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Centromere Position in Budding Yeast: Evidence for Anaphase A

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Although general features of chromosome movement during the cell cycle are conserved among all eukaryotic cells, particular aspects vary between organisms. Understanding the basis for these variations should provide significant insight into the mechanism of chromosome movement. In this context, establishing the types of chromosome movement in the budding yeast *Saccharomyces cerevisiae* is important since the complexes that mediate chromosome movement (microtubule organizing centers, spindles, and kinetochores) appear much simpler in this organism than in many other eukaryotic cells. We have used fluorescence in situ hybridization to begin an analysis of chromosome movement in budding yeast. Our results demonstrate that the position of yeast centromeres changes as a function of the cell cycle in a manner similar to other eukaryotes. Centromeres are skewed to the side of the nucleus containing the spindle pole in G1; away from the poles in mid-M and clustered near the poles in anaphase and telophase. The change in position of the centromeres relative to the spindle poles supports the existence of anaphase A in budding yeast. In addition, an anaphase A-like activity independent of anaphase B was demonstrated by following the change in centromere position in telophase-arrested cells upon depolymerization and subsequent repolymerization of microtubules. The roles of anaphase A activity and G1 centromere positioning in the segregation of budding yeast chromosomes are discussed. The fluorescence in situ hybridization methodology and experimental strategies described in this study provide powerful new tools to analyze mutants defective in specific kinesin-like molecules, spindle components, and centromere factors, thereby elucidating the mechanism of chromosome movement.

INTRODUCTION

During mitosis in many eukaryotic cells, chromosomes undergo a conserved set of movements that are mediated by two organelles, the bipolar mitotic spindle and the kinetochore, a complex chromosomal structure present at the centromere. The kinetochores on each pair of replicated chromosomes (sister chromatids) capture microtubules emanating from the spindle poles, and dynamic poleward and anti-poleward chromosomal movements ensue. Eventually, chromosomes congress to the midpoint of the spindle. When all chromosomes achieve congression (metaphase), the tightly paired sister chromatids separate and anaphase begins. Chromosomes segregate to the spindle poles concomitant with a shortening of kinetochore microtubules (anaphase A), whereas the spindle elongates due to separation of the spindle poles (anaphase B). Clearly, many forces act on the chromosomes to promote these movements and their regulation must be highly controlled. A variety of experiments demonstrate that the kinetochore plays a major role in mitotic chromosome movement and segregation (reviewed in Inoue and Salmon, 1995). In addition, kinesin-like proteins localized along the chromosomal arms and the flux of microtubule subunits at the spindle poles have been implicated in mediating mitotic chromosomal movements (Mitchison and Salmon, 1992; Afshar *et al.*, 1995; Vernos *et al.*, 1995; Wang and Adler, 1995).

Most studies of chromosome positioning have focused on movement during mitosis. However, early

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microscopic studies of eukaryotic cells revealed that even in interphase, chromosomes are not randomly positioned within the nucleus (Rabl, 1885; Wilson, 1925). These observations have been supported by recent fluorescent in situ hybridization (FISH) studies of centromere position in mammalian and *Schizosaccharomyces pombe* cells (Ferguson and Ward, 1992; Funabiki *et al.*, 1993; Vourc'h *et al.*, 1993). In G1 cells, centromeres are localized to the nuclear periphery whereas centromere distal regions tend to be more internally positioned. Depending on the cell type, centromeres can be loosely or tightly clustered. After leaving G1, centromeres are repositioned away from the nuclear periphery into internal regions of the nucleus. This repositioning can occur from early S-phase through G2. Neither the biological significance nor the underlying mechanism responsible for these movements is known.

However, chromosome movement in all eukaryotic cells is not identical. For example, in some organisms no metaphase is observed (Aist and Williams, 1972; Heath, 1980). Furthermore, in some cells chromosome segregation is achieved primarily by anaphase A whereas in others by anaphase B. The structure of complexes essential for chromosome movement such as kinetochores and spindle poles also varies significantly in cells from different eukaryotes. Significant insights into the mechanism of chromosome movement may be achieved by studying both the molecular composition of these complexes and the types of chromosome movement in several different eukaryotes. In this context, the study of chromosome movement in the budding yeast *Saccharomyces cerevisiae* is particularly interesting because its mitotic apparatus and kinetochores appear to be structurally less complex than those found in other eukaryotic cells. Hence, two questions arise, what types of chromosome movement are possible in budding yeast and do the macro structural differences reflect significant molecular or mechanistic differences?

The most obvious chromosome movement in budding yeast occurs during anaphase B when the spindle elongates from 1 to 2 μM to about 10 μM (Byers and Goetsch, 1975; Winey *et al.*, 1995). This property makes the study of anaphase B particularly amenable to genetic and molecular biological approaches and has enabled the identification of motor proteins important for spindle elongation as well as regulators of anaphase (Hoyt *et al.*, 1992; Roof *et al.*, 1992; Eshel *et al.*, 1993; Li *et al.*, 1993; Lamb *et al.*, 1994; Pellman *et al.*, 1995; Saunders *et al.*, 1995; Yamamoto *et al.*, 1996). From these studies it is clear that despite the structural differences between yeast and mammalian spindles, many aspects of the mechanism of anaphase B are conserved.

In contrast to anaphase B, the existence of anaphase A chromosome movement in budding yeast has been much more difficult to establish because of an inability to visualize individual yeast chromosomes or kinetochores by conventional microscopy. In fact, it is reasonable to postulate that budding yeast lacks anaphase A altogether since the extensive spindle elongation during anaphase B appears to be sufficient to accomplish chromosome segregation. However, three lines of evidence are consistent with the existence of anaphase A in budding yeast. First, partially reconstituted kinetochores exhibit, in vitro, an anaphase A-like activity (Hyman *et al.*, 1992). Second, although the budding yeast kinetochore is thought to be simple, established and potential yeast kinetochore components have a similarity to mammalian proteins (Meluh and Koshland, 1995; Brown, 1995; Stoler *et al.*, 1995; Connelly and Hieter, 1996). Based on this similarity, yeast centromeres are likely to have functions in common with other eukaryotic centromeres, including possibly anaphase A activity. Third, in vivo evidence for anaphase A has come from three-dimensional reconstructions of the mitotic spindle (Winey *et al.*, 1995). Although budding yeast kinetochores cannot be visualized by electron microscopy, a numeric argument suggests that the bulk of microtubules of the yeast spindle are attached to kinetochores. These presumptive kinetochore microtubules shrink during anaphase, a phenomenon associated with anaphase A chromosome movement of most other eukaryotes. However, each of these three lines of evidence are subject to caveats: 1) the in vivo relevance of the kinetochore reconstitution experiments has not been established; 2) the conserved kinetochore components may participate in common activities other than anaphase A; and 3) kinetochore position and hence anaphase A can only be inferred from the spindle reconstruction. It is clear that additional methods are needed both to corroborate or disprove these initial findings as well as to facilitate in vivo analysis of overall chromosome movements.

We recently developed a FISH protocol for budding yeast which allows us to determine the location of specific chromosomal regions within the yeast nucleus. Our previous work with budding yeast demonstrated that during vegetative (mitotic) growth, centromeres tended to be loosely clustered toward one side of the nucleus in asynchronous populations, that sister chromatids are paired along their length until anaphase, and that chromosomes condense in a cell cycle-dependent manner (Guacci *et al.*, 1993, 1994). Here, we utilized our FISH technique to monitor the position of centromeres in vivo as a function of the cell cycle. A similar approach has been used to analyze centromere movement in fission yeast (Funabiki *et al.*, 1993). With this assay, we were able to demonstrate that budding yeast centromeres exhibit anaphase A movements and that chromosome position and orientation during the cell cycle is comparable to that in other eukaryotes. The implications of these chro-

Table 1. Yeast strains

Name	Genotype
BP5050	*Mata/Matα leu2/LEU2 ade2/ADE2 ade3/ADE3 his7/his7 can1/can1 sap3/sap3 gal1/gal1 HOM3/hom3 CYC2/cyc2*
DK5306	*Mata/Matα cdc4-1/cdc4-1 leu2/leu2 ade2/ade2 ade3/ade3 his7/his7 can1/can1 sap3/sap3 gal1/gal1*
DK201	*Mata/Matα cdc28-1/cdc28-1 leu2/LEU2 ade2/ADE2 ade3/ADE3 his7/his7 can1/can1 sap3/sap3 gal1/gal1 hom3/HOM3*
Dk210	*Mata/Matα cdc14-1/cdc14-1 leu2/LEU2 ade2/ADE2 ade3/ADE3 his7/his7 can1/can1 sap3/sap3 gal1/gal1 hom3/HOM3*
DK229	*Mata/Matα cdc20-1/cdc20-1 LEU2/leu2 ade2/ADE2 ade3/ADE3 his7/his7 can1/CAN1 sap3/SAP3 gal1/gal1 hom3/ HOM3 ura1/URA1*
DK230	*Mata/Matα cdc23-1/cdc23-1 LEU2/leu2 ade2/ADE2 ade3/ADE3 his7/his7 can1/CAN1 sap3/SAP3 gal1/gal1 hom3/ HOM3 ura1/URA1*

mosome movements in such a simple eukaryote are discussed.

MATERIALS AND METHODS

Reagents

Zymolyase T100 was obtained from ICN (Costa Mesa, CA) or Seikagaki (Rockville, MD). Polylysine was obtained from Sigma (St. Louis, MO) and prepared as a 1-mg/ml solution in distilled H_2O. Restriction enzymes and proteinase K were obtained from Boehringer Mannheim (Indianapolis, IN). RNase was obtained from Sigma and made DNase free as described previously (Maniatis *et al.*, 1982). Nick translations were carried out using the BioNick Labeling System from Life Technologies (Gaithersburg, MD). Slides for in situ hybridization were obtained from Roboz Surgical (Rockville, MD). YEPD media contained 1% yeast extract, 2% peptone, and 2% dextrose. Yeast strains are listed in Table 1.

FISH

FISH was performed as described previously in Guacci *et al.* (1994), with the following minor modifications. Following spheroplasting, an equal volume of 1% Triton X-100 was added directly to the spheroplasted suspensia, which was then mixed by inversion, incubated for 5 min at room temperature, and centrifuged at 10K for 5 s. Spheroplasts were resuspended in H_2O and added to the polylysine-coated slides. In addition, in some cases, cells on slides were denatured twice, both before and after proteinase K treatment.

Probes for FISH

Probes were labeled with digoxigenin by nick translation and hybridized probes were detected using fluorescein isothiocyanate-(FITC) conjugated antibodies as described previously (Guacci *et al.*, 1994). The source of DNA templates used to make probes and the position of the corresponding homologous sequences on the chromosome are as follows.

Centromere Proximal Probes. The chromosome I centromere proximal probe (*CEN1* probe) consists of a 40-kb contiguous unique DNA sequence made from a mixture of chromosome I inserts (Guacci *et al.*, 1994). The centromere proximal edge of this probe is 19.8 kb from *CEN1*. The chromosome IV centromere proximal probe (*CEN4* probe) is the cosmid 70938, which contains a 40.1-kb yeast DNA insert. The centromere proximal edge of this insert is located 8.8 kb from *CEN4*. The chromosome XVI centromere proximal probe (*CEN16* probe) is the cosmid 71042, which contains a 36.2-kb yeast DNA insert. The centromere proximal edge of this insert is located 23.5 kb from *CEN16*.

Centromere Distal Probes. Two centromere distal probes from chromosome XVI were used in this study. Cosmid 70912 (arm probe 1) contains a 41.5-kb yeast DNA. The centromere proximal edge of this insert is located 295.5 kb from *CEN16*. Cosmid 70982 (arm probe 2) contains a 37.2-kb yeast DNA insert. The centromere proximal edge of this insert is located 387.5 kb from *CEN16*.

The cosmids from chromosome IV and chromosome XVI were purchased from the American Tissue Culture Collection. The chromosomal positions of all probes were determined using sequencing information from the *S. cerevisiae* genome database.

Determination of the Relative Position of FISH Signals within the Nucleus

In the course of the FISH protocol, cells are fixed and then mounted onto slides. Because of their asymmetric shape, cells adhere to the surface of the slide in an oriented manner. The nucleus and chromosomal DNA mass in turn are oriented with respect to the cell body reflecting the nonrandom position of the nucleus in the cell. This nuclear orientation is evident in cells processed for indirect immunofluorescence, where the spindle pole bodies always lay near or at the periphery of the chromosomal mass (see below). Since the fixation and mounting of cells is the same for both FISH and indirect immunofluorescence, the nonrandom orientation of the mounted nuclei and chromosomal masses must be preserved for FISH as well. The nonrandom nuclear orientation is essential for the quantification used in our FISH assays, since random nuclear and DNA mass orientation would obscure any nonrandom distribution of centromeres or other chromosomal sequences.

Following FISH, two kinds of DNA masses can be distinguished by their shape, elongated ellipsoid DNA masses which are from anaphase cells and spherical DNA masses which are from cells at all other stages of the cell cycle. The quantitation of the position of FISH signals in these types of DNA masses are as follows.

Spherical DNA Masses (Nonanaphase Cells). Because the DNA masses were not precise spheres, we measured the minimum and maximum diameters of each DNA mass to generate an average diameter. We then used two assays to characterize the position of FISH signals.

Distance-to-Edge Assay. Cells were hybridized with a single probe which generates up to two FISH signals per chromosomal DNA mass, one from each homologue. We measured the distance that each FISH signal was separated from the nearest edge of the chromosomal DNA mass (distance-to-edge). To correct for the slight variation of the DNA mass size caused by the FISH protocol, the distance-to-edge for each FISH signal was divided by the average diameter of the corresponding DNA mass (normalized distance-to-edge). The normalized data were grouped into intervals spanning 0.1 diameters and plotted in histogram form. Since the center of the DNA mass is 0.5 diameters from an edge, no distance-to-edge should exceed 0.5 diameters since that would place it closer to the opposite side.

Cluster Assay. Cells were hybridized with a mixture of three probes from centromere proximal regions of chromosomes I, IV, and XVI which generates up to six FISH signals per chromosomal DNA mass, two from each probe. We measured the largest separation between any two FISH signals in each DNA mass (maximal separation). To correct for any DNA mass size variation, the maximal

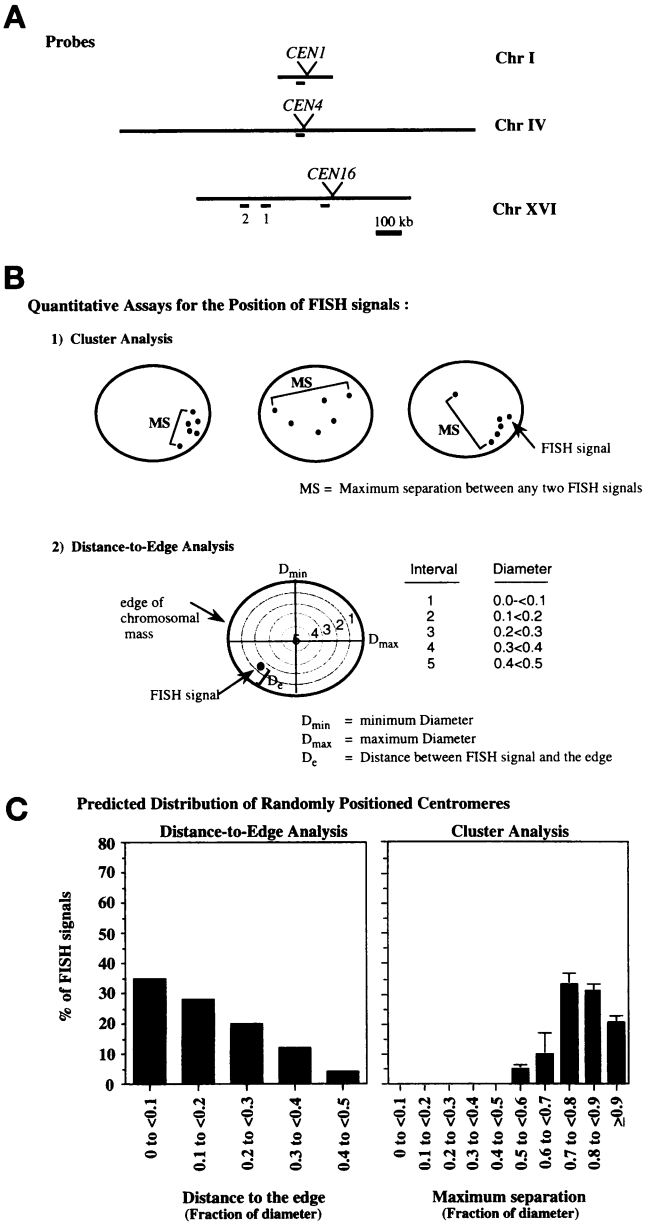

Figure 1. (A) Schematic showing the chromosomal DNA sequences used for FISH. Probes were made using DNA segments from three different yeast chromosomes. The length and position of the chromosomal sequences detected by the probes are drawn to scale (see MATERIALS AND METHODS). (B) Quantitation of centromere position using FISH. The diameter of a FISH signal and average nuclei were 0.3 and 4 μm, respectively. Part 1, clustering of FISH signals. Cells were hybridized with a mixture of three probes to generate up to six FISH signals per nucleus (see MATERIALS AND METHODS). We measured the maximum separation between any two of the six FISH signals (MS). The MS distance is dividing by the maximum diameter of the chromosomal mass to convert the data to MS as a fraction of the DNA mass diameter (see MATERIALS AND METHODS). Part 2, FISH signal distance to the periphery of the chromosomal mass. Cells were hybridized with one probe to generate two FISH signals per nucleus (see MATERIALS AND METHODS). We measured the distance between a FISH signal and separation was divided by the maximum diameter of the corresponding DNA mass (normalized maximal separation). We divided by maximum DNA mass diameter instead of average diameter to prevent cases where the maximal separation exceeds the diameter of the DNA mass. The normalized data were grouped into intervals spanning from 0.1 to 1.0 diameters and plotted in histogram form. ***Elongated DNA Masses (Anaphase Cells).*** The elongated DNA mass in anaphase cells enables us to orient our measurements with respect to its long axis. Since sister chromatids are moving to opposite ends of the nucleus, the DNA at each end of the long axis is leading the segregating DNA; therefore, it is designated the leading-edge of the elongated DNA mass. We measured the distance along the long axis of the elongated DNA mass (i.e., from leading edge to leading edge) and divided this value by two to yield the average diameter for each of the two separating DNA masses.

Distance-to-Leading Edge Assay. Diploid cells were hybridized with a single probe which generates up to two FISH signals in each of the two separating chromosomal DNA masses. We also measured the distance from the FISH signal to the nearest leading edge (distance-to-leading edge). The relative position of a given FISH signal was calculated by dividing its distance-to-leading edge by the diameter of a single DNA mass for the corresponding nucleus. This result is reported as a fraction of the diameter. The data were grouped into intervals spanning from 0.1 to 1.0 diameters and plotted in histogram form.

Image Collection and Measurement of Distances for FISH Analyses

Images were visualized using a standard Zeiss universal epifluorescence microscope. Propidium and FITC images were recorded digitally using a Hamamatsu CCD camera (2400) and the Image-1/AT processing system (Universal Imaging Corporation, Media, PA). This system allowed us to superimpose images. Images of the FISH signals were magnified 4000-fold (2000-fold optically and 2-fold digitally). Distances between the FISH signals and the pe-

Figure 1 (cont). the nearest edge of the chromosomal mass (D_e). The D_e is normalized to the size of the chromosomal mass by dividing by the average diameter, $(D_{min} + D_{max})/2$. Since most chromosomal masses are approximately spherical, the maximum diameter and average diameter are usually very similar. The normalized D_e is assigned to one of five intervals encompassing 0.1 diameters of the DNA mass (see MATERIALS AND METHODS). (C) Theoretical random distributions of FISH signals relative to other FISH signals (cluster analysis) and relative to the edge of the chromosomal mass (distance-to-edge analysis). In both calculations the chromosomal mass is assumed to be a spherical disk of uniform depth. Cluster analysis: To generate a random distribution of six FISH signals within the chromosomal mass, we used a Monte Carlo function to assign six random sets of x,y coordinates (representing the six FISH signals) within a circle (representing the chromosome mass) of radius r. This was done 40 times to mimic measurements of 40 independent chromosomal masses. An x,y coordinate was discarded only if it was at a distance of r or greater from the center of a circle. The distance between the signals with maximal separation (MS) was determined and divided by the diameter (2r). These normalized values expressed as a fraction of the diameter were placed into 1 of 10 intervals encompassing 0.1 diameters. The percentage of normalized MS in each interval was plotted in histogram form. This was repeated a second time with 40 new simulated chromosomal masses and the SD between the two trials is indicated by error bars. Distance-to-edge analysis: To generate a random distribution of centromeres relative to the periphery of the chromosomal mass, we determined the probability of a centromere signal being in one of the five intervals which is simply the surface area of the interval divided by the total surface area of the disk.

riphery of the nuclear DNA were measured using the morphometric programs of Image 1.

Indirect Immunofluorescence

Microtubules were detected in cells by indirect immunofluorescence as described previously (Yamamoto *et al.*, 1996).

Arrest of Cells at Discrete Stages of the Cell Cycle

Cells were grown in YEPD liquid at 23°C until cultures were mid-log phase and treated as follows.

Cdc Mutant Arrest. Cells were transferred to 37°C for 3 h to arrest cells.

Nocodazole (Nz) Treatment of Telophase-arrested Cells. Asynchronous cultures of diploid strain DK210 (*cdc14/cdc14*) were grown to mid-log phase and transferred to 37°C for 2.5 h to arrest cells in telophase. Nz was then added (15 μg/ml final concentration) and incubation was continued at 37°C for 1 h to depolymerize microtubules in arrested cells. Cells were washed free of Nz with YEPD (prewarmed to 37°C) and resuspended in fresh YEPD (prewarmed to 37°C), and the incubation was continued at 37°C for 1 h to allow microtubules to repolymerize in arrested cells.

RESULTS

Centromere Position Was Monitored Qualitatively and Quantitatively Using FISH

Using FISH, we identified the position of several different yeast centromeres within the nucleus during vegetative (mitotic) growth. Diploid cells were hybridized with a mixture of centromere probes from three chromosomes (I, IV, and XVI; Figure 1A). This enabled visualization of as many as six centromeres at once and yields qualitative information as to 1) centromere position relative to other centromeres, e.g., clustered or dispersed; and 2) centromere position relative to the total chromosomal mass, e.g., peripheral or internal. To quantitate centromere clustering, the maximum separation between any two FISH signals was measured and normalized (Figure 1B and MATERIALS AND METHODS). This method of quantitation is a minimal estimate of clustering as a distribution of five clustered and one dispersed centromere are scored the same as six dispersed centromeres (Figure 1B). To quantitate centromere position relative to the chromosomal DNA periphery, we hybridized diploid cells with a single centromere proximal probe from one chromosome (IV or XVI; Figure 1A). A single chromosomal probe was utilized because only two FISH signals are generated, making it easier to distinguish each signal. The distance between each FISH signal and the periphery of the chromosomal DNA mass (distance-to-edge) was measured and normalized (Figure 1B and MATERIALS AND METHODS). All distance measurements were normalized to the diameter of the chromosomal mass to minimize the effect of the small variability in the size of the chromosomal mass which was generated by a flattening of the nuclei during the FISH protocol. Under our conditions of fixing and mounting cells, the nuclei and chromosome mass are oriented on the surface of the slide reflecting the nonrandom position of the nucleus in the cell (see MATERIALS AND METHODS). This is critical since random fixation of chromosomal masses to the slide would tend to obscure nonrandom distributions of centromeres.

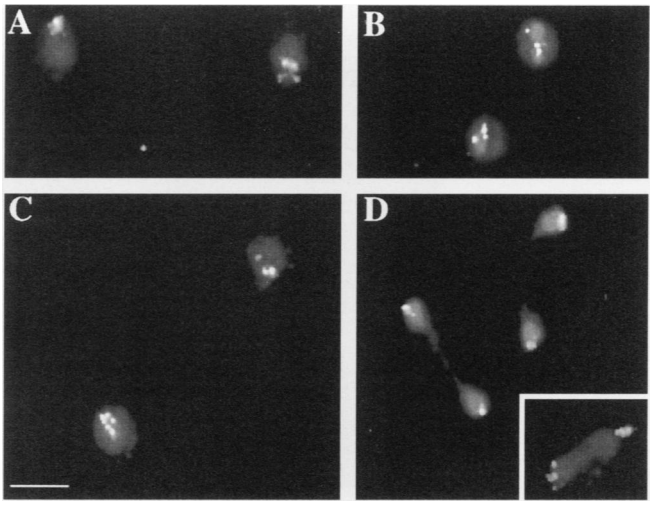

Figure 2. Centromere position in cells from asynchronously growing cultures. Mid-log cultures of diploid strain BP5050 were fixed and subjected to FISH using probes from centromere proximal sequences (see MATERIALS AND METHODS). Chromosomal DNA was stained with propidium iodide (gray), and hybridized digoxigenin-labeled DNA probes were detected by FITC-conjugated antibodies (white). (A–D) Qualitative assay of centromere position using a mixture of three centromere proximal probes (*CEN1*, *CEN4*, and *CEN16*, Figure 1A; see MATERIALS AND METHODS). Bar, 5μm.

To provide a reference for these analyses, the clustering of centromeres and centromere position relative to the edge of the chromosomal mass were modeled for the hypothetical case in which centromeres were located randomly within the nucleus (Figure 1C). In this calculation, we assumed that the flattened chromosome mass approximates a spherical disk with uniform depth. Although this idealized shape only approximates the real shape of the chromosome mass processed for FISH, the results from this modeling serve two purposes. First, they illustrate that for a random distribution of six centromeres, at least two centromeres are likely to be widely dispersed, i.e., to lay on nearly opposite sides of the chromosomal DNA mass. Second, they illustrate that in a random distribution of centromeres relative to the chromosomal mass, a centromere is more likely to be in a peripheral position because the peripheral intervals encompass a significant fraction of the chromosomal mass.

Centromeres Are Not Uniformly Positioned in Cells from Asynchronous Populations

Wild-type diploid strain BP5050 was grown at 23°C until mid-log phase, fixed, and then subjected to FISH.

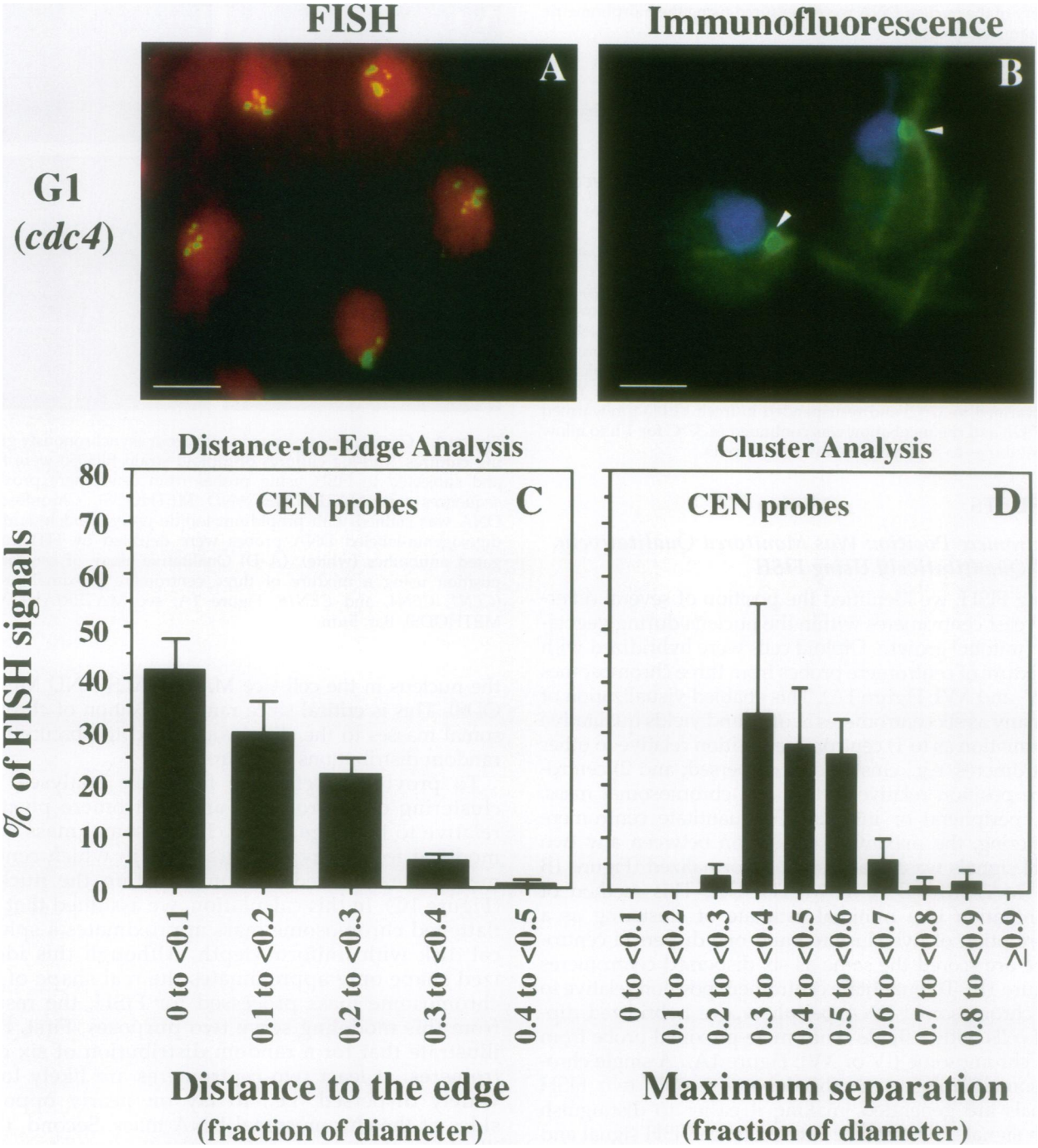

Figure 3. Centromere and spindle pole localization in G1-arrested cells. Cells from diploid strain BP5306 (*cdc4/cdc4*) were arrested in G1 and fixed. One aliquot was processed for FISH and a second aliquot was processed for immunofluorescence (see MATERIALS AND METHODS). (A) Micrograph showing centromere position in DNA masses following FISH. Cells were hybridized with a mixture of three centromere proximal probes (*CEN1*, *CEN4*, and *CEN16*; see MATERIALS AND METHODS). Chromosomal DNA was stained with propidium iodide (red), and hybridized digoxigenin-labeled DNA probes were detected by FITC-conjugated antibodies (yellow-green). DNA masses from five cells are shown. Bar, 5 μm. (B) Detection of microtubules and localization of spindle poles at the apex of microtubules following indirect immunofluorescence using antitubulin antibodies (see MATERIALS AND METHODS). Chromosomal DNA was stained with DAPI (blue), and microtubules were detected by FITC-conjugated antibodies (green). Two cells are shown. Arrowheads, position of the spindle poles. Bar, 2.5 μm. (C) Quantitation of centromere distance-to-edge. Cells were hybridized to a single probe (either *CEN4* or *CEN16*) to generate two FISH signals per DNA mass (Figure 1A and MATERIALS AND METHODS). The distance between any one FISH signal and the nearest edge of the DNA mass was determined, normalized to the average diameter, and placed into one of five intervals each

The gross morphology of the chromosomal DNA in most cells from an asynchronous population is roughly spherical (Pringle and Hartwell, 1981). In this population, the clustering of centromeres and their position relative to the chromosomal mass varied significantly (Figure 2). Some nuclei did have centromeres that were clustered and peripheral (Figure 2A), whereas others were dispersed and internal (Figure 2B). We were unable to correlate a specific pattern with a particular point in the cell cycle because the FISH protocol destroys bud morphology and spindle structure, the nonchromosomal landmarks normally used to distinguish cell cycle stages in asynchronous cultures. In contrast, anaphase cells could still be identified by the elongated ellipsoid shape of their chromosomal DNA (Pringle and Hartwell, 1981). In these anaphase nuclei, the centromeres were positioned more uniformly near the ends of the elongated chromosomal mass (Figure 2D and below). These observations suggested that centromere position was different between anaphase and other stages of the cell cycle.

Centromeres Are Clustered Loosely Near the Spindle Pole in G1-arrested Cells

To determine whether centromere position changes in a cell cycle-dependent manner, we compared centromere position in cells arrested at different stages of the cell cycle. This was made possible by the availability of *cdc* mutants, which after shifting to 37°C (nonpermissive temperature) arrest as uniform populations at distinct stages of the cell cycle (see MATERIALS AND METHODS). Following arrest of the *cdc* mutants, one aliquot of each culture was subjected to FISH to determine centromere position qualitatively and quantitatively as described above, while a second aliquot was processed for indirect immunofluorescence to visualize microtubules (see MATERIALS AND METHODS).

To examine the pattern of centromere position in G1 cells, we examined strains bearing either a *cdc28–1* or *cdc4* mutation since such strains arrest in G1 after incubation at the nonpermissive temperature (Pringle and Hartwell, 1981). In diploid strains DK201 (*cdc28–1/cdc28–1*) and BP5306 (*cdc4/cdc4*), centromeres appeared to be restricted to one half of the chromosomal mass (Figure 3A and our unpublished results). This impression was documented by our quantitative analysis. Measurements of centromere distance-to-edge indeed revealed a bias toward the nuclear periphery similar to that predicted for a random distribution (compare Figures 3C and 1C). In contrast, cluster analysis revealed that centromeres were grouped significantly more tightly than the random distribution (compare Figures 3D and 1C). Taken together, the peripheral bias and the clustering explain the restriction of centromeres to one half of the chromosomal mass. As expected, visualization of the microtubules in G1-arrested cells showed a monopolar aster (Figure 3B). The spindle pole body (SPB; the yeast microtubule organizing center) was evident as the vertex of tubulin staining and was always positioned at the nuclear periphery (Figure 3B), consistent with the fact that the yeast SPB is embedded in the nuclear envelope and that the nuclei are fixed to the slide in a specific orientation.

We wanted to determine whether the asymmetrically positioned centromeres were biased toward the side of the nucleus harboring the SPB. We previously showed that the rDNA in G1 cells is present as a diffuse cap at the periphery of one side of the nuclear DNA mass (Guacci *et al.*, 1993, 1994). The nucleolus in budding yeast cells forms a crescent-shaped cap which lies on the opposite side of the nucleus from the SPB in 77% of G1 cells (Yang *et al.*, 1989). Taken together, these results imply that the rDNA, which resides in the nucleolus, and the SPB are on the opposite side of the nucleus in most G1 cells. Thus, the rDNA can serve as a presumptive marker to identify the side of the nuclear DNA opposite the spindle pole. To compare the position of the rDNA to that of centromeres in G1-arrested cells, diploid strain BP5306 (*cdc4/cdc4*) was arrested and then subjected to FISH using a probe mixture containing a probe specific for the rDNA and a probe specific for two centromeres (*CEN4* and *CEN16*; Figure 1A). In 80% of the nuclei, the centromeric FISH signals were localized to one side of the DNA mass whereas the rDNA FISH signals were on the opposite side. These results strongly suggest that centromere position in G1 cells is biased toward the side of the chromosomal mass proximal to the spindle pole.

Centromeres in Mid-M Cells Are Positioned Away from the Periphery of the Chromosomal Mass and Away from the Spindle Poles

To assess centromere position in cells arrested in mitosis but prior to anaphase (short spindle and a single DNA mass), we examined strains bearing either a *cdc20* or a *cdc23* mutation (Pringle and Hartwell, 1981).

Figure 3 (cont). encompassing 0.1 diameters (Figure 1B and MATERIALS AND METHODS). Data from *CEN4* and *CEN16* probes were identical and were combined. The normalized data are reported as the percentage of FISH signals. The histogram and SDs (error bars) were generated using more than 200 measurements from three independent experiments. (D) Quantitation of centromere clustering. Cells were hybridized with a mixture of three centromere proximal probes (*CEN1*, *CEN4*, and *CEN16*) to generate up to six FISH signals per DNA mass (Figure 1A and MATERIALS AND METHODS). The maximal separation between any two FISH signals in a DNA mass was measured and normalized to the diameter of chromosomal mass (Figure 1B and MATERIALS AND METHODS). The normalized data are reported as the percentage of FISH signals with maximum separation in each of 10 intervals spanning 0.1 diameters and are displayed in histogram form. The histogram and SDs (error bars) were generated using ~80 measurements from two independent experiments.

Sister chromatids are condensed and paired (at the centromeres and along their length) in *cdc20*- and *cdc23*-arrested cells; therefore, we refer to this arrest point as mid-M (Guacci *et al.*, 1994 and our unpublished results). In mid-M-arrested diploid strains DK229 (*cdc20/cdc20*) and DK230 (*cdc23/cdc23*), centromeres appeared to be neither clustered nor positioned at the nuclear DNA periphery (Figure 4A and our unpublished results). Quantitation of centromere position revealed that centromeres were dispersed and distributed throughout the interior of the chromosomal mass (Figure 5, A, B, and E). This centromere distribution is likely to be representative of normal mid-M cells since it was observed in two different mutants that cause mid-M arrest. In addition, an internal centromere localization pattern was also detected in a minority of nuclei from asynchronous wild-type cultures (see above), presumably corresponding to mid-M cells from the cycling population. The spindle in mid-M-arrested cells traverses the chromosomal mass so the spindle poles are at or near the periphery of the DNA mass (Figure 4B). Although we cannot assess the orientation of the spindle relative to the centromeres in each nucleus, we conclude that centromeres are usually away from the poles in mid-M cells because, unlike the spindle poles, they are not confined to the periphery of the chromosomal DNA mass.

The internal centromere distribution in mid-M cells is specific to centromere proximal sequences. When the position of a centromere distal sequence (Figure 1A, probe 1) was analyzed by FISH in *cdc20*-arrested cells, the FISH signals were closer to the periphery of the DNA mass than the *CEN16* probe (compare Figure 5, E and F). Furthermore, there was a good correlation between the physical distance that a given DNA sequence was from the centromere on its chromosome and the distance-to-edge of the corresponding FISH signal. When cells were hybridized with centromere distal arm probe 2, 81% of the FISH signals were in the interval closest to the DNA periphery (0 to <0.1 distance-to-edge). When cells were hybridized with the more centromere proximal arm probe 1, only 43% of the FISH signals were located in this interval.

Centromeres in Anaphase and Telophase Cells Are Positioned Near the Periphery of the Chromosomal Mass and Proximal to the Spindle Poles Providing Evidence for Anaphase A

Next, we analyzed centromere position in cells arrested in mitosis but after anaphase. We utilized *cdc14* mutant cells, which arrest in late M with a fully elongated spindle and separated DNA masses (Pringle and Hartwell, 1981; Figure 4D). We have previously shown that sister chromatids are segregated and decondensed in *cdc14*-arrested cells; therefore, this arrest point will be referred to as telophase (Guacci *et al.*, 1994). In telophase-arrested diploid strain DK210 (*cdc14/cdc14*), centromeres were positioned very close to the DNA periphery and were tightly clustered (Figures 4C and 5, C and D). In a subset of cells, the two daughter nuclei are still connected by a small amount of chromosomal DNA, making it possible to identify the two leading edges of the segregating chromosomes (Figure 4C, inset). In these cells, centromeres were at the leading edges. This position correlates with that of the spindle poles in *cdc14*-arrested cells analyzed by indirect immunofluorescence (Figure 4D). Thus, centromere position is dramatically altered as cells progress through mitosis, changing from being dispersed and distal to the spindle poles in mid-M to clustered and in close proximity to the spindle poles by telophase (Figures 4 and 5). We also compared the absolute distance-to-edge and found that the average distance-to-edge in mid-M cells was 1.14 μm $\pm$ 0.20 and 1.22 μm $\pm$ 0.08 for *cdc23* and *cdc20* cells, respectively, whereas in telophase (*cdc14*) cells, it was only 0.36 μm $\pm$ 0.06 (SEM). These decreases in relative and absolute distances demonstrate centromere movement to the poles, thereby supporting the existence of anaphase A chromosome movement in budding yeast.

An analysis of cycling wild-type cells further corroborated the positioning of centromeres near spindle poles in anaphase. As described above, anaphase cells can be recognized by the elongated appearance of the chromosomal DNA. This shape presumably reflects ongoing segregation of sister chromatids to the spindle poles with the long axis corresponding to the position of the spindle (Figure 6A). The leading edges of the elongated DNA mass are near the spindle poles (Figure 6A, arrowheads). In all anaphase nuclei detected, FISH signals from centromeres were uniformly clustered either at or near the leading edges (Figure 6B). Quantitation of *CEN4* and *CEN16* positions confirmed these observations (Figure 6D). This was true both in cells with either slightly elongated or dramatically elongated bilobed DNA masses (Figure 2D). These results coupled with those from *cdc14*- arrested cells suggest that centromeres are clustered near the spindle poles from early anaphase to telophase.

As a consequence of centromere-mediated anaphase movement, chromosomal arms should trail behind the centromeres. To test this prediction, we assessed the relative orientation of chromosomes in anaphase cells by comparing the distance-to-leading edge for centromere distal probes to those for centromere proximal probes. To this end, asynchronous cultures of diploid strain BP5050 were subjected to FISH using one of two centromere distal probes from chromosomes XVI (Figure 1A, probes 1 and 2). As described above, we identified anaphase cells from the population by their elongated DNA morphology. When hybridized to a single centromere distal probe, four FISH signals were detected in most anaphase cells (Figure 6C). This re-

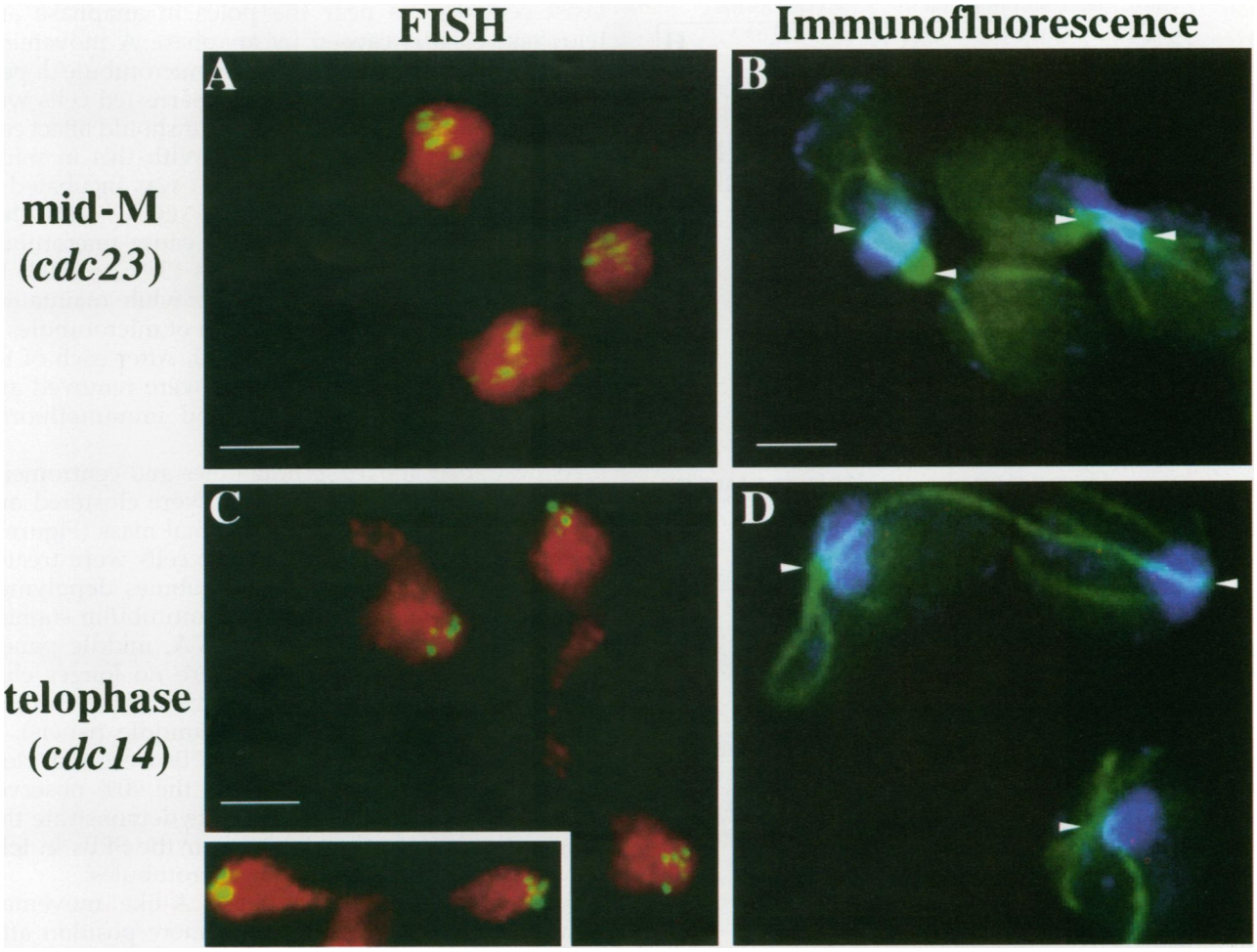

Figure 4. Centromeres and spindle poles visualized in cells arrested in mid-M and telophase. Diploid strains DK230 (*cdc23/cdc23*) and DK210 (*cdc14/cdc14*) were arrested in mid-M and telophase, respectively, and fixed. One aliquot of each strain was processed for FISH and a second aliquot was processed for immunofluorescence (see MATERIALS AND METHODS). (A and C) Micrographs showing centromere position in DNA masses following FISH. Cells were hybridized with a mixture of three centromere proximal probes (*CEN1*, *CEN4*, and *CEN16*; see MATERIALS AND METHODS). Chromosomal DNA was stained with propidium iodide (red), and hybridized digoxigenin-labeled DNA probes were detected by FITC-conjugated antibodies (yellow-green). Bars, 5 μm. (B and D) Detection of mitotic spindles and localization of spindle poles at the apex of microtubules following immunofluorescence using antitubulin antibodies (see MATERIALS AND METHODS). Chromosomal DNA was stained with DAPI (blue), and microtubules were detected by FITC-conjugated antibodies (green). Bars, 2.5 μm. (A and B) Mid-M diploid cells (DK230; *cdc23/cdc23*). (A) DNA masses from three cells. (B) Two cells each with an undivided nucleus and a short spindle are shown. (C and D) Telophase diploid cells (DK210; *cdc14/cdc14*). During preparation for both indirect immunofluorescence and FISH, the telophase configuration (two cell bodies, each with a chromosomal mass connected by a spindle) is often broken, giving rise to individual cell bodies with a single nucleus and a half spindle. (C) Chromosomal masses from one broken telophase cell (left) and two complete telophase cells (upper and lower right and inset) in which a thin line of DNA connects the two segregated DNA masses are shown. (D) One intact telophase cell (top) with an elongated spindle and two segregated chromosomal masses and one broken telophase cell (bottom) with a half spindle. Arrowheads, position of the spindle poles.

sult is expected since sister chromatids of each homologue have separated but are still contained in a single, albeit elongated DNA mass (Guacci *et al.*, 1994). Most FISH signals from centromere distal arm probe 1 were found well away from the leading edge (Figure 6, C and E). Furthermore, there was a good correlation between the physical distance of a given DNA sequence from its cognate centromere and the distance-to-leading edge of the corresponding FISH signals. For example, FISH signals from arm probe 2, which is almost 100 kb more centromere distal than arm probe 1, were further from the leading edge whereas FISH signals from probes 100 more centromere proximal than arm probe 1 were positioned closer to the leading edge (Guacci *et al.*, 1994 and our unpublished results). These results show that during anaphase in yeast, chromosomes are oriented in a manner similar to anaphase chromosomes in other eukaryotes, with cen-

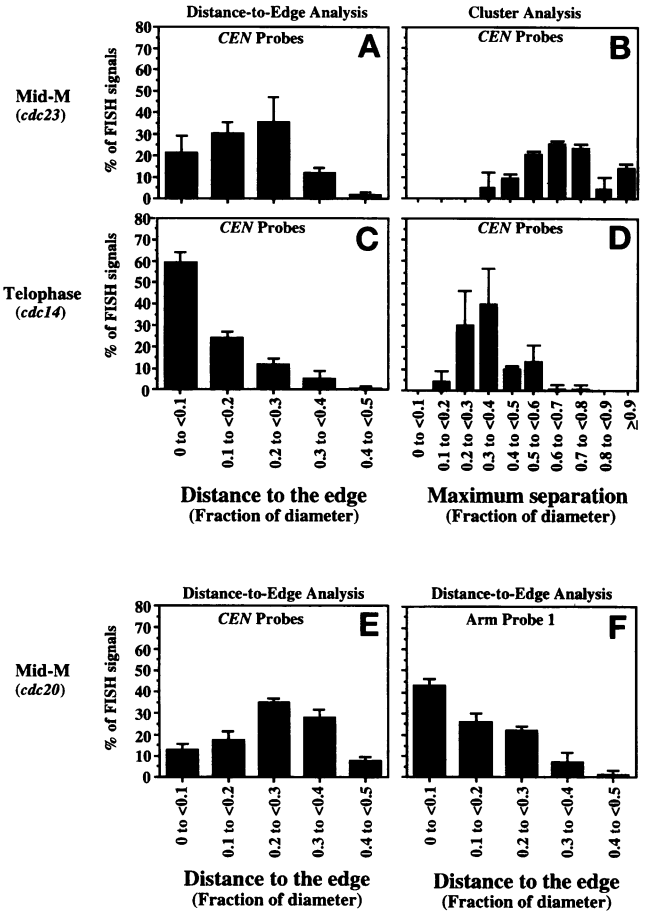

Figure 5. Quantitation of the position of sequences in mid-M and telophase cells. Diploid strains DK230 (*cdc23/cdc23*), DK229 (*cdc20/cdc20*), and DK210 (*cdc14/cdc14*) were arrested in either mid-M or telophase and subjected to FISH (see MATERIALS AND METHODS). (A–E) Centromere proximal probes. Data from *CEN4* and *CEN16* probes were identical and were combined. (F) Centromere distal probe. (A, C, E, and F) Quantitation of FISH signal distance-to-edge. Analyses were performed as described in the Figure 3C legend and in MATERIALS AND METHODS, except for (F) where arm probe 1 was used instead of the *CEN* probes. For the centromere distal probe, the histogram and SDs (error bars) were generated using more than 200 measurements from two to six independent experiments. (B and D) Quantitation of centromere clustering. Analysis was performed as described in the Figure 3D legend and in MATERIALS AND METHODS. The histogram and SDs (error bars) were generated using ~80 measurements from two independent experiments.

tromeres leading and close to spindle poles while chromosomal arms trail behind.

Telophase Clustering of Centromeres Is Microtubule Dependent and Can Occur after Anaphase B Spindle Elongation, Providing Further Evidence for Anaphase A-Like Activity

In other eukaryotes, the movement of centromeres to the poles is microtubule dependent. If the clustering of yeast centromeres near the poles in anaphase and telophase cells is caused by anaphase A movement, then this clustering should also be microtubule dependent. Thus, treatment of telophase-arrested cells with a microtubule depolymerizing agent should affect centromere clustering and position. With this in mind, diploid strain DK210 (*cdc14/cdc14*) was incubated at 37°C to arrest cells in telophase (37°C) and then treated with Nz while at 37°C to cause microtubule depolymerization in arrested cells (37°C + Nz). Finally, cells were washed free of Nz while maintained at 37°C, allowing repolymerization of microtubules in arrested cells (37°C + Nz + wash). After each of the three treatments, aliquots of cells were removed and processed separately for FISH and immunofluorescence.

As described above, spindle poles and centromeres in telophase-arrested cells (37°C) were clustered and close to the edge of the chromosomal mass (Figure 7, A and B, top panels). When these cells were treated with Nz (37°C + Nz), the microtubules depolymerized leaving only a single dot of antitubulin staining associated with the SPB (Figure 7A, middle panel). Furthermore, the centromeres were no longer clustered nor biased toward the periphery of the chromosomal mass (Figure 7, A and B, middle panels). In these Nz-treated cells, 74% of the SPBs were at or close to the DNA periphery, similar to the 90% observed prior to Nz treatment. These results demonstrate that the positioning of centromeres near the SPBs in telophase is indeed dependent on microtubules.

Further support for anaphase A-like movement came from our analysis of centromere position after arrested cells were washed free of Nz (37°C + Nz + wash). The microtubules repolymerized but did not reform the long spindles originally present in the telophase-arrested cells. Instead, monopolar spindles were observed and the SPB of each half spindle was found at the DNA periphery in 90% of the cells (Figure 7A, bottom panel). Most centromeres were again found clustered together near the edge of the DNA mass (Figure 7A and B, bottom panel). In fact, quantitative analysis showed that centromere position after microtubule repolymerization was indistinguishable from that observed prior to microtubule depolymerization (Figure 7B, compare top and bottom panels). The average diameter of the chromosome mass increased slightly during the course of treatment for cells processed for FISH as well as for indirect immunofluorescence. For FISH, the average DNA mass sizes were 3.7 μm for 37°C, 4.1 μm for 37°C + Nz, and 4.3 μm for 37°C + Nz + wash. Thus, the changes in centromere distribution that occur upon Nz treatment cannot be due to the increased size of the DNA mass since the largest DNA mass size is detected after removal of Nz, at which time the centromere distributions are identical to those prior to Nz treatment.

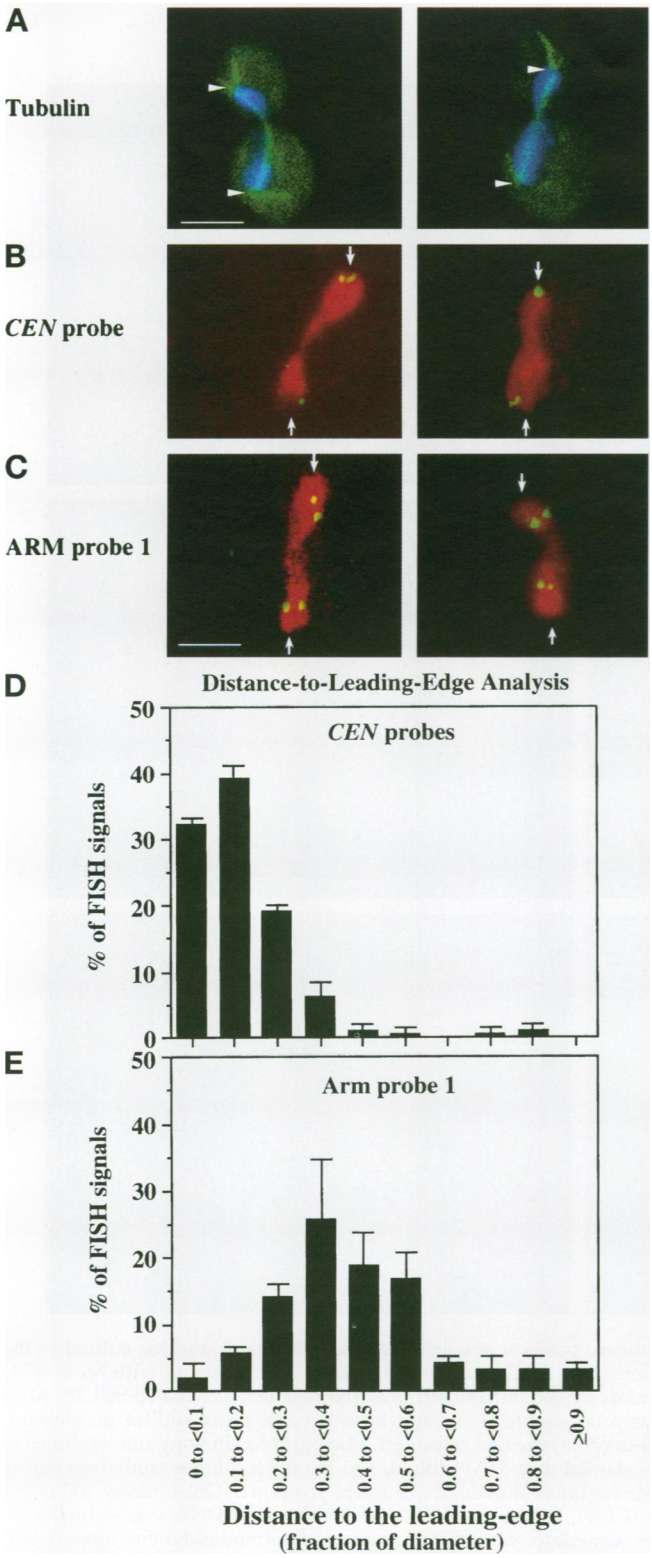

Figure 6. Centromere position in anaphase cells observed in a cycling population. Mid-log cultures of diploid strain BP5050 were fixed, and one aliquot was processed for FISH whereas a second aliquot was processed for immunofluorescence (see MATERIALS AND METHODS). (A) Micrographs showing mitotic spindles in anaphase cells subjected to indirect immunofluorescence. Chromosomal DNA is stained with DAPI (blue) whereas the mitotic spindle is detected with antitubulin and FITC-conjugated antibodies (green; see MATERIALS AND METHODS). Two anaphase cells are shown. Arrowheads, position of the spindle poles. Bar, 2.5 μm. (B and C) Micrographs of anaphase DNA masses following FISH. Chromosomal DNA was stained with propidium iodide (red), and hybridized digoxigenin-labeled DNA probes were detected by FITC-conjugated antibodies (yellow-green). Arrows, leading edges. Bar, 5 μm. (B) Centromere proximal probe (*CEN16*). Two anaphase DNA masses are shown. (C) Centromere distal probe (arm probe 1, Figure 1). Two anaphase DNA masses are shown. (D and E) Quantitation of FISH signal distance-to-leading edge. Cells were hybridized to a single probe and analyzed similar those described in the Figure 3C legend, except that distance was measured to the nearest leading edge (see MATERIALS AND METHODS). The normalized data are reported as the percentage of FISH signals with distances-to-leading-edge in each of 10 intervals spanning 0.1 diameters and are displayed in histogram form. (D) Centromere proximal probes (*CEN4* and *CEN16*). (E) Centromere distal probe 1. The histogram and SDs (error bars) were generated using ~200 measurements from two independent experiments.

Furthermore, these changes in centromere distribution were not due to an alteration in the nuclear orientation on the slides since, under all conditions, most nuclei had their SPBs near or at the periphery of the DNA mass.

To explain the changes in centromere distribution, we suggest that once the monopolar spindles form, the kinetochores capture microtubules and move to the poles. This would explain both the peripheral localization and the clustering of the centromeres. Although occasional nuclei are observed where an individual centromere fails to recluster (Figure 7A, bottom right panel, chromosome mass in upper left corner), the similarity of centromere positioning after microtubule repolymerization to centromere positioning prior to microtubule disassembly suggests that the capturing and movement of centromeres to the poles is efficient. The microtubule dependence of centromere reclustering provides additional evidence for a microtubule-dependent anaphase A activity in yeast which can occur in the absence of additional anaphase B spindle elongation.

DISCUSSION

We used FISH to monitor the position of centromere proximal and distal regions of chromosomes in cycling populations of yeast and in populations uniformly arrested at distinct stages of the cell cycle. In G1 cells, centromeres were loosely clustered toward the side of the chromosomal DNA mass containing the spindle pole. Similar results have been observed in fission yeast and mammalian cells (Ferguson *et al.*, 1992; Funabiki *et al.*, 1993; Vourc'h *et al.*, 1993). In mitosis prior to anaphase (mid-M), centromeres were

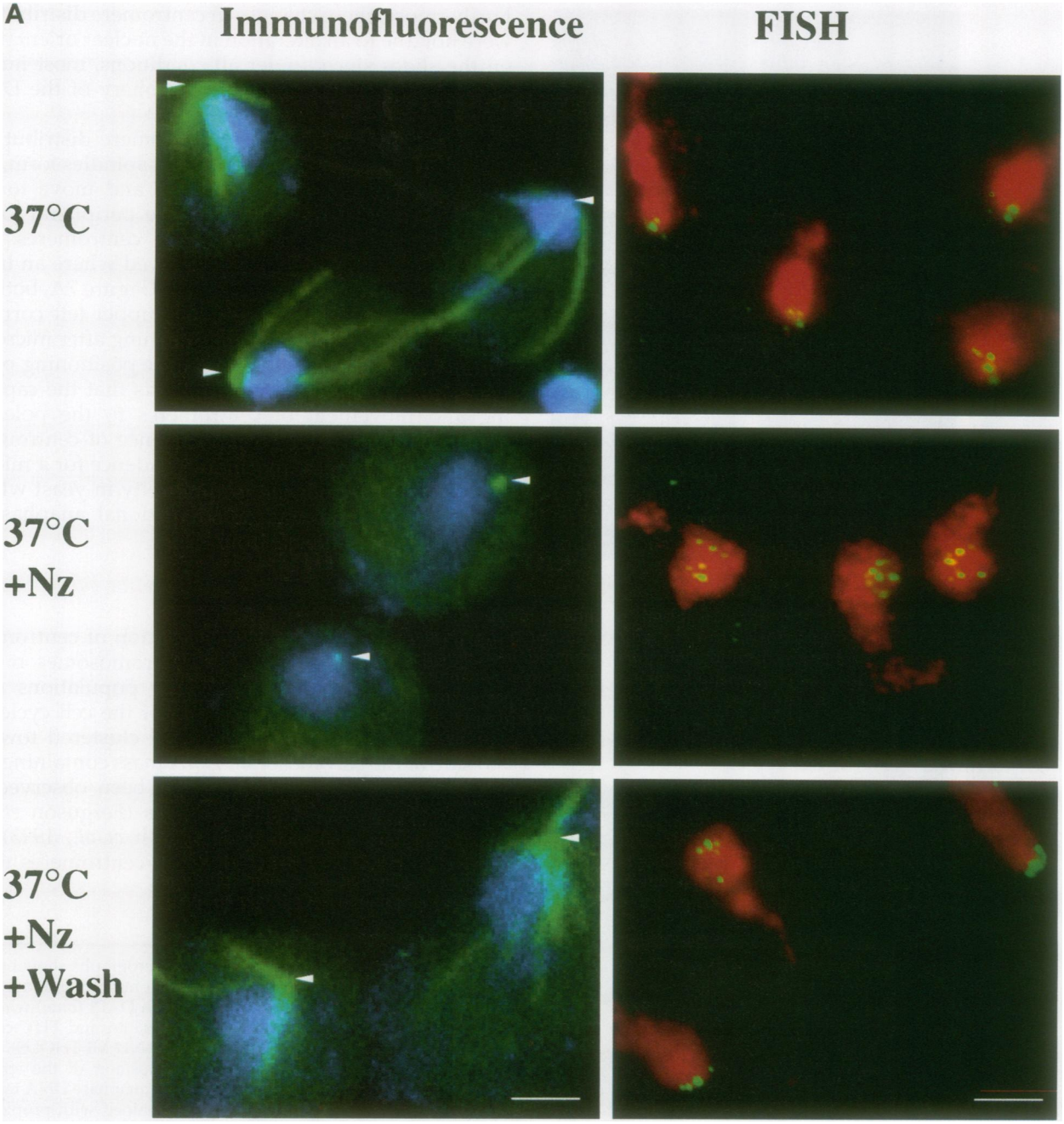

Figure 7. The effect of microtubule disassembly and assembly on centromere position in telophase-arrested cells. A mid-log culture of the diploid strain DK210 (*cdc14/cdc14*) was subjected to the following regimen: The culture was shifted to 37°C (37°C), treated with Nz at 37°C (37°C + Nz), and finally washed free of Nz at 37°C (37°C + Nz + wash) as described in MATERIALS AND METHODS and RESULTS. After each step, two aliquots of cells were removed and fixed. One aliquot was processed for FISH and the other was processed for immunofluorescence. (A) Centromeres and spindle poles visualized in cells arrested in *cdc14*-arrested cells. Left, Micrographs showing microtubules in cells subjected to indirect immunofluorescence. Chromosomal DNA was stained with DAPI (blue), and the microtubules were detected by antitubulin and FITC-conjugated antibodies (green). Bar, 2.5 μm. Right, Micrographs showing centromere position in DNA masses following FISH. Cells were hybridized with a mixture of three centromere probes (*CEN1*, *CEN4*, and *CEN16*). Chromosomal DNA was stained with propidium iodide (red), and hybridized digoxigenin-labeled DNA probes were detected by FITC-conjugated antibodies (yellow-green). Bar, 5 μm. (B) Quantitation of centromere position in cells treated as described in A. Left panels, Quantitation of distance-to-edge. Analyses were performed as described in the Figure 3C legend and MATERIALS AND METHODS. Data from *CEN4* and *CEN16* probes were identical and were combined. The histogram and SDs (error bars) were generated using 250–400 measurements from three to six independent experiments. Right panels, Quantitation of centromere clustering. Analyses was performed as described in the Figure 3D legend and MATERIALS AND METHODS. The histogram and SDs (error bars) were generated using ~80 measurements from two independent experiments.

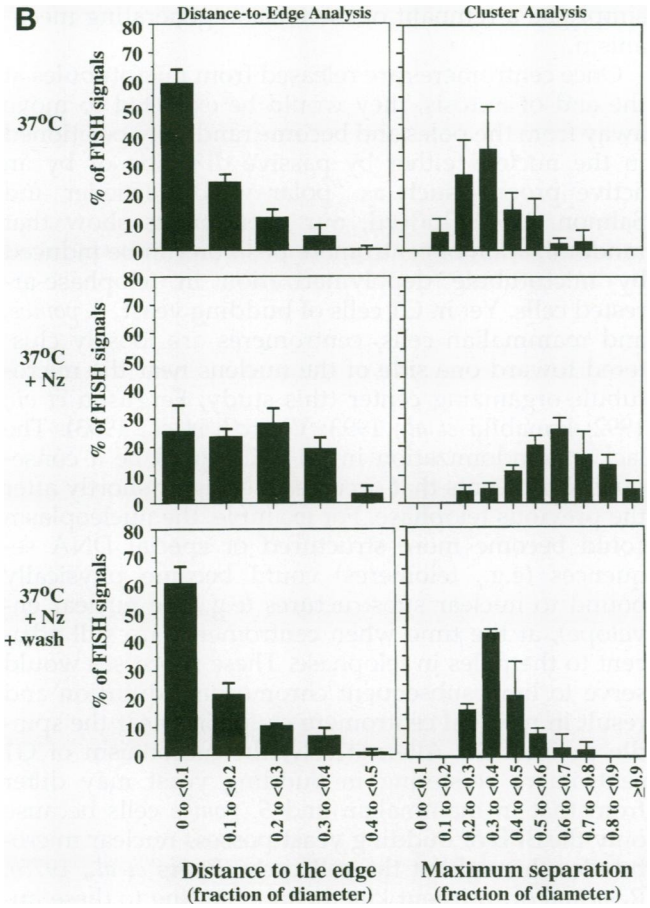

Figure 7 (cont).

biased toward internal regions of the chromosomal DNA mass and thus away from spindle poles whereas centromere distal sequences were more peripheral. In anaphase and telophase cells, centromeres were clustered tightly near the leading edge of the segregating chromosomal DNA masses and proximal to the poles while centromere-distal sequences trailed behind. Thus, the orientation of yeast chromosomes on the mitotic spindle before and during anaphase are strikingly similar to the orientation of mitotic chromosomes in other eukaryotic cells. Furthermore, the cell cycle-dependent changes in centromere position in budding yeast reported here are comparable to those observed in mammalian and *S. pombe* cells, suggesting that basic chromosomal movement and positioning are conserved in eukaryotes (Ferguson *et al.*, 1992; Funabiki *et al.*, 1993; Vourc'h *et al.*, 1993).

It has long been debated as to whether yeast chromosomes achieve metaphase. In a very small subset of cycling cells, the centromeres were arranged in a line within the chromosomal DNA mass (Guacci and Koshland, unpublished results). This linear pattern is consistent with a metaphase configuration (i.e., centromeres perpendicular to the spindle and equidistant-distant to the poles). However, since we cannot directly determine spindle orientation, this linear centromere arrangement could equally well reflect a prometaphase configuration (i.e., centromeres along the spindle length). In human cells and possibly fission yeast, the inactivation of the anaphase-promoting complex (APC) leads to a metaphase arrest (Funabiki *et al.*, 1993; Tugendreich *et al.*, 1995). Yet, in budding yeast the inactivation of either of two APC components (Cdc23p or Cdc16p) did not dramatically enrich for cells exhibiting a linear arrangement of centromeres (this study and our unpublished results). Thus, budding yeast apparently lacks a true metaphase as suggested by indirect methods in previous studies (Goh and Kilmartin, 1993; Winey *et al.*, 1995). Nonetheless, the absence of metaphase in budding yeast does not preclude the existence of poleward and antipoleward chromosome movement prior to anaphase. Indeed, such activities may be essential for bipolar spindle attachment (see below).

Although *S. cerevisiae* does not exhibit a classic metaphase, this study shows that budding yeast centromeres undergo anaphase A movements. We observed that in mid-M, paired sister centromeres are usually internally positioned while the spindle poles lie at the periphery of the chromosome mass. Occasionally, paired sister centromeres in mid-M are at the periphery, where, if proximal to one spindle pole, they must be located the entire length of the spindle (1–2 μm) away from the opposite pole. Given this broad range of centromere distances to spindle poles in mid-M cells, we would expect that in the absence of anaphase A, centromeres would remain at various distances from the poles as anaphase B spindle elongation occurs. Instead, we observed that in anaphase and telophase cells, centromeres are uniformly positioned at the leading edges of the chromosomal DNA mass and close to the spindle poles. Furthermore, in cells with even slightly elongated DNA masses (i.e., early anaphase B cells), centromeres are already clustered near the leading edge. These results are consistent with the movement of centromeres from positions away from the spindle poles in mid-M to positions in close proximity to the poles in anaphase, which by definition is anaphase A.

This conclusion is subject to the caveat that centromere position will be skewed partially toward the leading edge as a consequence of anaphase B spindle elongation. However, several additional observations from our studies and others argue for an anaphase A activity. In this study, we show that centromeric clustering to the periphery of the chromosomal mass near the spindle poles in telophase-arrested *cdc14* cells can be randomized by microtubule depolymerization. Remarkably, when microtubules are allowed to reform, centromeres recluster to the periphery of the chromo-

somal mass. This reclustering occurred without additional anaphase B spindle elongation. Since spindle poles are at the periphery, the simplest interpretation of these observations is that upon reformation of microtubules, kinetochores recapture microtubules and move by an anaphase A activity toward the poles in the absence of anaphase B. Furthermore, anaphase A was suggested previously from three-dimensional reconstructions of yeast spindles in which inferred kinetochore microtubules were shown to shorten during anaphase (Winey *et al.*, 1995). In addition, when green fluorescent protein-fusion proteins were tethered adjacent to a single yeast centromere, that centromere was shown to move toward the spindle pole at anaphase (Straight and Murray, personal communication). Taken together, these results provide strong evidence for anaphase A movement of yeast centromeres.

Our ability to monitor anaphase A activity in telophase-arrested yeast cells may help to elucidate an unexplored aspect of anaphase regulation. Previous studies of mitosis in other eukaryotes has been limited by an inability to assay anaphase A activity after centromeres reach the poles. It is possible that this activity is shutoff immediately after the centromeres reach the pole or may persist until spindle disassembly at the end of mitosis. Here, we show that anaphase A activity persists in telophase-arrested *cdc14* cells. The *cdc14* cells might be arrested prior to the point in mitosis where anaphase A activity is normally turned off. Alternatively, Cdc14p itself could play a role in centromere function or regulation so that inactivation of Cdc14p results in the inability to turn off anaphase A. Interestingly, *CDC14* encodes a putative phosphatase and phosphorylation has been implicated in the binding of the yeast centromere DNA-binding complex to DNA (Lechner and Carbon, 1991; Wan *et al.*, 1992).

The contribution of anaphase A to the separation of sister chromatids is limited to the length of the spindle prior to its elongation, which in budding yeast is only 1 to 2 μm (Byers *et al.*, 1975). The contribution of anaphase B to sister chromatid separation is the length of the fully elongated spindle, which in budding yeast is up to 10 μm (Byers *et al.*, 1975; Winey *et al.*, 1995). Why does budding yeast have anaphase A when anaphase B would seem to be sufficient to achieve chromosome segregation? In other eukaryotes, anaphase A-like activity is used to generate poleward forces on sister chromatids prior to the initiation of anaphase. This is thought to be an essential part of a tension-sensing mechanism that ensures that every sister chromatid pair achieves a bipolar attachment before the initiation of anaphase (reviewed in Gorbsky, 1995). In budding yeast, anaphase A activity may be used to generate poleward forces on paired sister chromatids to ensure a bipolar attachment. Subsequent anaphase A activity observed after the onset of anaphase may simply be a remnant of the tension-generating mechanism.

Once centromeres are released from microtubules at the end of mitosis, they would be expected to move away from the poles and become randomly positioned in the nucleus either by passive diffusion or by an active process such as "polar winds" (Rieder and Salmon, 1994). Indeed, our experiments show that randomization of centromere position can be induced by microtubule depolymerization in telophase-arrested cells. Yet in G1 cells of budding yeast, *S. pombe*, and mammalian cells, centromeres are loosely clustered toward one side of the nucleus near the microtubule-organizing center (this study; Ferguson *et al.*, 1992; Funabiki *et al.*, 1993; Vourc'h *et al.*, 1993). The lack of randomization in G1 cells could be a consequence of events that occurred during or shortly after the previous telophase. For example, the nucleoplasm could become more structured or special DNA sequences (e.g., telomeres) could become physically bound to nuclear substructures (e.g., the nuclear envelope), at the time when centromeres are still adjacent to the poles in telophase. These processes would serve to limit subsequent chromosome diffusion and result in residual centromere clustering near the spindle pole in G1. Alternatively, the mechanism of G1 centromere clustering in budding yeast may differ from that in mammalian and *S. pombe* cells because only the SPB of budding yeast possess nuclear microtubules throughout the cell cycle (Byers *et al.*, 1975). Residual or transient kinetochore binding to these microtubules after mitosis and into the next G1 could serve to maintain a skewed centromere distribution. It will be interesting to determine the biological significance of maintaining centromere positioning near the spindle pole. Prior to the end of mitosis, such positioning may serve to prevent chromosomes from straying across the plane of cytokinesis. During G1, it may facilitate the ability of centromeres to capture microtubules in the ensuing mitosis. This may be especially important for budding yeast since each kinetochore captures a single microtubule (Peterson and Ris, 1976).

Here, we have shown that budding yeast centromeres and chromosome arms undergo movements during the cell cycle like those seen in other eukaryotes. In other eukaryotes these movements are orchestrated in part by the kinetochore, by microtubule disassembly at the spindle pole, and by kinesin-like molecules associated with the arms (reviewed in Mitchison *et al.*, 1992; Afshar *et al.*, 1995; Pluta *et al.*, 1995; Vernos *et al.*, 1995; Wang *et al.*, 1995). How are these movements accomplished in budding yeast where the cytological structure of the kinetochore, spindle pole, and chromosome arms are so simple compared with the corresponding structures in many other eukaryotes. One possibility is that budding yeast

has evolved very different molecules to mediate these movements. Alternatively, these cytological differences are misleading, and the similar chromosome movements are the consequence of common if not identical mechanisms. For example, it is possible the "complex" mammalian kinetochores are actually modular structures composed of many individual "simple" yeast-like kinetochores (Fitzgerald-Hayes *et al.*, 1982; Brinkley *et al.*, 1992). The validity of these models will be established by continuing the molecular analysis of chromosome movement in budding yeast. Indeed, one powerful approach to this molecular analysis will be to analyze mutants defective in specific kinesin-like molecules, spindle components, and centromere factors using the FISH methodology and experimental strategies described in this study.

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