Centromere clustering is a major determinant of yeast interphase nuclear organization

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SUMMARY
During interphase in the budding yeast, Saccharomyces cerevisiae, centromeres are clustered near one pole of the nucleus as a rosette with the spindle pole body at its hub. Opposite to the centromeric pole is the nucleolus. Chromosome arms extend outwards from the centromeric pole and are preferentially directed towards the opposite pole. Centromere clustering is reduced by the ndc10 mutation, which affects a kinetochore protein, and by the microtubule poison nocodazole. This suggests that clustering is actively maintained or enforced by the association of centromeres with microtubules throughout interphase. Unlike the Rabl-orientation known from many higher eukaryotes, centromere clustering in yeast is not only a relic of anaphase chromosome polarization, because it can be reconstituted without the passage of cells through anaphase. Within the rosette, homologous centromeres are not arranged in a particular order that would suggest somatic pairing or genome separation.

Key words: Yeast, Nuclear architecture, Chromosome arrangement, Interphase, Centromere, SPB, Nucleolus, Saccharomyces cerevisiae

INTRODUCTION
It is now becoming widely accepted that interphase nuclei possess a high degree of spatial organization (see, e.g., Gilson et al., 1993; Lamond and Earnshaw, 1998; Marshall et al., 1997a). Order is dictated both by functional needs related to interphase metabolism and by spatial constraints. Several facets of nonrandom chromosome positioning have been recognized. Among them are the association of transcription- and replication-active chromosomal sites in foci, the existence of distinct individual chromosome domains, the preferential localization of chromosome ends at the periphery of the nucleus and the sequestering of ribosomal DNA tracts to separate compartments, namely the nucleoli. Also, nonrandom relative positioning of the two parental sets of chromosomes in diploids (like somatic pairing or genome separation) was observed, but not all aspects of it are understood. In many eukaryotes, the so-called Rabl-orientation of chromosomes prevails at interphase (for reviews see, e.g., Fussell, 1987; Dong and Jiang, 1998; Jin et al., 1998; Zickler and Kleckner, 1998). There, as a relic of anaphase movement, centromeres cluster near one pole of the nucleus whereas chromosome arms are arranged more or less in parallel and extend toward the opposite pole. For the budding yeast it has been shown previously that centromeres are clustered in a region near the spindle-pole body throughout interphase and that telomeres reside outside the centromere cluster (Goh and Kilmartin, 1993; Guacci et al., 1997a; Hayashi et al., 1998; Jin et al., 1998). However, it remains to be demonstrated, whether the outer chromosomal regions are distributed at random within the remaining nuclear space or whether they occupy more centromere-distant areas corresponding to increasing chromosome arm lengths, as classical Rabl-orientation would predict. It is also of interest, whether the centromere clustering observed in budding yeast, which has an intranuclear mitotic division, occurs by the same mechanism, i.e. anaphase polarization, as Rabl-orientation in most higher eukaryotes.

Apart from being a mere mechanical consequence of anaphase chromosome movement, the parallel centromere-telomere orientation of chromosomes could serve a functional role. For example, it has been proposed that Rabl-orientation is a major factor contributing to the vicinity (and possible interaction) of homologous chromosome regions by assigning them positions at the same latitude with respect to the centromeric pole within the nucleus (Jin et al., 1998). It had also been suggested that this specific arrangement of chromosomes at interphase could persist into meiotic prophase and facilitate meiotic homologous alignment (Fussell, 1987; Loidl, 1990; Zickler and Kleckner, 1998). Here we have applied fluorescence in situ hybridization (FISH) to centromeres and other chromosome regions in combination with immunostaining of the spindle pole body and microtubules to study the nonrandom interphase arrangement of chromosome arms in the budding yeast and its causes and consequences.

MATERIALS AND METHODS
Yeast strains and growth conditions
The yeast strains used in this paper are listed in Table 1. For most
experiments, the diploid strain SK1 (Kane and Roth, 1974) was used. Strain NK857, a haploid derivative of SK1, was kindly provided by N. Kleckner (Harvard University, Boston, MA). Strains DK4533-7-2 (cdc23-1 Ts-), 4202-15-3a (bar1-1) and JK418 (ndc10-1 Ts-) were gifts from D. Koshland (Carnegie Institution of Washington, Baltimore, MD), L. Hartwell (Fred Hutchinson Cancer Res. Ctr., Seattle, WA), and J. V. Kilmartin (MRC, Cambridge, UK), respectively. For mitotic and meiotic growth conditions see Jin et al. (1998).

Cell preparation

Cells were prepared in three different ways. For good preservation of their outer shapes and astral microtubules, cells were fixed and permeabilized conventionally (procedure A). For some FISH applications, cells were treated with a detergent prior to fixation (procedure B) to exploit the enhanced cytological resolution offered by spreading (Jin et al., 1998). Since this treatment tends to disrupt cells, we applied another method where nuclei are moderately spread (procedure C) to exploit the enhanced cytological resolution offered by application of a detergent after the fixation treatment. This semi-spreading (procedure C) is a good compromise for obtaining a good final concentration of 4%. Fixation took place at room temperature for 30 to 60 minutes. Cells were washed with 2% KAc and 10 mg/ml Dithiotreitol and 14 μl of a Zymolyase 100T (Seikagaku Co., Tokyo) stock solution (10 mg/ml) were added. Digestion was performed for 20 minutes at 37°C. After digestion, cells were washed with 2% KAc and collected by centrifugation (2000 rpm for 4 minutes). The pellet was resuspended in 500 μl 2% KAc, and 10 μl 0.5 M dithioretol and 14 μl of a Zymolyase 100T stock solution (10 mg/ml) were added. Digestion was performed for 20 minutes at 37°C. After digestion, cells were washed with 2% KAc and recovered in 100 to 150 μl of the same medium. This suspension was stored on ice (for up to 1 day) until used for the preparation of the slides.

For procedures A and C, 5-ml samples were taken from cultures with a density of ~1×10^7 cells/ml and formaldehyde was added to a final concentration of 4%. Fixation took place at room temperature for 30 to 60 minutes. Cells were washed with 2% KAc and collected by centrifugation (2000 rpm for 4 minutes). The pellet was resuspended in 500 μl 2% KAc, and 10 μl 0.5 M dithioretol and 14 μl of a Zymolyase 100T stock solution (10 mg/ml) were added. Digestion was performed for 20 minutes at 37°C. After digestion, cells were washed with 2% KAc and recovered in 100 to 150 μl of the same medium. This suspension was stored on ice (for up to 1 day) until used for the preparation of the slides.

For procedure A, slides were polylysine-coated (0.1% solution) to reduce the loss of cells, whereas for procedures B and C after spreading, cells readily stick to the slides due to their larger surface. 20 μl of the cell suspension were dropped onto a slide, spread out evenly on its surface with the help of a glass rod and left for 2-3 minutes under humid conditions. For immunostaining, slides were processed further without allowing the suspension to dry out completely.

For procedure C, 20 μl of a cell suspension produced as above were put on a slide and mixed with 4-fold amounts of both detergent (1% aqueous solution of Lipsol; LIP Ltd, Shipley, UK) and fixative (4% paraformaldehyde and 3.4% sucrose in distilled water). The mixture was then spread out with a glass rod and left to solidify in a chemical hood. The addition of sucrose has the advantage that the mixture is hygroscopic and does not dry out completely. Therefore these preparations can be used for immunostaining even after storage for several days in the refrigerator (see Loidl et al., 1998a).

For procedure B, unixed cells which had been spheroplasted with Zymolyase, were mixed with detergent and fixative on a slide as described above. For details of this spreading protocol see, e.g., Loidl et al. (1991, 1998a).

Immunostaining

Specific labelling of the spindle pole body (SPB) was obtained with monoclonal rabbit anti-Spc72p antibody (Knop and Schiebel, 1998; kindly provided by E. Schiebel, Beatson Institute of Cancer Research, Glasgow, UK). Microtubules and the SPB were immunolabelled with YOL1/34 monoclonal rat anti-yeast tubulin antibody (Kilmartin et al., 1982; purchased from Serotec, Kidlington, UK) according to a standard protocol (see, e.g., Pringle et al., 1991). Slides were washed twice for 5 minutes in 1× PBS (130 mM NaCl, 7 mM Na2HPO4, 3 mM NaH2PO4, pH 7.5), excessive liquid was drained and the slides were incubated with a drop of primary antibody (diluted 1:200 in 1× PBS) under a coverslip at 4°C overnight. After two 5 minutes washes in 1× PBS, slides were incubated with FITC- or TRITC-conjugated secondary antibody for 90 minutes at room temperature. The slides were then washed 2 × 5 minutes in 1× PBS and mounted under a coverslip in Vectashield anti-fading medium (Vector Laboratories Inc., Burlingame, CA) supplemented with 0.5 μg/ml DAPI (4′,6-diamidino-2-phenylindole) as DNA-specific counterstain.

Images of immunostained cells were taken and their coordinates were recorded. For subsequent FISH, the coverslip was rinsed off with 1× PBS, cells were postfixed for 5-10 minutes in paraformaldehyde fixative (see above) and then subjected to the standard FISH procedure (see below). In most cases, immunostaining was retained after FISH. In some instances, however, it had faded and FISH images had to be merged electronically with the corresponding images previously taken from the same coordinates of immunostained nuclei (Loidl et al., 1998b).

Fluorescence in situ hybridization (FISH)

The complete set of centromeres was highlighted by FISH with a pan-centromeric probe (Jin et al., 1998). rDNA repeats were labelled with a probe against fungal 25S rDNA (Scherthan et al., 1992). FISH probes for painting the left arm of chromosome IV (IVL) and for a region on XIR were produced by PCR using the Expand Long Template PCR System (Boehringer Mannheim GmbH, Mannheim, Germany) according to the manufacturer’s instructions. Appropriate primers were selected from the Saccharomyces Genome Database (Cherry et al., 1998). Care was taken to amplify only regions without major repeated DNA elements. For template sizes of around 8 kb we applied the following conditions: 2 minutes at 94°C; 10 cycles with 10 seconds at 94°C, 30 seconds at 98°C, 6.5 minutes at 68°C; 20 cycles with 10 seconds at 94°C, 30 seconds at 98°C, 6.5 minutes at 68°C with a prolongation of 20 seconds per cycle and a final extension of 7 minutes. The amplified PCR products were purified using the EluQuick-System (Schleicher & Schuell, Dassel, Germany).

For various loci on the left arm of chromosome III, the right arm of chromosome IV and the left arm of chromosome VII, cosmids or λ clones #70884, #70103, #70884 and #70779 from the American Type Culture Collection (ATCC; Gaithersburg, MD) were used as hybridization probes. The chromosomal localization of these and the other FISH probes used are shown in Fig. 1.

The probes were labelled by nick translation with Biotin-21-dUTP (Clontech Laboratories Inc., Palo Alto, CA), Cy3-dUTP (red), Cy5-dUTP (far-red; Amersham, Little Chalfont, England) or Fluorescein-

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### Table 1. Yeast strains used in this study

<table>
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<th>Name/number</th>
<th>Relevant genotype</th>
<th>Source/reference</th>
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<tr>
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<td>MATa/α, HO/HO</td>
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<td>N. Kleckner</td>
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<tr>
<td>DK4533-7-2</td>
<td>MATa, cdc23-1(Ts-), leu2, ade2, ade3, can1, sap3, ura1, his7, gal1</td>
<td>D. Koshland</td>
</tr>
<tr>
<td>#872</td>
<td>MATa/α, cdc23-1(Ts+)/cdc23-1(Ts-)</td>
<td>This study; derived from DK4533-7-2 by 5x backcross to NK857</td>
</tr>
<tr>
<td>4202-15-3a</td>
<td>MATa, ade2-1(oche), his4-580 (amber), lys2-2(oche), trp1 (amber), tyr1 (ochre), SUP4-3 (ts amber suppressor), bar1-1</td>
<td>L. Hartwell</td>
</tr>
<tr>
<td>JK418</td>
<td>MATa, ho, ura3, leu2, TRP1, ndc10-1(Ts-)</td>
<td>Goh and Kilmartin (1993)</td>
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Centromeres form a rosette with the spindle pole body inside

A high proportion of interphase (i.e., monopolar spindle-stage) nuclei with clustered centromeres (>50% in some series of preparations) showed a circular arrangement of centromeres both in spread (procedure B, see Materials and Methods, Fig. 2i) and semispread (procedure C) preparations (Fig. 2e; see also Jin et al., 1998). The space inside the centromere rosette showed reduced DAPI-staining, possibly due to lower chromatin density (Fig. 2g). Immunostaining with an antibody against the SPB revealed that the SPB was located in the center of the rosette in 27 out of 36 nuclei (75%; Fig. 2h) and in 6 nuclei it was inside but not in the middle of the centromere ring. Only in 3 nuclei was the SPB outside the rosette. From this we conclude that in living nuclei the SPB is located at the hub of the rosette. An area slightly larger than the SPB was highlighted with YOL1/34 monoclonal antibody against the α subunit of tubulin under conditions (procedure B) which disrupt astral microtubules (Fig. 2i). This is in accordance with the reported binding of this antibody to microtubules attached to the SPB (Kilmartin et al., 1982).

Centromere clustering is enforced by the attachment of centromeres to microtubules

Centromere clustering is only slightly reduced when nuclei are arrested at interphase for an extended period. A bar1 strain (which is highly sensitive to yeast mating pheromone; see Barkai et al., 1998) was arrested in G1 (95% unbudded cells, n=200) by exposure to a-factor (15 μg/ml; for experimental details see also Jin et al., 1998) and showed only a weak reduction of clustering from 82% to 72% after 3 hours (Fig. 3), possibly by diffusion of the chromosomes (see, e.g.,
Marshall et al., 1997b). Thus, during the short ~90 minutes interval between mitoses under normal growth conditions, diffusion should not produce a notable loss of centromere clustering. Only in cultures which had been kept under stationary conditions for 24 hours or longer, was centromere clustering found to be reduced to between 30 and 40% (Jin et al., 1998).

Interphase clustering of the centromeres could be due to their passive persistence at the positions they occupied at telophase, even after degradation of the mitotic spindle in the absence of a disruptive force. Alternatively, it could be actively maintained by the permanent association of centromeres with components of the spindle apparatus or the nuclear scaffold (see Marshall and Sedat, 1999). We performed experiments to...
see if the loss of microtubules or of the microtubule-kinetochore association has an influence on centromere clustering. In one experiment, we shifted the temperature-sensitive ndc10-1 mutation and by the microtubule poison nocodazole. (a) exponentially growing cultures were transferred from permissive temperature (23°C) to restrictive temperature (37°C) and aliquots were taken at the indicated time-points. A rapid reduction of centromere clustering was found both in spread (procedure B) and semispread (procedure C) ndc10-1 nuclei. Clustering remained high in wild-type nuclei which indicates that the effect is not due to increased temperature per se. (b) Also nocodazole-treated (15 μg/ml) nuclei showed a clear reduction in centromere clustering. Cells of a bar1 mutant MAFa strain were arrested at interphase (G1) by exposure to α-factor (see Results) and showed only minimal reduction of centromere clustering over the same period. Thus, centromere clusters do not simply disperse in the absence of the mitotic spindle, but the dispersal is due to the ndc10-1 mutation and the nocodazole treatment. Each experiment was performed three times and 100 nuclei were scored for each time-point in each run.

We also treated logarithmic cultures of the strain SK1 with nocodazole, an inhibitor of microtubule polymerization, at a concentration of 15 μg/ml. The effect of nocodazole treatment was less pronounced than that of the ndc10 mutation. On average, we found a reduction in the number of nuclei with clustered centromeres from over 80% to 40-50% after 60 to 90 minutes exposure (Fig. 3b). Similarly, Guacci et al. (1997a) and Marshall et al. (1997b) had observed an increase of distances between centromeres III in nocodazole-treated cells.

Thus, from the partial disruption of clusters by ndc10 and nocodazole we conclude that interphase centromere clustering is not due to the passive persistence of the anaphase configuration (which would only be possible in the absence of diffusional motion – see Discussion), but that it is enforced by the active maintenance of a SPB-kinetochore connection via microtubules.

**Centromere clustering is independent of anaphase chromosome polarization**

We wanted to test whether the formation of centromere clusters requires the mitotic spindle or if it can be brought about by the action of intranuclear microtubules which are present during interphase. To this end we destroyed centromere clusters and observed under which conditions they reform. We took advantage of the rapid and highly synchronous resolution of the centromere clusters, which occurs due to a major reorganization of the nucleus at meiotic prophase (Fig. 2f; Jin et al., 1998; Trelles-Sticken et al., 1999).

The strain SK1 was transferred to sporulation medium and samples were taken at regular intervals to determine the time when centromere clustering was lowest. Cultures which had reached that point were transferred to rich medium which causes them to leave the meiotic pathway and to return to mitotic growth (RTG; see, e.g., Zenvirth et al., 1997). Centromere clustering was found to reappear around 60 minutes after RTG (Fig. 4a). Phase contrast microscopy and immunostaining of microtubules from the same samples revealed that large buds and long bipolar anaphase spindles reappeared about 30 minutes later (Fig. 4a), suggesting that centromere clustering occurs prior to anaphase. (A few meiotic nuclei were also present due to meioses occurring in presporulation medium and to cells which failed to return to the mitotic cycle. However, their frequency was too low (<5%) to explain the observed centromere clustering by meiotic divisions.)

To define the temporal relationship between centromere clustering and spindle formation more precisely, we used a temperature-sensitive cdc23 mutant. In this mutant the cell cycle is arrested at metaphase because the sister chromatids cannot be separated due to a defect in the anaphase promoting complex (Iringer et al., 1995). Although cdc23 mutant cells do not do well in meiosis even at the permissive temperature (23°C), between 40 and 55% of the nuclei lost centromere clustering upon transfer to sporulation medium. Centromere clusters reappeared after transfer to rich medium at the restrictive temperature (37°C; Fig. 4b). After only 1 hour upon RTG, centromere clustering reached almost premeiotic levels. Although the meiosis-induced reduction in centromere clustering was low (due to the poor performance of cdc23 cells in sporulation medium), this reduction and reappearance of centromere clustering was consistent in 3 independent
experiments. To dismiss the possibility that mutant cells had escaped from arrest, we examined aliquots of the cultures after RTG for the presence of anaphase spindles and elongated anaphase nuclei in anti-tubulin and DAPI stained nuclei. No anaphase spindles were found in 500 nuclei, and only 18% of the cells had elongated nuclei. But strikingly, even those had their centromeres organized in a single cluster without any sign of beginning anaphase separation. This indicates that metaphase arrest had occurred in the mutant. Thus, we conclude that the poleward movement or the assembly of centromeres at the poles of dividing nuclei during anaphase/telophase is not required for centromere clustering. Rather, it occurs prior to or concomitantly with the formation of the bipolar spindle.

**A relaxed centromere-telomere polarization prevails in interphase nuclei**

We asked if chromosome arrangement in yeast interphase nuclei bears the signs of Rabl-orientation, i.e. the ± straight orientation of chromosome arms from the centromeric to the opposite pole. In DAPI stained nuclei, the nucleolus appeared as a weakly stained area opposite the dark spot that marks the region surrounding the SPB, confirming that centromeres and nucleolus mark two opposite poles in the yeast nucleus (see Yang et al., 1989; Guacci et al., 1997a; Oakes et al., 1998). The reduced DAPI staining of the nucleolus suggests that it contains little DNA; only thin DAPI-positive threads were sometimes visible within this area (Fig. 2g). FISH with a probe against rDNA highlighted relatively strongly condensed tracts of RDN repeats inside the nucleolus (Fig. 2i,j). Other chromosomal regions seem to be excluded from it (see also Guacci et al., 1994). The ca. 1000 kb long RDN array on chromosome XII, which comprises the rDNA repeats, begins ~300 kb away from the centromere (see Cherry et al., 1998). This physical distance of 300 kb between centromere XII and RDN is sufficient to span the intranuclear distance between the centromeric pole and the nucleolus. In diploid nuclei, the two RDN arrays sometimes appear separate (Fig. 2g,i) and at other times associated (Fig. 2j). The occasional association is either due to their joint formation of a nucleolus or to somatic pairing of the rDNA sequences (see below). Quite often, the RDN tract within the nucleolus appears loop-shaped (Fig. 2i) suggesting that the chromosome arm XIIR is folded back toward the centromeric pole (see also Guacci et al., 1994). Simultaneous hybridization to centromeres, rDNA and a locus distal to RDN confirmed that the distal portion of XIIR occupies the region between the centromeres and the nucleolus (Fig. 2j).

Since the U-turn of XIIR may be an exception because of the presence of the nucleolus, we checked the orientation of the more typical 450 kb chromosome arm IVL by ‘chromosome painting’. For that purpose, a mixture of ten PCR-amplified DNA fragments with a length between 8 kb and 10 kb was used as a hybridization probe (see Fig. 1). The probes closest to the centromere and the telomere were labelled with Cy3-dUTP and Cy5-dUTP, respectively, whereas all interstitial loci were labelled with fluoroscein-dUTP. The specificity of the probes was verified in spread and semispread pachytene nuclei (not shown) where individual bivalents...
appear as rods with compact painting signals (Loidl et al., 1995). In interphase nuclei, a continuous hybridization signal or the maximum number of eight interstitial signals was rarely observed, which may be due to differential condensation along the chromosomes or the occasional failure of the small individual probes to generate a detectable signal. Nevertheless, the array of signals allowed the course of the chromosome arm to be determined. In 25 out of 36 (69%) semispread nuclei (procedure C) from logarithmically growing cultures, the interstitial FISH signals were between the centromeric and the telomeric signals, although not normally in a straight line. This suggests that, while a general centromere-telomere polarization prevails, the chromosome arms follow a more or less meandering path (Fig. 2k). In the remaining 31% of nuclei, the centromeric and telomeric FISH signals were interspersed among the interstitial ones. Since this arrangement of signals may also result if a nucleus is viewed from the top (from the centromeric or opposite pole), it is estimated that less than 30% of nuclei fail to exhibit centromere-telomere polarization of chromosome arm I/V.

To quantify centromere-telomere polarization of chromosome arms, we measured the intranuclear distances between loci at the centromeres and at different physical distances from the centromere. In the case of a random course of chromosome arms, no significant differences in the intranuclear centromere-telomere distances should be observed, whereas a more linear orientation (with or without meandering of the interstitial DNA) would result in a positive correlation of physical and intranuclear distances (Fig. 5a). Multicolor FISH with probes specific for a region including the centromere of chromosome IV and for regions 273 kb and 1041 kb (Fig. 1) away on its long arm was carried out (Fig. 2l). In 75% of nuclei, intranuclear distances from the centromere were larger for the distal than for the proximal locus (Fig. 5b). However, in only 10% of nuclei the distal locus had a more than twice as large intranuclear distance from the centromere than the proximal locus, although its physical distance from the centromere is about four times as large. This suggests that the proximal part of the chromosome arm points away from the centromere more directly, whereas the distal part tends to loop back or to meander.

The arrangement of homologous centromeres within the rosette

To detect a possible nonrandom arrangement of homologous chromosomes within the centromere rosettes of diploid interphase nuclei (see above), centromeres of chromosomes I, IV, VIII and XVI were labelled differentially from the rest of centromeres (Fig. 2m) and the angular separation between homologs was determined. Angles between 0° (signals fused) and 180° (signals positioned on opposite sides of the rosette) were measured (see Materials and Methods) in 50 nuclei for each of the four chromosomes. As can be seen from Fig. 6, angles between 0° and 180° were present at approximately equal frequencies. 108 of the 200 homologous pairs of centromeres were separated by an angle of less than 90°, which is close to the 50% that would be expected if there is no preferential location of two homologous centromeres either in the same or the opposite halves of the ring (Fig. 6). This result refutes both, somatic pairing of centromeric or centromere-near chromosome regions and separation of the parental genomes. (The separation of parental chromosomes into two haploid sets on opposite sides of prometaphase rosettes has been claimed to occur in human fibroblasts and HeLa cells (Nagele et al., 1995) but was disputed recently (Allison and Nestor, 1999).) However, our observation of a random distribution of homologous centromeres within the rosette does not rule out the possibility that regions at more distal positions of chromosome arms show preferential homologous associations (Keeney and Kleckner, 1996; Kleckner, 1998; Burgess et al., 1999).

**DISCUSSION**

**Chromosome arrangement at interphase**

Here we describe the highly organized spatial distribution of chromosomes in interphase nuclei of the budding yeast. Its most prominent feature is the clustering of centromeres. Goh and Kilmartin (1993), who immunostained Ndc10p, a putative kinetochore protein, found a label mostly in the region of the SPB, and proposed that centromeres may be attached to intranuclear microtubules and cluster near the SPB even in
interphase. This centromere clustering was confirmed by Guacci et al. (1997a) and Jin et al. (1998) using FISH labelling of centromeres.

The circular arrangement of centromeres which we report here, can also be observed in intact nuclei (see also Goh and Kilmartin, 1993; Jin et al., 1998). It may be due to the presence of a core bundle of ‘continuous’ microtubules which is surrounded by a shell of kinetochore microtubules (Winey et al., 1995). Therefore, it may be imagined that centromeres form a rosette around this microtubule bundle, and that chromosome arms are displaced from the interior of the nucleus and confined to the peripheral domain during mitosis. Since intranuclear microtubules are present throughout the whole cell cycle (Byers and Goetsch, 1975), this arrangement is possibly maintained in interphase nuclei (Murray and Szostak, 1985; Goh and Kilmartin, 1993; Guacci et al., 1997a).

Whilst the classical Rabl-orientation is believed to be a consequence of the anaphase movement of the centromeres toward the spindle poles (Fussell, 1987; Dong and Jiang, 1998; Zickler and Kleckner, 1998), it does not seem to be the only means by which centromeres cluster. In several animals some degree of centromere clustering has been observed in the absence of Rabl-orientation (for references see Jin et al., 1998). Here we have shown for yeast that if centromere clustering is disrupted experimentally, it is reconstituted even in the absence of anaphase. This reformation of clusters seems to be executed by intranuclear microtubules which are present throughout interphase (see above). Also the sensitivity of clustering to a mutant Ndc10 centromeric protein and to the spindle poison nocodazole supports the existence of a microtubule-dependent process which stabilizes centromere clusters throughout interphase. In the absence of a stabilizing force, centromere clusters would be probably disrupted by Brownian motion inside the nucleus. However, Marshall et al. (1997b) observed only limited diffusion of centromere-near loci, and they suggested that it was constrained by elements of the cytoskeleton. This active maintenance of centromere clustering would suggest that it serves a function perhaps in the context of a general higher organization within the yeast nucleus. It is possible that centromere clustering is functionally equivalent to the prometaphase congression of centromeres at the cellular equator, which occurs in higher eukaryotes.

In the classical Rabl-orientation, centromere clustering is accompanied by the largely parallel orientation of chromosome arms which is the consequence of the trailing of the arms at anaphase. Since in yeast centromere clustering is brought about (at least in part) by a different mechanism, it was of interest to see how arms are oriented relative to the centromeres. Although we found a general centromere-telomere polarization (Fig. 2k), chromosome arms seem to meander and to loop back occasionally. This is particularly true for chromosome arm XII which carries the rDNA repeats. The RDN array occupies a position ~300 kb away from the centromere. Distal to it there is another 610 kb of chromosome XII DNA. Since the nucleolus occupies a region opposite to the centromere cluster, the RDN array forms a U-turn within the nucleolus, and the distal parts of chromosome XII are located in the zone between the centromeric and the nucleolar pole of the nucleus (Fig. 2j; see also Guacci et al., 1994, 1997a,b). Thus, because of its different mode of origin and a relatively relaxed centromere-telomere polarization, we prefer to designate the observed interphase chromosome arrangement in yeast as Rabl-like arrangement.

We found that if there is no or only a short bipolar spindle (<3 μm) present, centromeres form a single cluster. It splits in two when the spindle lengthens (Fig. 2a-d). This is in accordance with Straight et al. (1997) who observed in living cells a sudden separation of sister centromeres when the spindle was between 2.5 and 3 μm long. Centromeres then performed anaphase A movement towards the poles in less than 26 seconds. The shortness of this stage and probably a highly synchronous behaviour of all centromeres could explain why we did not observe a notable number of nuclei with centromeres scattered between the two spindle poles. On the other hand, Guacci et al. (1997a) found that in cdc20 and cdc23 mutant cells arrested in metaphase, centromeres were dispersed and detached from the spindle poles. It is therefore possible that also in unarrested metaphase centromeres transiently oscillate along the short bipolar spindle in the course of initial orientation. Whereas a limited number of individual centromeres may be seen to be widely separated from each other (Guacci et al., 1997a), the entirety of centromeres may still form a cluster of about 2 μm in diameter, as in our case.
The question of somatic pairing*

The Rabl-like orientation has an effect on the colocalization of homologous chromosome regions in diploids since, with reference to the centromeric pole, homologous regions will be found at similar latitudes of the nucleus. In addition to this effect, Keeney and Kleckner (1996), Kleckner (1998) and Burgess et al. (1999) have reported a small preference for somatic pairing. However, in our analysis of the relative distribution of homologous centromeres within the centromere rosette we found no tendency of a preferential arrangement. It is highly improbable that the order of centromeres within the circle is upset by the spreading exerted during preparation, therefore we can safely assume that the observed lack of an ordered arrangement reflects the natural state. In a previous paper we have reported an experiment in which the distances between the centromeres of chromosome IV were on average slightly shorter than the distances between the centromere of chromosome IV and a centromere-near region on chromosome III (Fig. 3 in Jin et al., 1998). However, this slight difference may have been caused by the fact that the probe on chromosome III maps to a region ~30 kb from the centromere and thus resides slightly outside the rosette, at a different nuclear latitude.

The random arrangement of homologous centromeres which we report here, does not preclude local homologous interactions further down the chromosome arms. However, in view of the relatively weak preference for homologous colocalization (own unpublished results; Guacci et al., 1994; Keeney and Kleckner, 1996; Kleckner, 1998; Burgess et al., 1999), we are inclined to believe that it is not necessary to invoke specific mechanisms for homologous interaction in vegetative yeast cells. Chromosome sorting due to spatial constraints, in addition to the Rabl-like organization, could occur within the nucleus. For instance, not only distances from the centromeres, but also the position of loci on long vs. short arms could be crucial. Since telomeres are located at the nuclear periphery (Klein et al., 1992; Gotta et al., 1996), loci on short arms would be located near the nuclear surface, whereas interstitial regions on long arms at a similar latitude would tend to occupy the interior of the nucleus. This would create a tendency of arms of similar lengths (and thus of homologs) to colocalize.

The meiotic clustering switch: a discussion

We have shown previously that centromere clustering is resolved in meiotic prophase (Jin et al., 1998). At around the same time, telomeres cluster in a region near the SPB, causing chromosomes to loop back on themselves and to form the so-called bouquet (Trelles-Sticken et al., 1999). This centromere-telomere clustering switch seems to be conserved between organisms as diverge as higher plants (Schwarzacher, 1997; Moore, 1998), Schizosaccharomyces pombe (Chikashige et al., 1997), and the budding yeast. The Rabl-orientation or Rabl-like orientation and the bouquet are similar in that both are characterized by a roughly parallel arrangement of chromosome arms between a centromeric and a telomeric domain. However, as was pointed out by Zickler and Kleckner (1988), the meiotic bouquet configuration is not a simple reinforcement of the Rabl-orientation because centromeres and telomeres switch positions with respect to the microtubule organizing center. Thus, the clustering switch is a major nuclear restructuring event during which chromosomes turn around by 180 degrees. Even so, Rabl-orientation could provide a nonrandom chromosome disposition which facilitates bouquet formation (Fussell, 1987) or some other aspect of meiotic nuclear reorganization (Loidl, 1990).

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