hybridization to examine the position of homologous sequences on sister chromatids in budding yeast. Pairing was observed at every locus examined including loci adjacent to centromeres from two different chromosomes and four centromere distal loci from two different chromosomes. Therefore, it is likely that all sister chromatids are paired near their centromeres and along most of their arms in budding yeast. Consistent with this conclusion was our observation that sister chromatids also appear paired along the entire length of the *rDNA* locus (500 kb). When sister chromatids are examined in intact cells undergoing mitosis from larger eukaryotes, they appear paired along their entire length (Cooke et al., 1987; Sumner, 1991). Therefore, the extent of pairing between sister chromatids in yeast and other eukaryotic cells appears similar. However, when larger eukaryotic cells are arrested in mitosis with microtubule depolymerizing reagents, the pairing of sister chromatids is limited to a region near or at the centromere. Under similar conditions, pairing persists along the entire length of sister chromatids in yeast (this study). The source of this difference may be elucidated when the mechanism and regulation of pairing is better understood. Finally, the proximity of sister chromatids in yeast and the absence of somatic pairing of homologs (this study) provides an explanation for why sister chromatids are preferred over homologs as substrates for recombinational repair during G2 (Kadyk and Hartwell, 1993).

The question arises as to position of physical contacts between sister chromatids to account for their extensive pairing. From cytological studies of sister chromatids in larger eukaryotes, extensive pairing of sister chromatids has been attributed to the physical interaction of heterochromatin associated with large blocks of repetitive DNA sequences (Lica, et al., 1986; Sumner, 1991; Carmena, et al., 1993). However, natural yeast chromosomes have little repetitive DNA except as part of telomeres and part of the *rDNA* cluster, and yet sister chromatids are paired along their entire length (this study). These results hinted at the possibility that sister chromatid pairing in yeast may occur at positions other than at large blocks of repetitive DNA or associated heterochromatin. This possibility was confirmed by our observation that efficient pairing occurred between sister chromatids of a minichromosome (minichromatids) that completely lacked repetitive DNA. It is likely that in larger eukaryotes sister chromatid pairing is also not limited to large blocks of repetitive DNA/heterochromatin, given that many basic cell biological processes have recently been shown to be conserved between yeast and other eukaryotes.

A




B

| Arrest | Mean separation in μm for: probes 1 & 2 | probes 1 & 3 |
|---|---|---|
| *cdc28* | 0.67 (0.04) | 0.96 (0.08) |
| *cdc4* | 0.61 (0.04) | 0.93 (0.07) |
| *cdc20* | 0.46 (0.05) | 0.68 (0.05) |
| wt + noco | 0.35 (0.04) | 0.59 (0.04) |
| *cdc15* | 0.62 (0.06) | 0.93 (0.07) |
| *cdc14* | 0.72 (0.06) | 1.11 (0.09) |

*Figure 9.* Quantitation of chromosome XVI condensation in cells arrested at different stages of the cell cycle. Haploid cells were arrested at various stages of the cell cycle and analyzed by in situ hybridization using pairs of probes that were separated by 145 (Fig. 1, probes *1* and *2*) or 250 kb (Fig. 1, probes *1* and *3*). Images were magnified 4,000-fold and the distance between the signals was determined as described in the methods. The small percentage of nuclei with only one signal was assumed to be the result of two signals that were not resolved. These signals were assigned a value of 0-μm separation. This assumption appeared valid since the number of nuclei with one signal increased as the distance between two probes decreased (data not shown). (*A*) The distance between signals produced by probes 1 and 2 was measured in nuclei from cells arrested with different *cdc* mutations or nocodazole. Depending upon the distance between the signals produced by the probes, each nucleus was placed into one of seven groups. These groups consisted of 0.4-μm intervals that began at 0 (the minimal separation) and ended at 2.8 μm (the average diameter of the nucleus). The percentage of total nuclei in each interval was plotted. (*B*) The distance between signals produced by probes 1 and 2 or by probes 1 and 3 was measured in cells arrested with *cdc* mutations or nocodazole. From a sample size of ~50 nuclei from each strain, the mean separation and the standard error of the mean (in parentheses) were calculated. The strains used for this analysis were: DK4522-282 (*cdc28*), DK4523-042 (*cdc4*), H20cla5 (*cdc20*), DK4521-001 treated with nocodazole (noco), DK4536-151 (*cdc15*) and DK5203-3-4 (*cdc14*).

One possible mechanism to mediate pairing of sister chromatids is to link them physically by the catenation of sister DNA molecules which occurs as a byproduct of DNA replication (Murray and Szostak, 1985). However, most sister minichromatids of the pDK243 minichromosome were paired but not catenated in nocodazole-arrested cells (this study; Koshland and Hartwell, 1987). These results strongly suggest that catenation of sister DNA molecules must not be required to maintain chromatid pairing. This conclusion is supported by two other observations. In budding yeast cells lacking topoisomerase II, sister chromatids of small natural chromosomes can segregate from each other without breaking (Spell, R., and C. Holm, 1994), and in fission yeast cells lacking topoisomerase II, sister centromeres can separate from each other (Funabiki et al., 1993). If catenation is not necessary for maintaining sister chromatid pairing, why does residual catenation between sister DNA molecules of larger chromosomes persist until anaphase? It may be caused by the ability of topoisomerase II to reversibly catenate and decatenate two DNA molecules that are being held in close proximity by some other pairing mechanism (Holm et al., 1985). However, the possibility that catenation may play a role in the establishment of sister chromatid pairing has not been eliminated by our study.

As an alternative to catenation, we favor the idea that pairing of sister chromatids is maintained by proteins like the mammalian INCENP and CLiP proteins that localize between sister chromatids prior to anaphase (Cooke et al., 1987; Earnshaw and Cooke, 1989; Rattner et al., 1988). If pairing is mediated by such proteins, it is clearly of interest to determine whether they bind to DNA or to chromosomal proteins and whether the position of their binding to sister chromatids is specified directly or indirectly by particular DNA sequences. As it is possible to isolate minichromosomes from yeast as chromatin (Pederson et al., 1986; Kingsbury and Koshland, 1991) and to manipulate their DNA backbone, further investigation of the pairing between sister minichromatids both genetically and biochemically should provide a powerful approach to address these questions.

### *Chromosome Condensation*

In this study we used FISH to analyze the basal level of chromosome condensation in G1 (*cdc28* and *cdc4*) and postanaphase- (*cdc14* and *cdc15*) arrested yeast. From this study we can estimate compaction ratios of chromosome XVI in these arrested cells. The compaction ratio is defined as the predicted length that a DNA segment would be in B form (0.34 nm/bp × the number of kilo bases between the midpoints of two probes) divided by the observed length of the chromosome segment (mean physical separation in μm between FISH signals generated by the two probes) (Fig. 9 *B*). The compaction ratio for yeast chromatin in interphase is ~80; this value is very similar to the compaction ratios of 70 to 100 estimated from FISH studies of interphase chromosomes from human and Chinese hamster cells (Lawrence et al., 1988; Trask et al., 1989). This similarity suggests that chromatin may be packaged similarly in interphase cells of yeast and of other eukaryotes. This is interesting in light of the fact that a H1-like molecule has not yet been identified in budding yeast.

Conflicting evidence has been reported for whether chromosomes of budding yeast condense extensively during mitosis (Wintersberger et al., 1975; Peterson and Ris, 1976; Gordon, 1977; Peterson and Pinon, 1978). In this study we used FISH to analyze mitotic chromosome condensation, using a pool of four probes from discrete loci along a 400-kb region of chromosome XVI and another probe to the entire 500 kb of the *rDNA* cluster on chromosome XII. Qualitatively the level of condensation of the *rDNA* locus appears to change as evidenced by the alteration in the *rDNA* FISH signal from amorphous to discrete bars or loops. Similarly

the level of condensation of chromosome XVI also appeared to change as evidenced by the alteration in the spacing of the chromosome XVI FISH signals from dispersed to more tightly associated. These changes appeared to be cell cycle regulated as both chromosome XII and chromosome XVI apparently became more condensed only in anaphase cells or of subset *cdc* mutants that cause cells to arrest in G2-M.

We determined that the compaction ratio for chromosome XVI was 140 in nocodazole-arrested cells and 115 in *cdc20*-arrested cells. These values are at most two fold greater than the compaction ratio for decondensed chromosomes from cells arrested in G1 or late M. These differences in compaction ratios are small and it is possible that swelling or spreading of DNA that may occur during the preparation of the cells could distort chromosome shape and thus the resultant measurements. However, we strongly suggest that the differences are real and reflect chromosome condensation in mitosis. First, the timing of condensation of chromosome XVI correlated with the condensation of the *rDNA* locus with one exception (see below). Second, this apparent condensation occurred only in G2-M and was maximal in cells arrested by a microtubule depolymerization drug as has been observed in *S. pombe* and in many other eukaryotes (Taylor, 1959; Umesono et al., 1983; Funabiki et al., 1993). Third, the same differences in compaction ratios were observed using two sets of probes that spanned different lengths of the genome. Fourth, FISH analysis of human cells with probe pairs 130- and 340-kb apart showed only a 5–10-fold increase in compaction ratio for mitotic chromosomes compared to interphase chromosomes (Lawrence et al., 1988) demonstrating that small increases in compaction ratios can reflect significant condensation. Taken together, these results suggest that chromosomes in budding yeast condense in mitosis although less than chromosomes in other eukaryotes.

Given the possible small amount of mitotic condensation for budding yeast chromosomes, one could wonder whether mitotic condensation is essential. We estimate that the genomic length of the longest yeast chromosome arm is ~1 megabase (Carle and Olson, 1985; Mortimer et al., 1989). Based upon the compaction ratio of 80 for interphase chromosomes (this study), we estimate its physical length to be at least 4.0 $\mu$m in the decondensed state. Since the spindle elongates only to 6–8-$\mu$m long (Byers and Goetsch, 1975), a small amount of mitotic condensation may be necessary to ensure that by the end of anaphase the lagging ends of large chromosomes are moved far enough away from the plane of division so as not to be cleaved by nuclear division and or cytokinesis. In addition, we speculate that a small amount of condensation may be essential in all eukaryotes because coupling of the process of condensation with topoisomerase II activity could help to drive out most of the tangles between chromosomes or sister chromatids prior to anaphase.

In *cdc15*-arrested cells the *rDNA* locus on chromosome XII appeared condensed while chromosome XVI was decondensed. These results show that the condensation state of two regions of the yeast genome can differ within the same nucleus and is consistent with the idea that there may be a hierarchy of timing of condensation for different regions of chromosomes in budding yeast. In fact real time imaging of chromosomes in *Drosophila* embryos suggest that some regions can condense earlier or decondense later than others (Hiraoka et al., 1989). One possibility is that the condensation of the *rDNA* locus is subjected to different or additional controls. These controls may be necessary because of the highly repetitive structure of the *rDNA* locus or as a means to control the amount of translation by regulating the amount of rRNA in the cell. The *CDC15* gene encodes a kinase (Schweitzer and Philippsen, 1991). It will be interesting to determine whether the phenotype of the *cdc15* mutant reflects a direct role for this kinase is modulating different states of condensation.

### *Sister Chromatid Pairing and Condensation Provide New Landmarks for Characterizing Cell Cycle Progression of Mutants in Budding Yeast*

In many eukaryotes the condensation and decondensation of chromosomes have been important landmarks for monitoring entry and exit from mitosis. Similarly, the separation of paired sister chromatids has been a landmark for entry into anaphase. The absence of these landmarks in budding yeast previously has limited the analysis of mutants and inhibitors affecting progression through the cell cycle. Using FISH we have been able to use these landmarks to subdivide mutants that were shown previously to arrest somewhere between S and M (prior to spindle elongation). By these criteria, cells arrest in late S-G2 by the *cdc9* mutation (decondensed *rDNA* and paired sister chromatids), in G2-M transition by *cdc13* and *cdc16* mutations (partially condensed *rDNA* and paired chromatids) and in M by nocodazole and *cdc20* mutation (condensed *rDNA* and paired sisters). However, these interpretations are subject to the caveat that the defects in any of these gene products may directly prevent or limit the ability of *rDNA* to condense or sister chromatids to separate, which of itself would be informative.

### *Future Applications*

The applications of our FISH method are limited by the extensive proteolysis step that makes co-localization of proteins and DNA difficult. We are currently trying modifications which will enable such co-localization. However, because of our ability to detect sequences as short as 4 kb, we envision numerous immediate applications of FISH for the study of budding yeast. First, we are currently using this method to identify mutants that precociously separate or fail to condense their sister chromatids as a means to identify new structural and regulatory molecules that mediate these processes. Second, the regulation of the cell cycle is very complex as evidenced by the increasing number of *cdc2* (and *cdc2*-like) kinases, phosphatases, and cyclins. The use of condensation and pairing of sister chromatids as landmarks to study mutants defective in important cell cycle regulators may provide new insights that will help to elucidate their role in cell cycle progression. Third, classical cytological studies and more recent FISH studies have shown that centromeres and telomeres occupy specific spatial positions either relative to each other, to the nuclear periphery or to the mitotic spindle (Rabl, 1885; Wilson, 1925; Vourc'h et al., 1993; Funabiki et al., 1993). Preliminary results using FISH suggests this may also be the case in budding yeast (Guacci, V., E. Hogan, and D. Koshland, unpublished observations). The mechanism for maintaining these spatial arrangements and their functional importance are unresolved. With the advent of FISH, budding yeast is a particularly attractive system for

pursuing these studies because of the availability of antibodies to centromere and telomere proteins as well as appropriate cis and trans mutants. Finally, FISH can be used to address the role of the spatial organization of genes in gene expression. For example it will interesting to determine whether the expression or switching of the mating type cassettes is dependent upon their position within the nucleus.

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