SEQUENTIAL ALTERATIONS IN THE NUCLEAR CHROMATIN REGION DURING MITOSIS OF THE FISSION YEAST SCHIZOSACCHAROMYCES POMBE: VIDEO FLUORESCENCE MICROSCOPY OF SYNCHRONOUSLY GROWING WILD-TYPE AND COLD-SENSITIVE cdc MUTANTS BY USING A DNA-BINDING FLUORESCENT PROBE

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SUMMARY

Video-connected fluorescence microscopy was introduced to study the yeast nuclear chromatin region. It was defined as the nuclear area where a DNA-binding fluorescent probe 4',6-diamidino-2-phenylindole specifically bound and fluoresced. The 3-dimensional feature of the mitotic chromatin region was deduced by analysing the successive video images of a cell viewed at different angles. By investigating synchronous culture of the wild-type fission yeast Schizosaccharomyces pombe, we found sequential structural alterations in the chromatin region during mitosis. The steps found include the compaction of the chromatin region from the regular hemispherical form, the formation of a U-shaped intermediate and the rapid segregation into 2 daughter hemispherical forms. Six cs cdc mutants, apparently blocked in mitosis, were observed by fluorescence microscopy. Under the restrictive conditions their chromatin regions exhibited either hemispherical, compact, disk-like, U-shaped or partially segregated chromatin regions. Two mutants showed anomalous nuclear locations. The results of the temperature shift-up experiments of the highly reversible KM52 and KM108 strains supported the above scheme of sequential alterations in the chromatin region.

INTRODUCTION

The nuclear division of yeast has been studied extensively using the cdc mutants (Hartwell, 1974; Nurse, Thuriaux & Nasmyth, 1976) and by electron microscopy (Robinow & Marak, 1966; Moens & Rapport, 1971; McCully & Robinow, 1971; Byers & Goetsch, 1974, 1975; Peterson & Ris, 1976). A single spindle pole body (SPB) present in the nuclear membrane bears microtubules radiating into the nucleus. The duplication of the SPB and the migration of the two bodies to opposite sides of the nucleus result in the complete spindle. The spindle consists of continuous and discontinuous microtubules. The discontinuous microtubules are thought to associate with kinetochores and to be involved in chromosome separation. Thus the yeast mitotic apparatus is similar to that of higher eukaryotes, and may be an excellent system for studying the mitotic cell division cycle at the molecular level (Hartwell, 1974).
Much less is known about the chromosome structure. Although histones and nucleosomes are present in yeast (Lohr & Van Holde, 1975; Thomas & Furber, 1976), chromosome condensation during mitosis has not been found (e.g. see Gordon, 1977). Only 20 nm chromatin-like fibres were observed in thin-sectioned nuclei (Peterson & Ris, 1976; Gordon, 1977). Therefore, it has been difficult to understand the behaviour of the chromosomes in the stages of mitosis.

We have undertaken to study the nuclear division of the fission yeast, *Schizosaccharomyces pombe* (reviewed by Mitchison, 1970; Gutz, Heslot, Leupold & Loprieno, 1974), focusing specifically on the chromosome structure during mitosis. In order to study the morphological changes in the chromosomes during mitosis, we employed fluorescence microscopy using a DNA-binding fluorescent agent, 4',6-diamidino-2-phenylindole (DAPI) (Dann, Bergen, Demant & Voltz, 1971; Williamson & Fennel, 1975). We found that DAPI gave detailed fluorescent images of the nuclear chromatin region simply by mixing it with the *S. pombe* cells. The fluorescence microscope was connected with a highly sensitive TV camera, so that the fluorescent images could be recorded on video tapes. The 3-dimensional shape of the nuclear chromatin region was deduced by analysing the successive video images of a cell seen at different angles.

The morphology of DAPI-stained nuclei in the interphase of *S. pombe* was conspicuous: a hemispherical region with 2 short rods protruding from the plane surface. In *Saccharomyces cerevisiae*, however, such protrusions were very tiny. Hence *S. pombe* had an advantage over *S. cerevisiae* with regard to the fine morphology of the nuclear chromatin region.

Alterations in the nuclear chromatin region during mitosis were investigated by observing 2 kinds of cells: (1) wild-type cells grown asynchronously or synchronously; (2) cold-sensitive *cdc* mutants apparently blocked in mitosis. Our results demonstrate sequential structural changes in the nuclear chromatin region during mitosis. The steps found include the compaction of the chromatin region from the regular hemispherical form, the formation of U-shaped intermediates and the rapid segregation into 2 daughter hemispherical forms.

**Materials and Methods**

**Fission yeast**

The wild-type *S. pombe* used in the present study was a haploid strain with the mating type h*~* (strain 972 h*~*). The basic handling techniques described by Mitchison (1970) and Gutz *et al.* (1974) were followed.

**Media**

Cells were grown in YPD medium, which contains per litre: 10 g yeast extract, 20 g polypeptone and 20 g glucose. YPD plate was the same as YPD medium except that 15 g agar was added.
Video fluorescence microscopy of yeast mitosis

Isolation of cold-sensitive mutants of the cell division cycle

Detailed isolation procedures and properties of cold-sensitive mutants (ct cdc) of the cell division cycle will be reported elsewhere. The wild-type strain 972 h− was mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine, and the survivors on the YPD plate at 37 °C were replica-plated at 22 and 37 °C. Cold-sensitive mutants were found at a frequency of about 0.3%. Cell elongation at the restrictive temperature (Bonatti, Simili & Abbondandolo, 1972; Nurse et al. 1976) was used as a criterion for the cdc mutant. In the present work, we used 6 mutants, the nuclei of all of which did not divide at 22 °C. Their cold-sensitive and cell-elongation phenotypes were linked genetically and segregated 2:2 in tetrads. Preliminary genetic analyses showed that the 6 mutants belonged to different complementation groups.

Selection synchrony

We followed the procedures described by Mitchison & Carter (1975). About $1 \times 10^{10}$ wild-type cells were collected from a 1-litre exponentially growing culture by centrifugation, and resuspended in YPD medium at a final volume of 4 ml. The suspension was layered on two 40 ml linear gradients of 10% to 40% sucrose in YPD medium, and centrifuged in swing-out buckets at 500 g for 5 min at room temperature. The top layer of cells (2.5 ml) was then removed from each sample and they were suspended together in 100 ml of fresh YPD medium and grown at 30 °C with shaking. Ten-ml samples of synchronized culture were removed at intervals and kept on ice. An aliquot of the sample was taken, and the cell number and the cell plate index (number of cells with cell plates per number of total cells) were counted. The rest of the sample cells were washed with cold distilled water, incubated with DAPI and observed under a fluorescence microscope as described in the next section.

Fluorescence microscopy

Apparatus. An Olympus fluorescence microscope (BH-RFL) with an Osram 100 W high-pressure mercury arc lamp was used. Usually a 100× oil-immersion objective lens (UVFL) and a 5× photographic lens were employed. An ultrasonic silicon intensifier target (SIT) camera (Ikegami CTC9000) (Hotani, 1979) was connected to the microscope. Fluorescent images were displayed on a picture monitor screen (Ikegami, PH-96). The images were recorded with a video tape recorder (Matsushita Elec. Co. NV 6600). A time video coder (FOR-A, VTG 55B), which displays digital time counts at intervals of 1/30 s on the corner of the TV screen, was attached to the video system, in order to record the time sequence of the moving images. The magnification of the video images was calibrated with an objective micrometer. A 10 μm gauge was recorded at the start of each experiment.

Fluorescent staining. Cells grown in YPD medium were washed twice with cold distilled water. Fifty μl of cell suspension (about $10^{7}$/ml) was mixed with 5 μl DAPI (Boehringer Manheim, 1 μg/ml). A few minutes later, the cell suspension was placed on a glass slide with coverslip, and observed under the fluorescence microscope with ultraviolet illumination. The absorption filter was an Olympus L435. Williamson & Fennel (1975) reported that DAPI specifically binds cellular DNA (nuclear as well as mitochondrial) of fixed preparations of yeast cells. In our procedure, the mitochondrial fluorescence could be kept at a minimum if the specimen suspensions were observed within 30 min after staining, probably due to the slower penetration of DAPI into mitochondria.

The same procedure was used for ethidium bromide (2.5 μg/ml) except that the staining time was about 30 min. Though whole cytoplasm revealed orange fluorescence, nuclei with more intense fluorescence could be distinguished. Washed cells were first mixed with DAPI for 5 min at 2 °C, and then the mixtures were incubated with ethidium bromide for 30 min.

The DAPI staining did not reduce the viability of the cells. However, attempts to trace dividing cells in the DAPI-containing YPD medium have been unsuccessful. In YPD medium, DAPI seemed to accumulate rapidly in the cytoplasmic vacuoles and the nuclear fluorescence was obscured. In the washed cells such accumulation of DAPI into the vacuoles was negligible.
Culture conditions of the cs cdc mutants

The mutant cells were grown exponentially at 37 °C for 4 h, and then transferred to 22 °C with shaking. The phenotypes of the mutants at 22 °C were tested after prolonged incubation. Since each mutant had a different generation time at 37 °C (from the minimum, 150 min for KM52, to the maximum, 360 min for KM170; wild-type 130 min), the incubation time at 22 °C ranged from 4 to 10 h.

The temperature shift-up experiments were carried out as follows. The mutant cells (KM108 or KM52) grown asynchronously at 37 °C for 4 h were transferred to 22 °C, and shaken for 4 h (KM52) or for 10 h (KM108). The culture was diluted 10 times with fresh YPD medium and shaken at 37 °C. Aliquots of the culture were taken at intervals of 15 min and kept on ice. The number of cells was determined using the counting chamber and the morphology of the chromatin regions was observed by video-connected fluorescence microscopy.

RESULTS

Nuclear chromatin region of asynchronously growing wild-type cells

The fission yeast *S. pombe* has a rod-like shape with constant cell width (3.5 μm) and variable length (7–15 μm). The cell divides by fission instead of budding. The cell cycle, which is illustrated schematically in Fig. 1 (left row), has 2 different phases; the phase of cell elongation in the first three-quarters of the cell cycle (A–C) and the phase of constant cell length in the remaining one-quarter of the cell cycle. Nuclear division (C–E) and cell plate formation (F), followed by cell separation (G), take place during the period of constant cell length (Mitchison, 1970).

Exponentially growing asynchronous cells were washed with cold distilled water and stained with a DNA-binding fluorescent dye, DAPI, as described in Materials and methods. A fluorescent micrograph is shown in Fig. 2A. The nuclear chromatin region was visualized as a bright bluish fluorescent area. It has a semicircular outline (about 2 μm in size) with one or two short bars attached to the side of the chord. Though the images of the fluorescent chromatin region were variable from cell to cell, we suspected that they were produced by different projections of the same 3-dimensional object. This was in fact the case. When a cell rotated slowly between the coverslip and glass slide, continuous changes in the appearance of the nuclear chromatin region were revealed in the cell.

After observing many rotating cells, an example of which is shown in Fig. 2B, we concluded that the nuclear chromatin region is an approximate hemisphere having 2 short rods protruding from the plane surface. A clay model is shown in the inset of Fig. 2A. In this paper we call such a structure either the hemispherical form or the Martian type.

Most cells in an asynchronously growing culture had generally one, and less frequently two, hemispherical chromatin regions. Stationary cells also contained a single Martian type nuclear chromatin region. The direction of the 2 protrusions relative to the cell axis differed from cell to cell. Cells seen just after the completion of the chromosome separation were exceptional; the directions of the protrusions were frequently parallel to the cell axis.

Ethidium bromide staining revealed the *S. pombe* nucleus as an approximate sphere (2 μm in diameter). If, however, cells were stained first with DAPI and then with
ethidium bromide, the nuclei were separately stained: a blue hemisphere with 2 rods and a dense orange crescent (data not shown). These results and other evidence (Hiraoka & Yanagida, unpublished data) indicate that the former represents the DNA-rich region while the latter is rich in RNA. The two protrusions appear to be parts of the chromatin embedded in the nucleolus.

Fig. 1. Cell cycle of the fission yeast *S. pombe*. The generation time is about 2 h at 37 °C and 3 h at 22 °C in YPD medium. Illustrations on the left are based on the results of Mitchison (1970) and McCully & Robinow (1971). A cell elongates in \( \frac{1}{2} \) of the cell cycle (A–C). Nuclear division (C–E), cell plate formation (F) and cell separation (G) take place in the rest of cell cycle where the cell length remains constant. The nuclear membrane is preserved during mitosis (shown in Fig.). A spindle apparatus exists inside the nucleus (c–E). The fluorescent video images at the right showing the cells stained with a DNA-binding fluorescent agent DAPI (the nuclear chromatin regions are the white bodies), correspond to the stages of cell cycle at the left (see text). Bar, 10 μm.

Cells from asynchronously growing cultures were classified as 4 different types, as shown in Table 1. Type I cells contained a single chromatin region and were shorter in length. Types II and III contained 2 chromatin regions per cell and were longer than the type I cell. The cell plate is absent in type II and present in type III. The
nuclear chromatin regions of type I, II and III cells, which constitute 99% of the total cells in the asynchronous culture, were hemispherical.

A very small fraction of the cells (type IV) revealed chromatin regions significantly different from the hemispherical form. Some examples of the fluorescent video images are shown in Fig. 2c. They appear to be either shrunken, deformed or split. The cell

Fig. 2. Nuclear chromatin regions of wild-type cells stained with DAPI. A. Fluorescent micrograph of asynchronously growing wild-type cells. Most of the nuclear chromatin regions had a semicircular outline with 1 or 2 short bars attached to the side of the chord. The 3-dimensional shape is approximate in the clay model (inset). B. A series of fluorescent micrographs showing the successive changes in shape of the characteristic hemispherical (Martian) chromatin region. C. A small fraction (1%) of the asynchronously growing wild-type cells exhibited nuclear chromatin regions significantly different from the hemispherical type. Several examples of this class (type IV, see Table 1) are shown. The nuclear chromatin regions appear to be either shrunken, deformed or split. Bar, 10 μm.
length of this class was identical to that of types II and III. Type IV cells may be undergoing the mitotic process. If they truly represent the intermediates of mitosis, they should appear only during the period of nuclear division. We introduced the selection synchrony method (Mitchison & Carter, 1975) to determine at what stage the type IV cells appear during the cell cycle.

Table 1. Four classes of cells in asynchronously growing wild-type culture

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of chromatin regions/cell</th>
<th>Shape of chromatin region</th>
<th>Average cell length (μm)</th>
<th>% of total cells</th>
<th>Examples in Figs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>Hemispherical</td>
<td>10.2 ± 2.0</td>
<td>82</td>
<td>Fig. 1A, B, G</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>Hemispherical</td>
<td>13.5 ± 0.5</td>
<td>7</td>
<td>Fig. 1E</td>
</tr>
<tr>
<td>III</td>
<td>2 (with cell plate)</td>
<td>Hemispherical</td>
<td>13.5 ± 0.7</td>
<td>10</td>
<td>Fig. 1F</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>Compact, U-shaped and others</td>
<td>13.5 ± 0.5</td>
<td>1</td>
<td>Fig. 1C, D, G</td>
</tr>
</tbody>
</table>

Sequential shape change of the nuclear chromatin region in synchronized culture

Exponentially growing cells were concentrated and overlaid on a sucrose gradient. After centrifugation, the cells on the top of a diffuse band were collected and diluted with YPD medium, and continued to grow at 30 °C. By this procedure, short-sized cells were selected, which grew synchronously (Mitchison & Carter, 1975). Aliquots of the culture were taken at intervals for cell counting and fluorescence microscopy. As shown in Fig. 3A, the cell count (filled circles) doubled at 100 and 220 min. The peaks of the cell plate index (open circles) appeared at 90 and 210 min after the start of synchronous culture. Cells of each aliquot were stained with DAPI, and the fluorescent images were recorded on video tape. Cells were classified as one of types I-IV on the monitor TV screen. As seen in Fig. 3B, type I cells constituted nearly 100% at 0 min, decreased after 60 min, reached the minimum (30%) at 90 min and increased thereafter. On the other hand, cells of types II and III were 0% at 0 min, increased after 60 min, and reached the maxima at 90 min (type II, 15%; type III, 40%). Type IV cells were not found at 0 min, and increased after 60 min. At 75 and 90 min, they constituted as much as 15% of the total cells, and then decreased to less than 2% at 120 min; the type IV cells appeared only during mitosis, indicating that the type IV cells are intermediates in nuclear division. The number of type IV cells reached the maximum at 75 min, while types II and III arrived at the maxima 15 min later. Thus, the mitotic cycle of S. pombe may be sequenced as follows:

Type I → type IV → type II → type III → type I.

Type IV cells were classified further according to the size and shape of their nuclear chromatin regions. Although structural variations were continuous, 2 distinct subclasses were found to be dominant. One class contained the shrunken or compact
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chromatin region. The other contained the U-shaped chromatin region. Typical examples are shown in Fig. 4A and B, respectively. In the selection synchrony experiment (Fig. 3c), the number of the compact form reached the maximum (11%) at 75 min and the number of the U-shaped form attained the maximum (10%) at 90 min. This result suggested a sequential shape change in the nuclear chromatin region as follows:

Hemispherical form → compact form → U-shaped form → 2 separated hemispherical forms.

Fig. 3. Morphological alterations in the nuclear chromatin region in synchronous culture. The selection synchrony was carried out according to Mitchison & Carter (1975) and as described in Materials and methods. A. The number of cells (●) doubled around 100 and 220 min. The cell plate index (number of cells with cell plate per total number of cells) attained maxima at 90 and 210 min (○). B. Cells were stained with DAPI and classified into 4 (I-IV) types. The frequency of each type is expressed as a percentage. Type I (○) has a single hemispherical chromatin region. Type II (▲) has 2 chromatin regions at either end of the cell without a cell plate. Type III (△) has 2 chromatin regions, with a cell plate. Type IV (●); see Fig. 2c) has a non-hemispherical chromatin region. C. Frequencies of 2 major sub-classes of the type IV chromatin region are shown: the compact type (○) appeared first and reached a maximum at 75 min, and then the U-shaped form (●) at 90 min.
Fig. 4. Time-lapse fluorescent video images of type IV chromatin regions. DAPI-stained cells rotating around the cell axis were recorded by the video system so as to obtain 3-dimensional information on the nuclear chromatin region. The major sub-classes of type IV structures are shown in A and B, while the minor subclasses are shown in C–E. Bar, 10 μm. A. A rotating cell revealing the compact form of the chromatin region. A cell with the hemispherical chromatin region is shown at the right for comparison. B. A cell exhibiting a U-shaped chromatin region. Note that the cavity faces in the direction of the cell axis. A clay model is shown at the right. C. A disk-like chromatin region showing a flattened hemisphere with faint protrusions. D. A flattened and U-shaped chromatin region; a clay model is shown at the right. E. A segregating form.
Three-dimensional shape of the mitotic nuclear chromatin region

The major and minor subclasses of the type IV chromatin regions were analysed using the video system. Cells rotating around their axes were chosen to obtain 3-dimensional information on the nuclear chromatin region.

A series of video images derived from the compact form is shown in Fig. 4A. The size is smaller than that of the hemispherical form in type I cells (shown at the extreme right for comparison). The shape is a distorted ellipsoid or sphere, showing a bumpy surface; no protrusions were seen.

The time-lapse video images of a U-form chromatin region are shown in Fig. 4B. It was not a difficult task to deduce the 3-dimensional shape from the successive images of a rotating cell. A clay model is shown at the right (Fig. 4B). The structure is similar to a half-opened castanet. The 5th image in Fig. 4B represents a cell rotated about 180° from the first image around the cell axis. The large cavity made by 2 equal halves was stained with ethidium bromide in the double-staining method with DAPI, indicating that the cavity is rich in RNA. It is of interest to note that the position of the U-form appears to be fixed in the cell. We always found that the cavity faced towards the cell side (also see Fig. 2C).

Three examples of the minor subclasses of type IV are shown in Fig. 4C-E. The video images derived from a flattened hemisphere having 2 faint protrusions are shown in Fig. 4C. Another example shown in Fig. 4D is similar to the U-shaped form, but has a shallow cavity. The angle made by the 2 equal halves is not as sharp as that found in the U-shaped form in Fig. 4B. A clay model is shown at the right. Although they are low in frequency, they may be important in understanding the detailed process of chromosomal transfiguration. As will be described in the next section, a certain class of mutants revealed images similar to those of the type IV minor subclass.

The time-lapse video images shown in Fig. 4E represent a segregating nuclear chromatin region. The cells containing partially segregated chromatin regions were low in frequency, indicating that the chromosome segregation process is rapid. Tiny protrusions were already seen in the centre of 2 segregating chromatin regions. The space between the chromatin regions was found to be rich in RNA.

Nuclear chromatin regions of the cs cdc mutants under restrictive conditions

Some of the cold-sensitive (cs) cell division cycle (cdc) mutants isolated in our laboratory showed, under restrictive conditions, characteristic nuclear chromatin regions similar to those found in the type IV cells of the wild type. The mutant isolation procedure and the detailed properties of the isolated cs cdc mutants will be reported elsewhere. In brief, 500 cs mutants were screened from 200000 nitroso-guanidine-mutagenized colonies by replica-plating. Twenty five independent strains showed terminal phenotypes corresponding to the expected defects in nuclear division at 22 °C (Nurse et al. 1976); the cells elongated without nuclear division. Six of those strains (KM52, 108, 138, 170, 311, 376) were used in the present study. The DNA synthesis of the mutants under restrictive conditions was apparently normal. Preliminary genetic study showed that cs and cell-elongation phenotypes in each mutant
were caused by a single chromosomal mutation. All 6 mutants belonged to different complementation groups. The phenotypes of 6 mutants under the restrictive conditions are described below.

**KM138.** The mutant extended its cell length to 25 μm (on average) at 22 °C for 6 h. The nuclear chromatin region stained with DAPI was rather flattened, like a disk (Fig. 5A). The plane surface of the disk was usually perpendicular to the cell axis. The protrusions became very thin and were barely visible. A structure similar to this was found in a type IV minor subclass of the wild type (Fig. 4c). In KM138, the compaction of the chromatin region appeared to be attained although the shape was not ellipsoidal.

**KM376 and KM108.** Both the compact form and the U-shaped intermediate were found in these 2 mutants. The compact form was the major form in KM376 (Fig. 5b), while the U-form was dominant in KM108 (Fig. 5c). The ellipsoidal compact forms in KM376 were indistinguishable from those found in the wild type (Fig. 4a). The U-shaped forms in KM108 appeared to be somewhat different from those of the wild type (Fig. 4b); the cavity was narrow in the mutant chromatin region.

**KM311.** After 6 h at 22 °C, most of the mutant cells contained a pair of closely located chromatin regions (Fig. 5d, left). After prolonged incubation at 22 °C, the chromatin regions were separated to some extent and more than 50% of the cells branched (Fig. 5d, right). One peculiar observation made was that a chromatin region occasionally migrated into the branched part of the cell. KM311 may be defective in a step of chromosome segregation and separation.

**KM32.** This mutant was also not as normal in chromosome segregation as KM311, but it has an additional curious phenotype. After 6 h at 22 °C, many cells contained a nucleus displaced from the centre of the cell body (Fig. 5e, left). The chromatin region was compact and occasionally split in two. After 10 h at 22 °C, the displaced chromatin region was divided into 2 parts with a short distance between them (Fig. 5e, right). The cell plate was formed at the middle of the cell; the nuclear chromatin regions were present only on one side of the cell.

**KM170.** The hemispherical chromatin region observed in the type I cells of the wild type was found in the cells of this mutant after incubation at 22 °C for 10 h. However, the cells were longer (15 μm on average) than those of the wild type. The mutant might be defective in an early step of nuclear division, such as the step of compaction.

Transformation of the mutant nuclear chromatin region by temperature shift-up

To establish further the mitotic steps that took place in the wild-type synchronized culture, we investigated the structural changes in the chromatin regions in the cs cdc mutants after a shift from restrictive to permissive conditions. Fig. 6 shows the results of 2 experiments using mutants KM108 and KM32. The 2 mutants were still viable after prolonged incubation at 22 °C. They were first grown at 37 °C for 4 h asynchronously, then incubated at 22 °C so as to arrest normal growth, and again shifted back to 37 °C.

At 0 min after the shift-up, 83% of the KM108 cells comprised type IV cells.
Fig. 5. Nuclear chromatin regions in the cold-sensitive cdc mutants at the restrictive temperature. Mutant cells were grown at 37 °C, transferred to 22 °C and shaken for 4–10 h. Cells were stained with DAPI. A. KM138 at 22 °C for 10 h. The compact disk-like form of chromatin region was observed. B. KM376 at 22 °C for 10 h. The compact form of chromatin region was seen. C. KM108 at 22 °C for 10 h. Structures similar to the U-shaped form were found. D. KM311 at 22 °C for 6 h (left) and 10 h (right). The nuclear chromatin regions are separated by a short distance (left). Cell branching occurred at the middle of cell (right). E. KM52 at 22 °C for 4 h (left) and 10 h (right). The nuclear chromatin region was compact or U-shaped and often displaced from the centre of cell at 4 h. At 10 h at 22 °C, the chromatin regions are segregated on one side of the cell and the cell plate has formed. Bar, 10 μm.
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(68% U-shaped and 15% compact types). The number of type IV cells decreased as the type II (peak at 60 min) and type III (peak at 90 min) cells increased. The increase in type I cells occurred finally at 100 min. The doubling of the cell number took place around 110 min, and would be correlated with the decrease in type III cells. These results indicated a sequential change: type IV → II → III → I.

Fig. 6. Sequential alteration of the chromatin regions in KM108 and KM52 after a shift from the restrictive to the permissive temperature. Frequencies of the 4 cell types were measured and expressed as percentages. A, KM108; B, KM52. The cell number doubled at 110 min in KM108 and at 55 min in KM52 after the shift.

The response of KM52 to the temperature shift to 37 °C was rapid, as shown in Fig. 6B; the steep decrease in type IV cells that had compact or U-shaped chromatin regions occurred within 45 min. The number of type III cells increased and reached the maximum at 15 min after the shift while the increase in type I cells started only after 30 min. The decrease in type III cells coincided with the increase in type I cells. The cell number doubled at around 55 min. The apparent sequence was as follows: type IV → III → I. The number of type II cells remained at a low level during the transformation. The precursors of the cell plate probably accumulated in the cells.
under the restrictive conditions prior to the temperature shift-up, thus causing the rapid formation of cell plates.

The results described above show that, by raising the temperature, both KM108 and KM52, previously incubated at 22 °C, were switched synchronously from the arrested state to the normal growth pattern. Furthermore, the results support the scheme of sequential alterations in the nuclear chromatin region derived from experiments with the wild-type synchronous culture.

DISCUSSION

The use of an ultrasensitive video camera attached to the fluorescence microscope has several merits. A large number of DAPI-stained cells with a low intensity of fluorescence could be recorded in a short time and reproduced instantaneously. This facilitated the classification of several thousand cells (Figs. 3, 6). Cells moving in the suspension could be traced so that the time-lapse images, varied by using different viewing directions, helped to reconstruct the 3-dimensional features of the nuclear chromatin region (Figs. 2, 4). The conventional film-recording required an exposure time of at least 30 s and the specimens should be fixed on a glass slide. Because the cells used in the video analyses were still viable after staining (see Materials and methods), the structures observed were supposed to be the least damaged.

Our results show that the nuclear chromatin region alters its form during the period (about 15 min) of nuclear division of the fission yeast S. pombe. In the synchronized wild-type culture, different forms of the chromatin region appeared in an ordered sequence as follows. (1) A hemispherical form with 2 short protrusions; (2) an ellipsoidal compact form without protrusions; (3) a U-shaped intermediate; (4) a segregating form in which 2 chromatin regions could be distinguished; (5) 2 small hemispherical forms, located at either end of the cell. Rupture of the nuclear membrane and the formation of 2 daughter nuclei should occur just after this step (Fig. 1A; see also plate 39 of McCully & Robinow, 1971). The cell plate is formed thereafter.

Two minor types of nuclear chromatin regions were observed (type IV minor sub-classes). One was a disk-like structure with barely visible protrusions (Fig. 4c). The mutant KM138 cells under the restrictive conditions showed a structure similar to this (Fig. 5A). The other was a rod-like structure sometimes difficult to distinguish from the ellipsoidal compact form (data not shown). We were not able to determine their exact positions in the sequence described above, since their frequencies were so low (1-3 %), even in the synchronous culture. These 2 minor forms were included in the group of compact forms in the frequency analyses.

The hemispherical form was a constant feature of the nuclear chromatin region throughout the cell cycle of S. pombe except at mitosis (Fig. 3b). The hemisphere was small immediately after mitosis and seemed to increase in size during the S phase, which follows shortly after nuclear division (the G1 phase of S. pombe is very short; Mitchison & Creanor, 1971). Cells in the stationary phase also have hemispherical forms with protrusions.

The presence of 2 domains in a nucleus was demonstrated by double-staining with
DAPI and ethidium bromide; a bluish DAPI-stained hemisphere with 2 protrusions and an orange ethidium bromide-stained hemisphere, which covered the protrusions. Hiraoka & Yanagida (unpublished data) investigated the localization of DNA and RNA in isolated nuclei. Digestion of the isolated nuclei with DNase caused the disappearance of the DAPI-stained hemisphere while RNase had no effect on the DAPI-stained images. On the other hand, ethidium bromide-stained nuclei were round-shaped, and upon digestion with RNase the round nuclei changed to the hemispherical form. Digestion of the double-stained nuclei with RNase caused the disappearance of the orange area. These results demonstrated directly that the yeast nucleus consists of 2 regions: the DNA-rich chromatin region and the RNA-rich (nucleolus) region, and are consistent with previous cytological and electron microscopical studies (Robinow & Marak, 1966; Gordon, 1977).

We have never found the discrete chromatids observed by Fischer, Binder & Wintersberger (1975) and Robinow (1977), but we found that a certain kind of chromosomal compaction appeared during mitosis (about 50–70% reduction in the volume of the chromatin region). Gordon (1977) reported that the nuclear chromatin is dispersed throughout the cell cycle of *S. cerevisiae*. McCully & Robinow (1971) did not find any indication of chromosome condensation in thin sections of *S. pombe*. Because cells with compact chromatin regions were rarely found in asynchronous cultures (Table 1), they might have been missed in the previous electron microscopic studies. Alternatively, the distinction between the dispersed and more condensed states of chromatin may not be easy to see at the electron microscopic level. The compaction of the yeast chromatin region found in this study was not as striking as that of the higher eukaryotes. Histone H1 is considered to be involved in chromatin condensation. In yeast cells the presence of H1 is not certain, although the presence of nucleosomes consisting of 4 histones (H2A, H2B, H3, H4) is well established (Lohr & Van Holde, 1975; Thomas & Furber, 1976). Our results suggest that a chromatin-condensing factor may well be present during the early steps of nuclear division.

The structure of the U-shaped intermediate gave an insight into the mechanism of chromosome segregation. Transformation from the compact to the U-shaped form should include the steps in the production of the cavity or cleft in the centre of the chromatin region. The appearance of the small cleft was the initial sign of segregation. In the U-shaped form with a large cavity that always faced in the direction of the cell side, the 2 segregating chromatin regions were still partly associated. The appearance of the chromatin regions seen in Fig. 4B and E suggested that the associated chromosomal parts may be the small protrusions. The structure of the U-shaped intermediate is symmetrical. Double-staining with DAPI and ethidium bromide showed that the RNA-rich region was sandwiched between 2 segregating chromatin regions. Thus, the segregation process appeared to occur in a well-organized fashion.

The mitotic steps seem to be controlled by many gene functions: the 6 cs cdc mutants used in the present work revealed, under restrictive conditions, either hemispherical, compact, U-shaped or partially segregating chromatin regions. The block at the segregation process caused anomalous nuclear location (KM52) or cell
branching (KM311), which may indicate that nuclear elongation and division may be interrelated with the organization of the cytoskeleton.

A useful property of the KM108 and KM52 mutants was their highly reversible response to a shift from restrictive to the permissive conditions. The cell population from an asynchronous culture was arrested at the same stage by prolonged incubation under restrictive conditions and the response to the temperature shift-up was quite synchronous. The results of the temperature shift-up experiments (Fig. 6) support the sequential steps proposed from the analyses of the wild-type synchronous culture.

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REFERENCES


Video fluorescence microscopy of yeast mitosis


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