The use of cell division cycle mutants to investigate the control of microtubule distribution in the fission yeast *Schizosaccharomyces pombe*

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Summary

We have characterized the changes in microtubule organization that occur through the cell division cycle of the fission yeast *Schizosaccharomyces pombe* by indirect immunofluorescence microscopy. During interphase, groups of cytoplasmic microtubules, independent of the spindle pole body (SPB), form an array extending between the cell tips. These microtubules are involved in positioning the nucleus at the cell equator and in the establishment of cell polarity. At mitosis, the interphase array disappears and is replaced by an intranuclear spindle extending between the now duplicated SPBs. Elongation of the spindle sees the appearance of astral microtubules emanating from the cytoplasmic face of the SPBs. These persist until the end of anaphase whereupon the spindle microtubules depolymerize and two microtubule organizing centres (MTOCs) at the cell equator re-establish the interphase array. We have used the unique properties of various cell division cycle mutants to investigate further the function of these different microtubule arrays and their temporal and positional control.

Key words: microtubules, microtubule organizing centres, *Schizosaccharomyces pombe*, cell cycle, cell division cycle mutants.

Introduction

The dynamic properties of microtubules are fundamental to the passage of a eukaryotic cell through its cell division cycle. Changes in microtubule organization are most evident at the G_2/M boundary where a gross rearrangement sees the interphase microtubule array giving way to the mitotic spindle (Vandre & Borisy, 1986) and during mitotic anaphase where more subtle changes accompany the movement of the chromosomes to the poles (McIntosh, 1984). However, the fact that, under certain growth conditions at least, experimental manipulation of the degree of polymerization of cytoplasmic microtubules during interphase can provoke the precocious entry of cells into S phase (for review see Thyberg, 1984), suggests that the relationship between the cell cycle and the cytoskeleton may be deeper than is currently generally appreciated.

The nature of the controls that are envisaged to govern passage through the eukaryotic cell cycle is emerging primarily from the study of yeasts. In both the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*, the use of cell division cycle (*cdc*) mutants has allowed the identification of two major control points in the cell cycle (Pringle & Hartwell, 1981; Nurse, 1985). The first of these, termed 'start' (Nurse, 1981), defines the point in G_1 beyond which the cell is committed to the mitotic cell cycle and unable to undertake the alternative pathways of conjugation and sporulation. The second, in G_2, signals commitment to the nuclear division cycle (Hayles & Nurse, 1986). Although the two yeasts are widely separated in evolutionary terms (Huysmans *et al.* 1983), these control processes share a common molecular basis (Beach *et al.* 1982). Indeed, it has recently been shown that the conservation of this

cell cycle control function may extend to humans (Lee & Nurse, 1987; Draetta *et al.* 1987), implying that it is probably representative of the control mechanisms operating in most eukaryotes.

A major advance in the study of the yeast cytoskeleton was the development of techniques for the localization of cytoskeletal proteins in *S. cerevisiae* by fluorescence microscopy (Adams & Pringle, 1984; Kilmartin & Adams, 1984). Coupled with the application of molecular genetics to both *cdc* mutants and the genes for actin, tubulin and myosin (Gallwitz & Seidel, 1980; Toda *et al.* 1984; Hirokoa *et al.* 1984; Adachi *et al.* 1986; Neff *et al.* 1983; Schatz *et al.* 1986*a*,*b*; Watts *et al.* 1985) this work opened up a new avenue along which to approach the relationship between the cytoskeleton and the cell division cycle. The value of *S. cerevisiae* as an appropriate model for the study of the eukaryotic cell division cycle is, however, the subject of some debate (Nurse, 1985). We have therefore begun to develop a corresponding cytological analysis of the cytoskeleton in *S. pombe*. In an earlier report we determined the changes in F-actin through the cell cycle in this organism (Marks & Hyams, 1985). In the present paper we present a similar analysis for tubulin in both wild type and a number of *cdc*$^-$ mutants.

Abstracts of this work have appeared elsewhere (Hyams *et al.* 1985; Hagan & Hyams, 1986).

Materials and methods

Strains

The wild-type strain of *Schizosaccharomyces pombe 972* h$^-$ and the temperature-sensitive cell division cycle (*cdc*) mutant strains: *cdc2.33* h$^-$, *cdc2.L7* h$^-$, *cdc10.129* h$^-$, *cdc10.C4* h$^-$, *cdc11.136* h$^-$, *cdc14.118* h$^-$ and *cdc25.22* h$^-$ and the *wee1.50* h$^-$ mutant strains (Nasmyth & Nurse, 1981; Nurse *et al.* 1976) were kindly supplied by Dr P. Nurse. Strains were maintained on supplemented yeast extract plates (Mitchison, 1970). Single colonies checked for ploidy and, in the case of cell cycle mutants, temperature sensitivity, were used to inoculate 10-ml starter cultures 2 days prior to the inoculation of the main culture.

Cell culture

Asynchronous cultures of *972* h$^-$ were grown, to a density of 6×10^6 to 1×10^7 cells ml^{-1} (mid log phase) on a reciprocal shaker at 25 °C in modified minimal medium, EMM2 (Nurse, 1975). The temperature-sensitive mutant strains were grown to a cell density of 1×10^6 to 2×10^6 cells ml^{-1} (early log phase) at 25 °C and the culture transferred to a reciprocal shaker at 36 °C. Samples were taken at 3, 4, 5 and 6 h after the temperature shift (the generation time is 2·5 h at 36 °C).

Immunofluorescence microscopy

The procedures followed were based on those of Kilmartin & Adams (1984) and Adams & Pringle (1984). Fixation was achieved using either aldehydes or solvents.

(1) *Aldehyde fixation*. Cells were fixed by addition to the culture of 1/9 (v/v) of a freshly prepared 30 % (w/v) solution of formaldehyde in 100 mM-Pipes, 1 mM-EGTA, 1 mM-magnesium sulphate (PEM) and 50 % glutaraldehyde (BDH) to a final concentration of 0·2 %. The glutaraldehyde was added between 20 and 45 s after the formaldehyde. The culture was left in the presence of fixatives on a reciprocal shaker at the same temperature as the growing culture for 30–90 min. Fixation was stopped by three washes in PEM buffer. Cells were resuspended to a final density of 5×10^7 cells ml^{-1} in PEM containing 1·2 M-sorbitol (PEMS).

Digestion of the cell wall was achieved by the addition of 50 μl ml^{-1} of 5 % mutanase (Novozym 234; Novo-Enzyme Products, UK) and 50 μl ml^{-1} 1·25 % zymolyase (20 000 U, Seikagaku Kogyo Co. Ltd) both in PEMS. Digestion could be achieved using zymolyase alone, but the *cdc* mutants were more variable than wild-type cells in their sensitivity to either enzyme and the use of two different enzymes helped to standardize the process. As cells lost their walls they appeared more refractile under phase-contrast optics and the internal structures of the cell, including vesicles and nuclei, became apparent. Digestion was stopped when 50–70 % of the cells were judged to have lost their cell walls. Cells were then permeabilized by resuspension in PEM+1 % Triton X-100 for 30 s followed by three washes in PEM buffer. Following three 5-min washes with PEM containing 1 mg ml^{-1} sodium borohydride and a further three washes in PEM, the cells were resuspended in PEM+1 % bovine serum albumin (BSA), 0·1 % sodium azide and 100 mM-lysine hydrochloride (PEMBAL) for at least 30 min.

(2) *Solvent fixation*. A 30-ml sample of culture was filtered onto Whatman GF/C glass-fibre filters and the cells shaken off in 8 ml methanol at −20 °C. After 8 min the cells were removed from methanol by three washes in PEM at room temperature. Other organic solvents at −20 °C also proved to be efficient fixatives as follows; (a) acetone for 5 min, (b) acetone for 30 s followed by methanol for 5 min, (c) 5 % acetic acid, 95 % ethanol for 8 min. Cells were protoplasted as above with the exception that the concentration of the enzymes in the digestion mix was halved and digestion was terminated by washing in PEMS when only 5–10 % of the cells appeared to have lost their cell walls (the remainder lost their cell walls in the washing step). Cells were resuspended in PEMBAL buffer for 30 min subsequent to the post-permeabilization washes.

The mutant cells were fixed and washed before being left in PEM plus 0·1 % sodium azide overnight. Cells could be left at this stage for up to 4 days without any obvious deleterious effects on the microtubule architecture.

Antibody staining

The primary anti-tubulin monoclonal antibodies YOL1/34 and YL1/2 (Kilmartin *et al.* 1982) were both kindly supplied by Dr J. Kilmartin. Cells were pelleted from PEMBAL and resuspended in 5–7 volumes of 1 % antibody in PEMBAL. Following a 16-h incubation the cells were washed three times in PEMBAL and resuspended in 5–7 volumes of rhodamine isothiocyanate (RITC)-labelled rabbit anti-rat antibody (Miles Yeda) at 2 % in PEMBAL. The secondary antibody solution was preabsorbed with *S. pombe* protein extract prepared using the protocol of Simanis & Nurse (1986)

omitting the TX buffer washes and centrifuged at 1300 revs min^{-1} for 10 min. Incubation in the secondary antibody was for at least 4 h. Excess antibody was removed by three washes in PEMBAL.

For observation cells were air dried onto poly-L-lysine (1 mg ml^{-1})-coated coverslips and inverted onto Elvanol mounting medium containing 1 mg ml^{-1} para-phenylene diamine and 1 μg ml^{-1} 4′,6-diamidino-2-phenylindole (DAPI) (Williamson & Fennell, 1975). Slides were viewed with a Zeiss photomicroscope 3 fitted with epifluorescence optics and using a planchromat 63×1·25 NA objective optovar 1·25 to view DAPI/phase images and a 63×1·4 NA objective optovar 1·25 to view the tubulin images. Photographs were taken on Kodak Tri-X-Pan film rated at 400 ASA.

Results

Wild-type staining patterns

A typical asynchronous mid-log phase population of wild-type *S. pombe* cells prepared for indirect immunofluorescence microscopy using the primary anti-tubulin antibody YOL1/34 following aldehyde fixation is shown in Fig. 1A. By comparing the tubulin stained cells with the corresponding combined phase-contrast and DAPI images, to indicate the cell outlines and position of the nuclei, respectively (Fig. 1B), it is, possible to relate particular microtubule arrays with position in the cell cycle. In interphase, cytoplasmic microtubules are aligned along the long axis of the cell (cell 1). By focusing through it is clear that the majority of these microtubules run between the tips of the cells although this is not always evident in a photographic image, which displays an optical section through the cell. A rarer class of cytoplasmic microtubules do not connect with the cell tips at either one or both ends. Both categories apparently lie immediately below the plasma membrane. This is illustrated in Fig. 1C, in which a cell has attached perpendicularly to the coverslip: in this case four groups of tubulin-staining structures extend along the cell axis. Aldehyde fixation probably underestimates the abundance of this class of

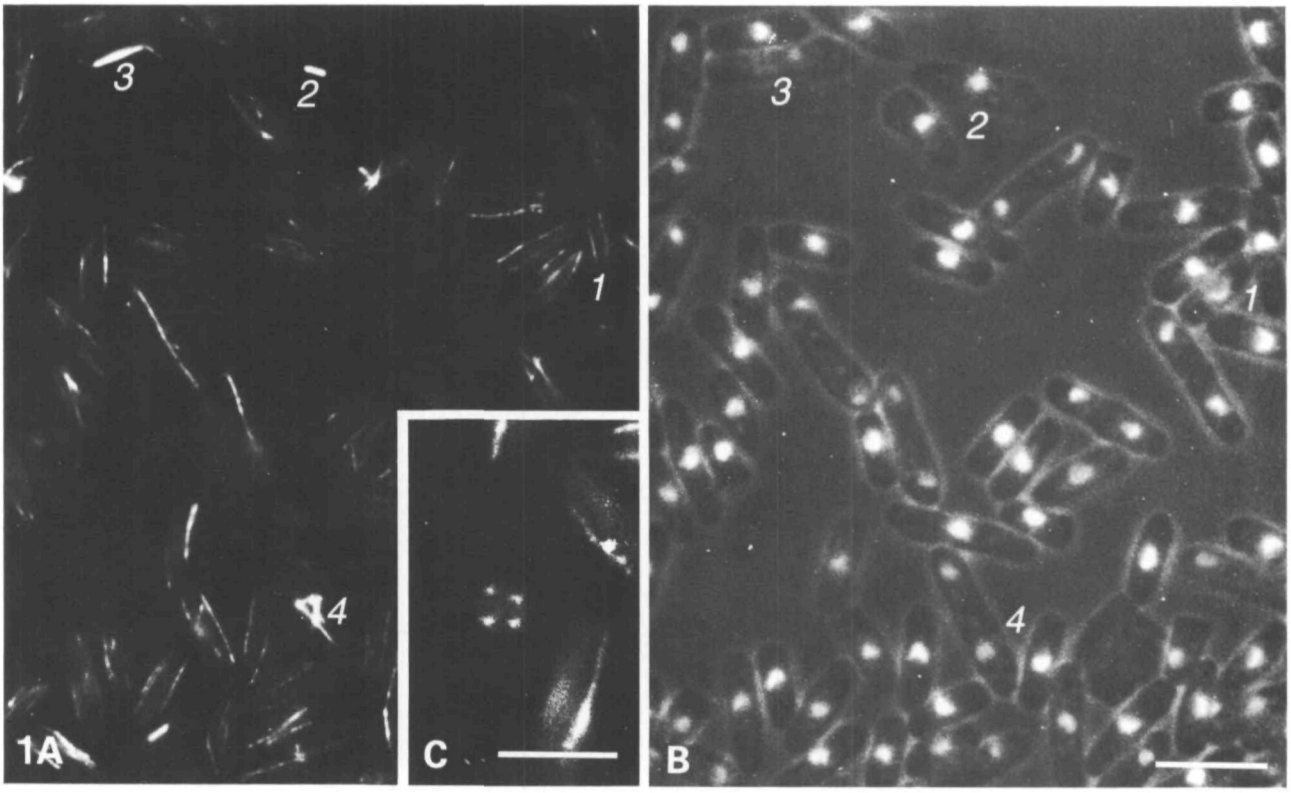

Fig. 1. Microtubule localization in wild-type *S. pombe* strain *972* h^- by indirect immunofluorescence microscopy after aldehyde fixation using the anti-α-tubulin antibody YOL1/34 (A) and combined DAPI fluorescence and phase-contrast images (B). All classes of staining are represented. Cell 1 is typical of interphase cells with cytoplasmic microtubules aligned along its long axis. Cell 2 has a typical early spindle, while cell 3 exhibits the anaphase B state where extra-nuclear microtubules have seeded onto the outer face of the spindle pole bodies. Cell 4 shows an example of the post-anaphase staining pattern with microtubules emanating from two points in the centre of the cell. Although there appear to be three foci in this cell the lower one is the result of the microtubules emanating from the two organizing centres crossing over in the centre of the cell. In no instance have three post-mitotic organizing centres been seen. Insert C shows a transverse optical section through a cell, demonstrating the proximity of the microtubules to the plasma membrane. Bar in B, 10 μm; Bar in C, 5 μm.

microtubules (see below). As cells enter mitosis (cell 2) cytoplasmic microtubules are replaced by an intranuclear mitotic spindle. As this elongates extranuclear microtubules become associated with the spindle poles (cell 3). Finally, at the end of anaphase a fourth class of staining becomes apparent, as typified by cell 4, in which microtubules emanate from two foci at the cell equator.

Aldehyde-fixed cells stained with a different anti-tubulin antibody (YL1/2) gave identical staining patterns (not shown). In cells fixed with solvents and probed with either YOL1/34 or YL1/2 antibodies cytoplasmic microtubules were somewhat better preserved, with up to eight linear elements per cell being visualized (Fig. 2). However, spindle preservation was more variable with this mode of fixation and cell shrinkage was induced, therefore aldehyde fixation was routinely employed.

In Fig. 3 individual cells have been arranged on the basis of their length and position in mitosis to illustrate changes in the microtubule organization through the cell division cycle. The transition from interphase (Fig. 3A–D) to mitosis (Fig. 3E–K) is signalled by the appearance of two anti-tubulin staining dots on the edge of the nucleus, presumably the daughter spindle pole bodies (SPBs), and the concomitant disappearance of the cytoplasmic interphase microtubules, although frequently what appears to be a single anti-tubulin staining structure is seen in these cells (Fig. 3E). In the majority of cases this structure is orientated towards the SPBs. In haploid *S. pombe* cells the DNA assumes a hemispherical configuration with the rest of the spherical nucleus being taken up by the nucleolus (Toda *et al.* 1981). Using this criterion the dots appear anywhere on the nuclear surface. Separation of the dots precedes the formation of a short spindle on one side of the nucleus (Fig. 3F,G). The spindle is somewhat brighter at the ends than in the middle (Fig. 3G,H). In wild-type cells grown at 25 °C the spindle elongates from an initial length of around 1·5–2·0 μm until, in most cases, it appears to extend the entire length of the cell (12–14 μm at 25 °C). After early anaphase no variance in staining intensity along the length of the spindle could be detected although astral microtubules tangential to the spindle poles appear (Fig. 3I,J). These may lie either in the same or opposite orientation, the alternatives, which are seen with equal frequency, being represented by Fig. 3I,J and the cell indicated by the arrow in Fig. 2C.

Concomitant with the arrival of the nuclei at the cell ends two dots of fluorescence are seen at the cell equator, predominantly in a different focal plane to each other (Fig. 3K). These are followed by a microtubule network that can be traced back to two points presumed to be equivalent to the dots at a later stage (Fig. 3L,M,N). As septation progresses the focal

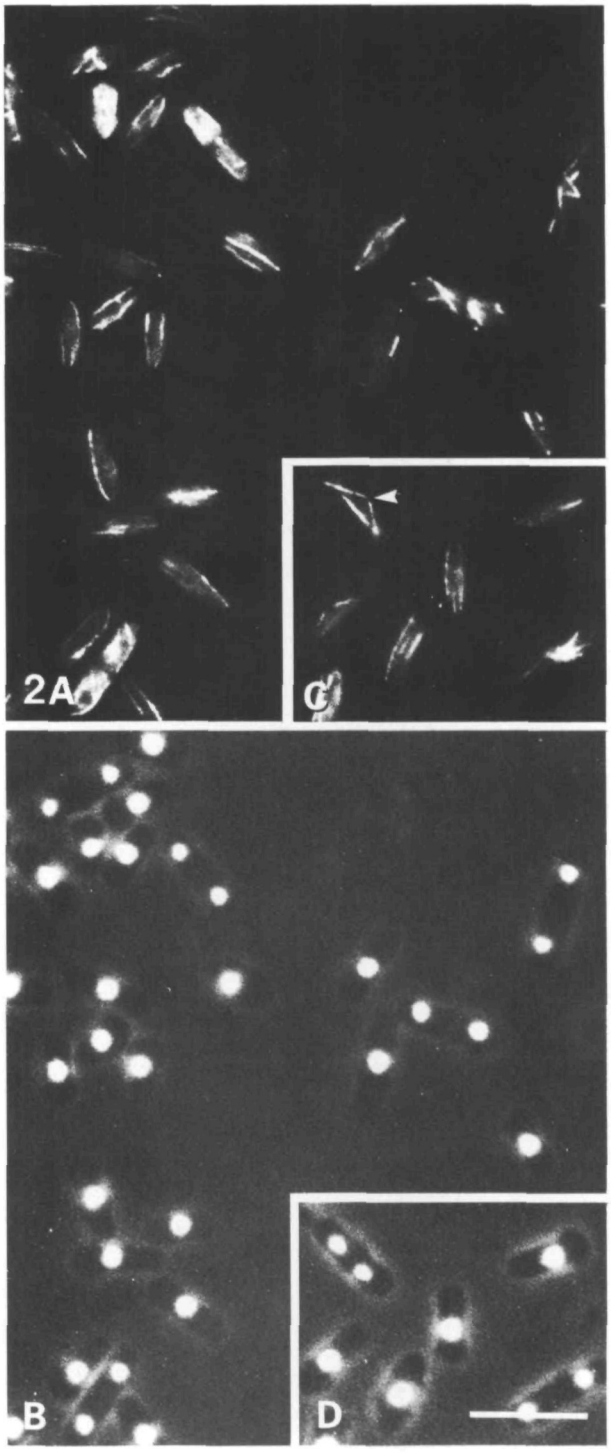

Fig. 2. Indirect immunofluorescence of tubulin distribution in wild-type *S. pombe*, strain 972 h$^-$ using the anti-α- and β-tubulin antibody YL1/2 (A,C) and combined DAPI fluorescence and phase-contrast images of the respective fields of cells (B,D), after fixation in methanol at −20 °C. All stages of the cell cycle are represented. The arrow in C indicates a cell with astral microtubules extending in opposing directions. Bar, 10 μm.

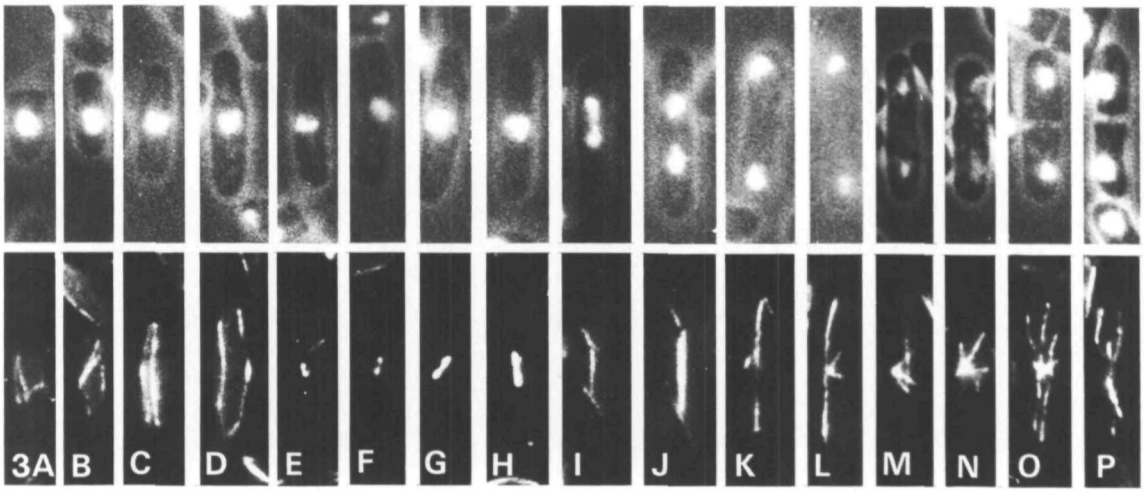

Fig. 3. A photographic summary of the tubulin distribution throughout the cell cycle of the same population of fission yeast cells fixed by the aldehyde method as shown in Fig. 1. The classification of the staining patterns is as described in the text. A,B,C,D are arranged in order of increasing size, as cell length can be used as a marker of the position in the cell cycle in *S. pombe* (Mitchison & Nurse, 1985). E shows the initial nuclear staining in mitotic cells. A residual cytoplasmic element orientated towards the replicated SPBs is shown. F,G,H, show the elongation of the dumbbell-shaped spindle prior to anaphase B. Anaphase B and the concomitant appearance of the astral microtubules is seen in I,J. The astral microtubules in these panels are in the same orientation (cf. Fig. 2C). The appearance of the post-anaphase array and the concomitant disappearance of the spindle is represented by the cells in K–N. The cells chosen for these frames have only one of the two MTOCs in the plane of focus to aid visualization of the structure. O,P show the later stages of the re-establishment of the interphase array, O being in mid-septation and P just preceding the completion of this process. Note that in O the two organizing centres are very close to each other in the same plane of focus. ×1500.

points for the microtubules of this 'post-anaphase array' appear to move to the centre of the cell (Fig. 3O) and end up, just prior to division, as a single staining body (Fig. 3P). These results are consistent with the existence of two microtubule organizing centres (MTOCs) in the centre of the cell at the end of mitosis. The limitations of fluorescence microscopy prevent us from determining whether the MTOCs fuse, simply overlie each other, or whether one or both are turned off prior to division, having re-established the interphase array.

In Fig. 4 we show the percentage of cells in an asynchronous wild-type culture at 25 °C (cell cycle time 4 h) possessing the categories of staining shown. Whilst the actual number of cells counted for the first two early mitotic classes is too low to attribute any absolute temporal value, the figures do indicate that the transition from the interphase to mitosis is rapid. Correcting the figures for position in the cell cycle by the method of Mitchison (1971) gives an approximate time of 25 min for the mitotic period in minimal medium at 25 °C.

Microtubule localization in cell cycle control mutants

The *cdc10* and *cdc2* gene products have previously been shown to play a role in governing commitment of the cell to the mitotic cell cycle (Nurse, 1985). The point of commitment, defined by mutants in these

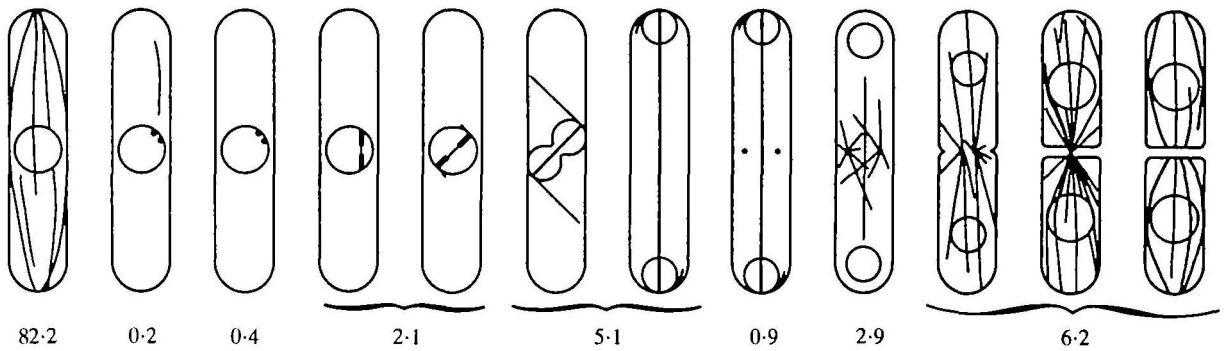

Fig. 4. A schematic representation of the changes in microtubule distribution during the cell cycle of *S. pombe*. The figures give the relative percentage of cells displaying each class of staining and come from counting 4253 cells in random fields of the wild-type strain *972* h^- grown at 25 °C and stained after aldehyde fixation.

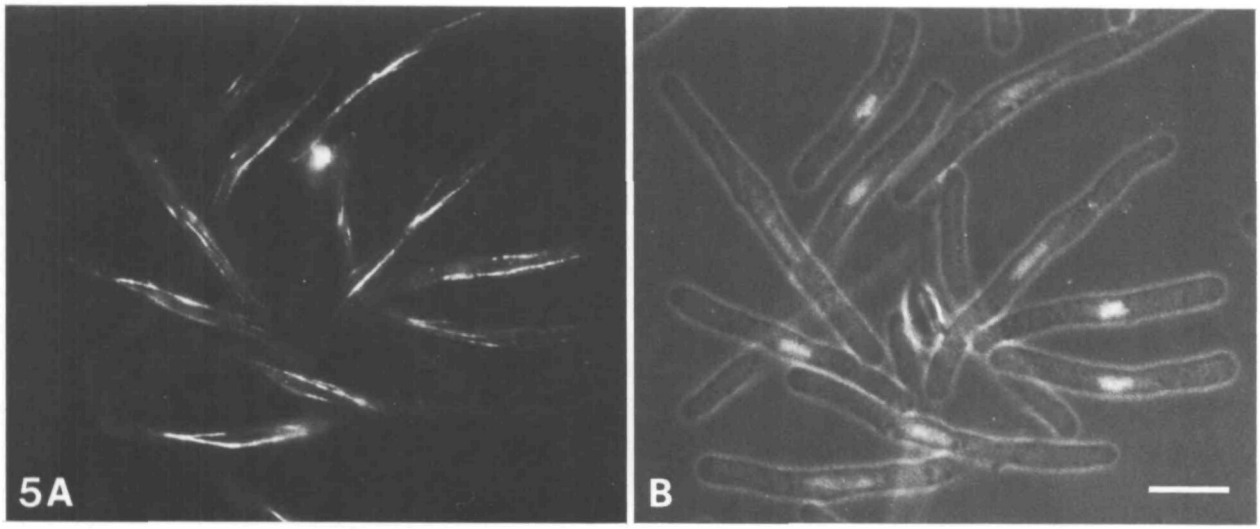

Fig. 5. Microtubule localization in aldehyde-fixed cells of the strain *cdc25.22* h⁻ after incubation at 36°C for 4·25 h. This pattern, with the cells displaying accentuated versions of the interphase array, is also typical of cells of the strains *cdc2.33* h⁻, *cdc2L.7* h⁻ after 4·25 h and *cdc10.C4* h⁻ and *cdc10.129* h⁻ after 6 h at the restrictive temperature. Bar, 10 μm.

genes, is referred to as start. Another execution point for the *cdc2* gene is at the G_2/M boundary, where the *cdc25*, *wee1* and *nim1* gene products are also operative in the control network (Russell & Nurse, 1987*a*,*b* and references therein). Cultures of the strain *cdc10.129* h⁻ and *cdc10.C4* h⁻ incubated at 36°C for 6 h, *cdc2.L7* h⁻, *cdc2.33* h⁻ and *cdc25.22* h⁻ for 4·25 h revealed homogeneous populations of elongated cells indicating a cell cycle specific block. Tubulin staining showed that all of the elongated cells possessed an interphase array as typified by the *cdc25.22* h⁻ cells shown in Fig. 5. The microtubules appeared to describe a slightly helical path between the cell tips dipping in and out of the plane of focus, behaviour seen to some extent in wild-type cells but accentuated by the increased length of these mutants.

Mutants defective in *wee1* function divide at a reduced size due to a curtailed G_2 period (Fantes, 1984). An asynchronously growing population of the temperature-sensitive strain, *wee1.50* h⁻ is shown in Fig. 6. Despite their small size the cells apparently display all of the wild-type arrays (Fig. 6A), the only anomaly being a low level of aberrant mitoses (about 1 %) where spindle elongation was seen to occur in the absence of nuclear division (Fig. 6C,D), resulting in the appearance of enucleate cells in the culture (Fig. 6E). It is interesting to note that the cell in Fig. 6C displaying this phenomenon shows a septum bisecting a spindle prior to the formation of a post-anaphase array.

Further controls are revealed by cells leaking through the temperature block

The temperature-sensitive mutant *cdc25.22* is an allele of the *cdc25* gene that blocks efficiently after 4·25 h at the restrictive temperature, although, under the conditions used here, when this is extended to 6 h around 20 % of the cells leak through into mitosis. Fig. 7 shows examples of such cells. Whereas in cells blocked at the *cdc25* execution point prior to mitosis nuclei remain centrally positioned (Fig. 5), the dividing nuclei can wander towards the cell ends. While the spindle is now eccentrically positioned, the post-anaphase array is still located in the centre of the cell (Fig. 7A,B). Fig. 7C,D shows a short spindle associated with astral microtubules that are longer than those seen in wild-type cells.

Microtubule behaviour in early septation mutants

Early septation mutants undergo a series of nuclear divisions in the absence of cell division (Nurse *et al.* 1976). In wild-type cells the divided nuclei, which have been separated to the ends of the cell by the elongating spindle, return to a position a quarter of the way along the cell, i.e. the centre of the two daughter cells. In *cdc14.118* h⁻ cells at the restrictive temperature the nuclei do not halt their progress at this point, but return to cluster in the centre of the cell (Fig. 8A). *cdc11.136* h⁻ cells show more varied nuclear positioning, with some cells showing centrally positioned nuclei and others having nuclei positioned either side of the cell equator. Fig. 8 shows this in *cdc11.136* h⁻ and *cdc14.118* h⁻ cells after 6 h at the restrictive temperature, which is equivalent to around three cell cycles under the conditions used in this study. The reason why early mitotic cells are chosen to illustrate the control over nuclear positioning is that in the absence of cell division there is no longer a marker to distinguish between interphase cells and those in which

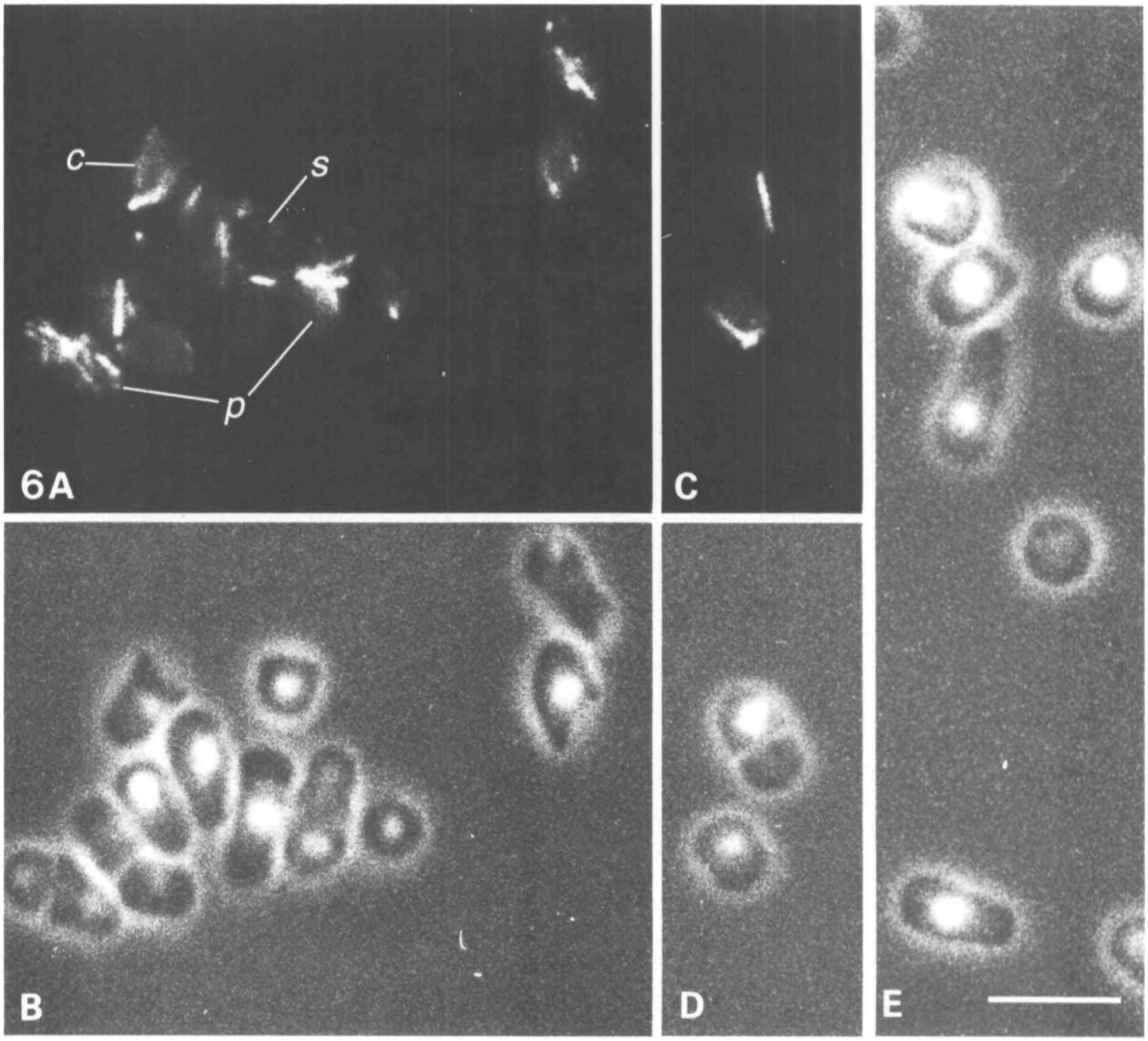

Fig. 6. Microtubule localization in aldehyde-fixed cells of the cell cycle control mutant *wee1.50* h⁻ after 6 h at 36°C. All of the wild-type patterns are visible in A,B; *c*, a cell containing cytoplasmic microtubules; *s*, a cell containing a spindle; *p*, two cells containing post-anaphase arrays. C,D show an example of the premature elongation of the spindle, pushing the single nucleus into one of the daughter cells. E shows an enucleate cell seen in the culture, presumably arising from a division such as that seen in C. Bar, 5 μm.

the nuclei are still in the process of relocation following mitosis.

A population of *cdc11.136* h⁻ cells stained after 4·5 h at the restrictive temperature reveals a number of mitotic conformations (Fig. 9). Early mitotic nuclei can be either centrally positioned as in cell 1 or separated as in cells 2 and 3. The post-anaphase array is similarly observed in two conformations; either centrally positioned as in cell 4 or spatially distinct (cell 5). On the other hand in all of the blocked *cdc14.118* h⁻ cells examined to date, after an identical incubation period, the post-anaphase array is always centrally located, as typified by the cell in Fig. 10E,F. The high degree of synchrony in the initiation of mitosis and the rate of spindle elongation in any one cell is seen by the uniform length of spindles at all stages of nuclear separation in Figs 8, 9, 10.

Discussion

The fission yeast *Schizosaccharomyces pombe* is assuming an increasingly prominent role in studies of both the cell division cycle and the cytoskeleton (for reviews see Simanis *et al.* 1986; Yanagida *et al.* 1985; Yanagida, 1987). In an attempt to bring together these hitherto distinct areas of research, we have used fluorescence microscopy to characterize the organization of cytoskeletal proteins through the cell division cycle of wild-type cells and in a number of *cdc* mutants. In an earlier study we showed that structural rearrangements of F-actin occurred at three distinct points in the

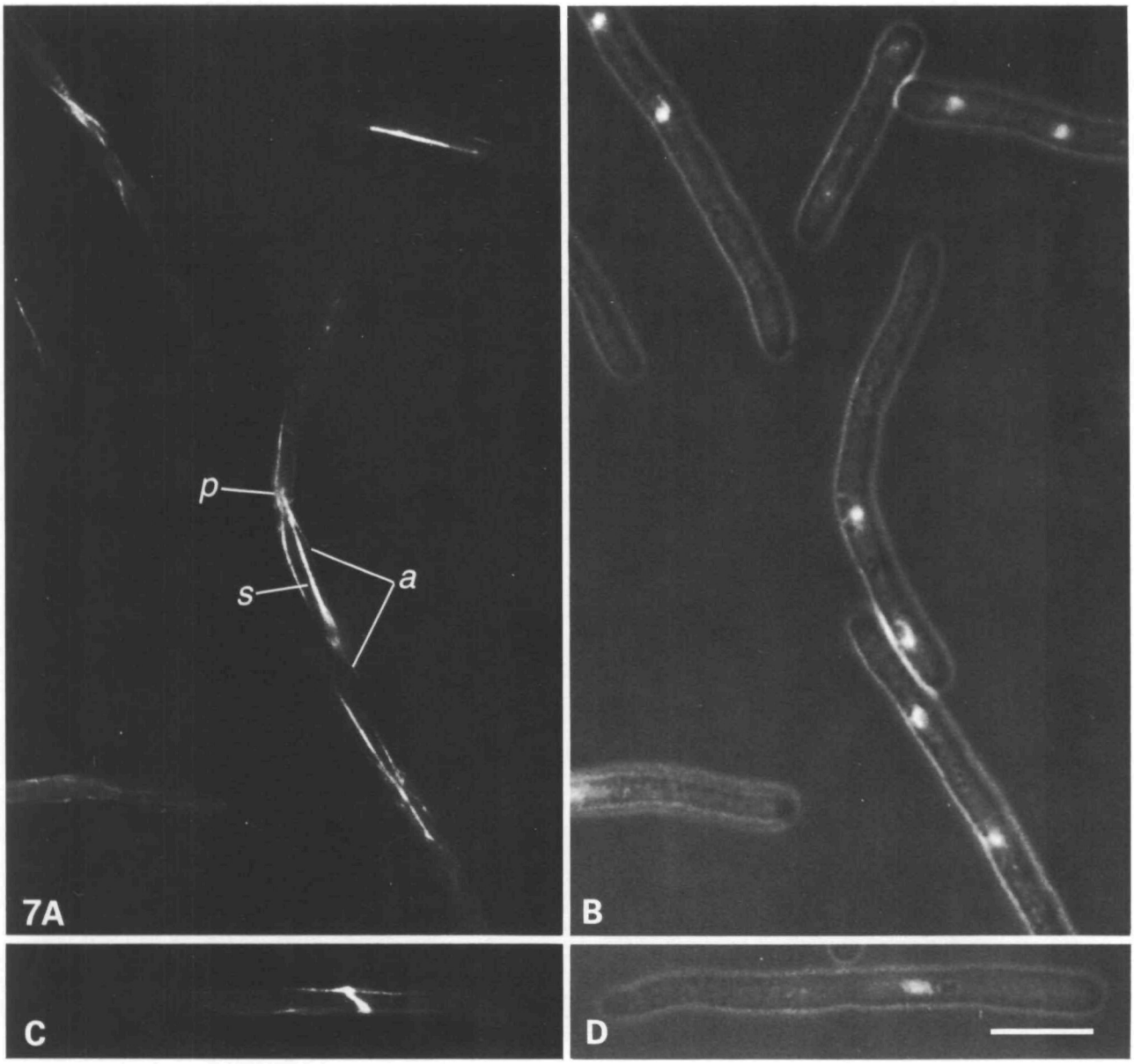

Fig. 7. Cells of the strain *cdc25.22* h⁻ after 6 h at the restrictive temperature stained after aldehyde fixation reveal further controls. The cell in the centre of A and B shows an eccentrically positioned dividing nucleus. The spindle (*s*) with its astral microtubules (*a*) and a centrally positioned post-anaphase array (*p*) can be seen. The cell shown in C,D has an early spindle with very long astral microtubules. Bar, 10 μm.

mitotic cell division cycle; at the transition from monopolar to bipolar cell growth in G_2, at the initiation of septation in M phase, and at the reinitiation of monopolar growth at the cell division/G_1 boundary. At each of these transitions, reorganization of actin was coincident with changes in the pattern of cell wall deposition (Marks & Hyams, 1985; Marks *et al.* 1986, 1987). Here we have shown that microtubules also exhibit marked rearrangements at defined points of the cell cycle, in this case at the G_2/M boundary and at mitotic telophase. Each of these appears to be under the control of distinct MTOCs.

Interphase in *S. pombe* occupies approximately 75 % of the cell division cycle, during which period cells roughly double in length from 7 to 14 μm (Mitchison, 1970). Throughout this growth phase from three to eight anti-tubulin staining elements extend between the cell apices. Identical patterns were obtained with the antibody YL1/2, which recognizes both α- and β-tubulins in *S. pombe*, and YOL1/34, which recognizes the two α-tubulins (Adachi *et al.* 1986; our unpublished results). Some variations in microtubule preservation was observed when ice-cold methanol, and other combinations of solvents, rather than the aldehydes,

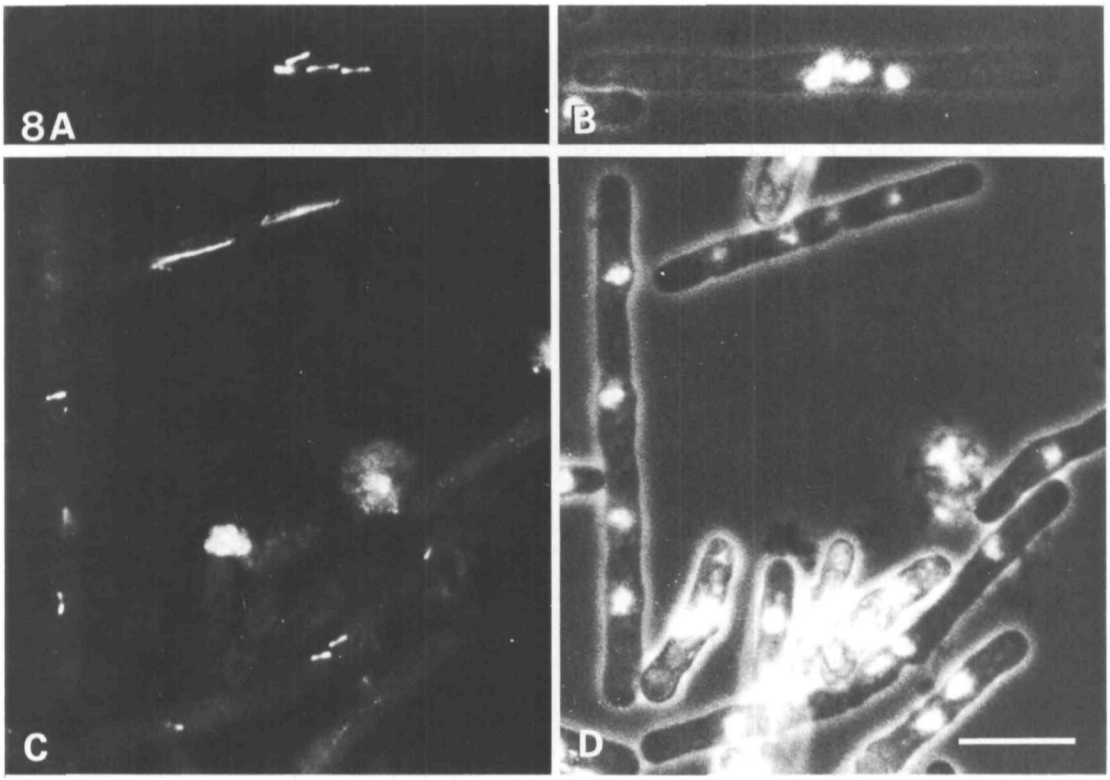

Fig. 8. Nuclear positioning in the early septation mutants *cdc14.118* h⁻ (A,B) and *cdc11.136* h⁻ (C,D) after 6 h at the restrictive temperature. In *cdc14* the nuclei are clustered in the cell centre, while in *cdc11.136* h⁻ cells they may be spaced at intervals along the cell axis (see also Fig. 9). Early mitotic cells are chosen to illustrate the differences of nuclear positions between these strains for the reasons outlined in the text. The large number of dead cells in the *cdc11* culture is typical of the early septation mutants and results from abortive attempts to form septa (I. M. Hagan, P. Riddle and J. S. Hyams, unpublished observations). Bar, 10 μm.

were used as fixatives. Cytoplasmic microtubules, for instance, were more abundant when solvents were employed, although spindles were less well preserved. Such variations reflect the difficulties involved in adapting immunofluorescence techniques to small, walled cells. However, all fixation regimes so far employed revealed the same pattern of changes in microtubule organization through the cell division cycle.

Although the ultrastructure of wild-type *S. pombe* has been extensively investigated, electron microscopy has only occasionally recorded cytoplasmic microtubules, nor were they evident in previous attempts to apply anti-tubulin immunofluorescence microscopy to fission yeast (Hirokoa *et al.* 1984; Hirano *et al.* 1986; Uemura & Yanagida, 1986). However, thin sections of the *cdc* mutant strain *cdc3.6* revealed bundles of three cytoplasmic microtubules, which are probably equivalent to the individual components of the interphase array shown here by immunofluorescence (Streiblova & Girbart, 1980).

Microtubule organization in elongated cells of the mitotic cell division cycle mutants *cdc2*⁻, *cdc10*⁻ and *cdc25*⁻ represents an accentuation of the interphase state. These results are consistent with the cell cycle regulatory role of these genes. Without the functions encoded by *cdc2* and *cdc10*, cells are unable to traverse the rate-limiting steps at the commitment point to the mitotic cell cycle (start), and the G_2/M boundary for *cdc2* and *cdc25* (Simanis *et al.* 1986).

What is the function of cytoplasmic microtubules in *S. pombe*? One strongly implied possibility is in the maintenance of organelle position. In the cold-sensitive β-tubulin mutant, *nda3*, the nucleus does not maintain a fixed location when cells are grown at the restrictive temperature (Toda *et al.* 1983; Umesono *et al.* 1983). A comparable situation is seen in this study where the mitotic nuclei in elongated cells of *cdc25.22* that have leaked through the cell cycle block wander considerable distances from the cell centre when cytoplasmic microtubules disappear. A relationship between nuclear positioning and microtubules had been shown previously in *Aspergillus nidulans* (Oakley & Morris, 1981), and in other fungi structural links between microtubules and the nucleus have been recorded (McKerracher & Heath, 1985).

Cytoplasmic microtubules may also play a role in the establishment of growth polarity in *S. pombe*, perhaps

through an, as yet, undefined influence over the organization of F-actin, which we have shown is intimately involved in cell wall deposition (Marks & Hyams, 1985; Marks *et al.* 1986, 1987). The disappearance of cytoplasmic microtubules at the G_2/M boundary coincides with the cessation of cell elongation as

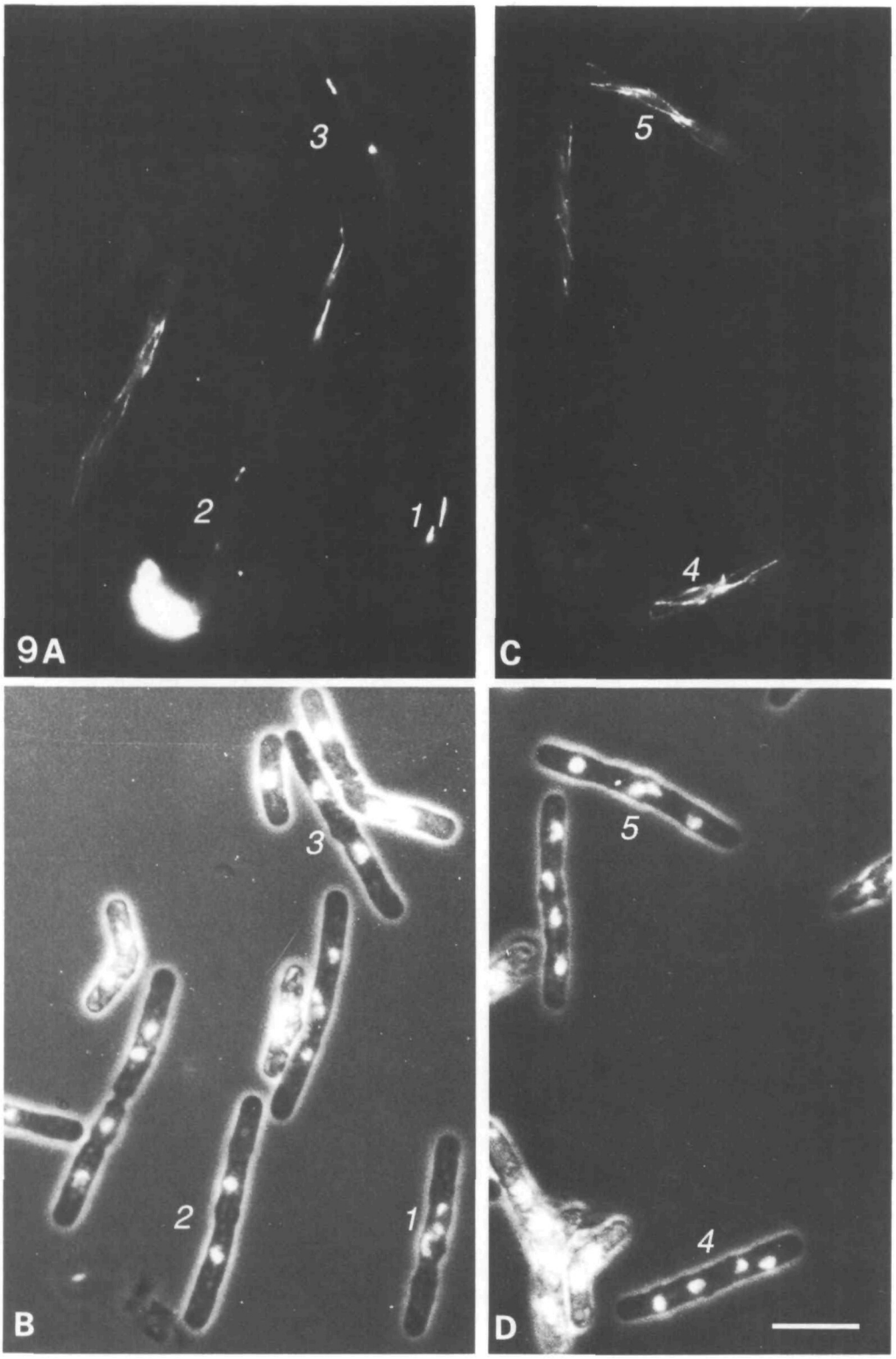

Fig. 9. Microtubule staining and DAPI/phase imaging of the strain *cdc11.136* h$^-$ after 4·5 h at the restrictive temperature. A,B, the varying nuclear positioning of the very early mitotic nuclei. Cell *1* has centrally positioned nuclei while in cells *2* and *3* the nuclei occupy different positions along the cell length. C,D show examples of the two types of post-anaphase array seen in this mutant strain (cells *4* and *5*). Bar, 10 μm.

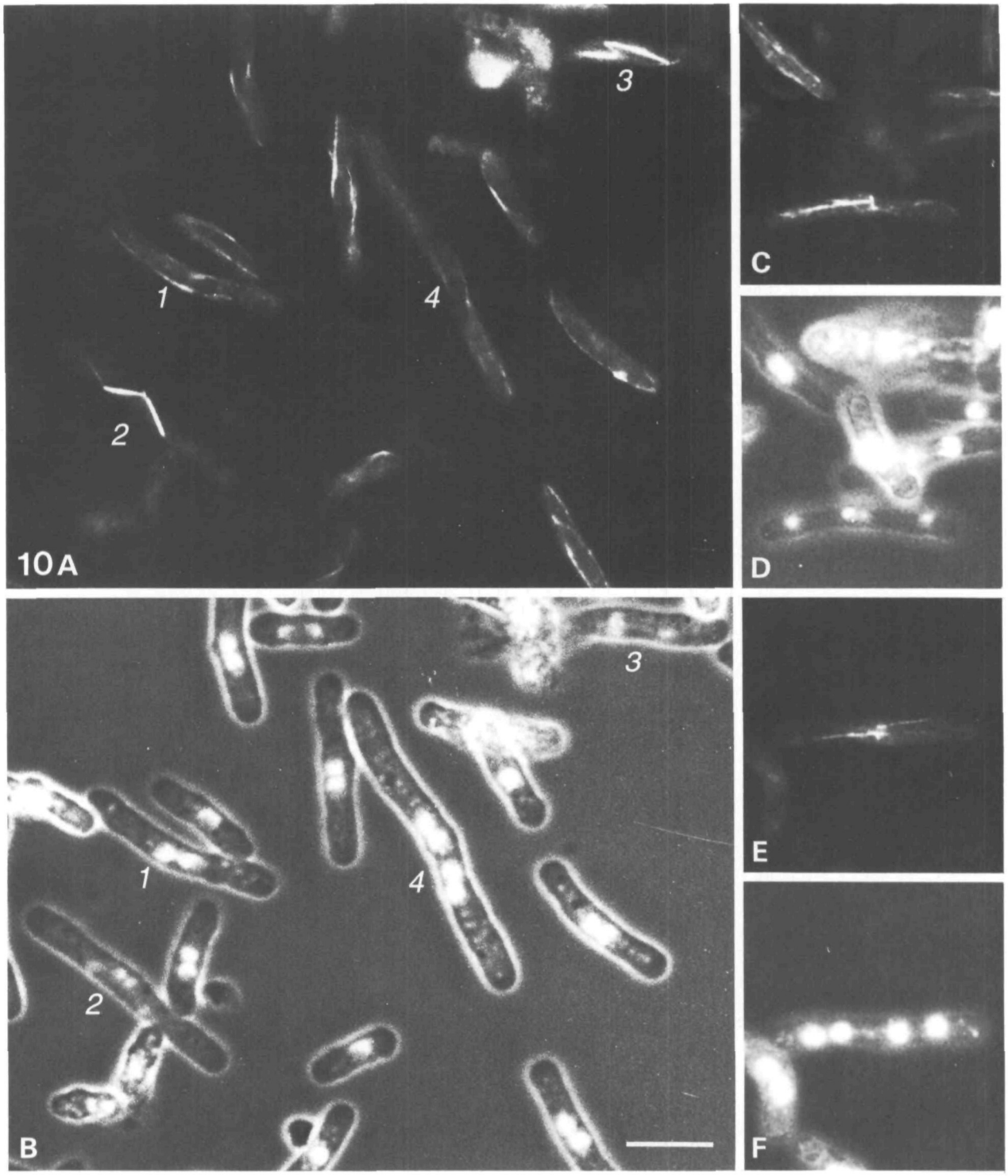

Fig. 10. Microtubule staining of the strain *cdc14.118* h⁻ at the restrictive temperature. A. A typical asynchronous population after 6 h at 36°C with a good demonstration of the nuclear positioning before (cell *1*), during (cells *2*, *3*) and after (cell *4*) a two-nuclei mitosis. The elongating spindles can either elongate in opposite directions down the cell (cell *2*) or overlap during this process (cell *3*). A typical late mitotic figure is seen in C,D with the joint spindle length just exceeding that of the cell. E,F show the kind of centrally positioned post-anaphase array seen in this mutant. The cells in C–F have been blocked for 4·5 h, analogous to the duration of the block used with the *cdc11.136* h⁻ cells shown in Fig. 9. Bar, 10 μm.

cells enter the constant volume stage (Mitchison, 1970). Disruption of microtubule function, either by growth of *nda3* at the restrictive temperature or treatment of wild-type cells with microtubule inhibitors leads to a loss of control of wall deposition resulting is distended or branched cells (Toda *et al.* 1983; Umesono *et al.* 1983; Walker, 1982; our unpublished observations). Also, the organization of cytoplasmic microtubules in *Schizosaccharomyces japonicus*, which has a less well defined shape than *S. pombe*, appears to be more random (C. Alfa & J. S. Hyams, unpublished results). By contrast bud enlargement in *S. cerevisiae* continues apparently normally in the absence of microtubules, despite the fact that nuclear positioning becomes random (Pringle *et al.* 1984). These differences between the two yeasts reflects their contrasting modes of growth and division. This is emphasized by the role of the SPB in each. In *S. pombe*, the association of microtubules with the SPB is apparently restricted to mitosis. As with other aspects of cytoskeletal organization, the arrangements of cytoplasmic microtubules in *S. pombe* more closely resembles that of the filamentous fungi (Hoch & Staples, 1985; Runeberg *et al.* 1986) than the budding yeasts, where microtubules extend from the cytoplasmic face of the SPB throughout bud development (Kilmartin & Adams, 1984). The different roles of the SPBs in the two yeasts may reflect the organization of the respective cell cycles, in particular the duration of mitosis (Nurse, 1985).

The end of interphase in *S. pombe* is marked by the disappearance of the cytoplasmic microtubules and the concomitant appearance of two tubulin-staining dots on the side of the nucleus, as microtubules are nucleated off the intranuclear face of the now duplicated SPB. Occasionally a cytoplasmic microtubule persists beyond this point, but the low frequency with which cells displaying this type of staining pattern are observed, emphasizes the rapidity of the interphase to mitosis transition (McCully & Robinow, 1971).

In contrast to the situation with interphase microtubules, our description of spindle formation and elongation is, by and large, consistent with both electron microscope and earlier immunofluorescence studies (McCully & Robinow, 1971; Hirokoa *et al.* 1984; Hirano *et al.* 1986; Uemura & Yanagida, 1986). The increased intensity of staining at the ends of short spindles in *S. pombe* suggests the existence of two sets of microtubules, one extending between the poles, the other between the poles and the kinetochores (Hirokoa *et al.* 1984). The fact that this differential staining is lost prior to spindle elongation suggests that anaphase A precedes anaphase B in *S. pombe*.

A recent study of microtubule rearrangements during mitosis in *S. pombe* using freeze-substitution showed that the elongating spindle frequently assumed an 'S' shape (Tanaka & Kanbe, 1986). In our study, examination of many thousands of dividing nuclei failed to reveal this conformation, neither is it seen in living cells (Robinow, 1981). Rather, the only curvature we have seen in spindles has been a bowing out of the spindle in a minority of dividing cells (unpublished results).

Tangential cytoplasmic microtubules associated with the SPB are first detected when the spindle completely spans the nucleus (Fig. 4) and are thus probably associated with anaphase B. Similar structures have previously been reported by electron microscopy of regenerating protoplasts (Hereward, 1974), in *cdc27.K3* at the restrictive temperature (King & Hyams, 1982) and in wild-type cells fixed by freeze-substitution (Tanaka & Kanbe, 1986), although in none of these cases were interphase microtubules preserved. This may argue for some preferential stability of the astral microtubules, possibly due to their association with the SPB. That the SPB has the potential to seed more (unpublished observations) and longer astral microtubules than it does during the course of a normal mitosis is demonstrated in cells of the strain *cdc25.22* that leak through their temperature-sensitive block. Astral microtubules have been suggested to have a role in nuclear separation during mitosis in fungi (Aist & Berns, 1981) and the structures in *S. pombe* may be performing a similar role.

The end of anaphase is coincident with the appearance, presumably by activation, of two MTOCs in the centre of the cell, which re-establish the interphase array. That the position of these post-anaphase MTOCs is independent of that of the mitotic spindle is shown most convincingly by *cdc25*$^-$ cells that have leaked through their temperature-dependent block. Here the post-anaphase array is still seen at the midpoint of the cell, the position of the pre-mitotic nucleus, despite the fact that the nucleus becomes displaced from this central location during mitosis. The early septation mutants provide further evidence for a relationship between the positioning of the two MTOCs and pre-mitotic nuclear location. In multinucleated cells of the strain *cdc14.118* h$^-$ the pre-mitotic nuclei are always centrally positioned as are the post-anaphase MTOCs, whilst in another early septation mutant, *cdc11.136* h$^-$, the nuclei show variable positioning as do the post-anaphase arrays. The presence of two sets of organizing centres in mitotic binucleate *cdc11.136* cells shows that the duplication of these structures is co-ordinated with the nuclear division cycle. At present it is not apparent whether the nucleus determines the position of the post-anaphase array, or whether both respond to a similar control.

When cells of the strain *wee1.50* h$^-$ were grown at the restrictive temperature a low level of aberrant mitoses were seen (about 1 %), with spindle formation

and elongation occurring before other aspects of nuclear organization have progressed to such a stage as to allow equal segregation of the chromosomes. This behaviour may explain the previously noted cell loss in *wee1.50* diploid cell cultures (Fraser & Nurse, 1979). Interestingly, *cdc2.1w* cells, which divide at a comparable size to *wee1.50* cells (7·7 *versus* 7·9 μm; Thuriaux *et al.* 1978), also undergo aberrant mitoses, whilst the larger *cdc2.3w* cells (9 μm at division; Fantes, 1981) do not (unpublished observations). This behaviour appears to be a less extreme case of the so-called 'mitotic catastrophe' seen upon over-expression of the mitotic inducer $cdc25^+$ in *wee1.50* cells (Russell & Nurse, 1986). An interesting feature of such cells is that septum formation can occur in advance of the formation of the post-anaphase array. These events, which occur coincidentally in wild-type cells, therefore appear to be independently controlled.

In this paper and in our earlier study (Marks & Hyams, 1985) we have shown that progress through the mitotic cell cycle of *S. pombe* involves structural rearrangements of the cytoskeleton. These define new landmarks, which more accurately identify the execution points of existing *cdc* mutants and may allow ways of characterizing new mutants at other cell cycle transition points. Analysis of certain *cdc* mutants at the molecular level has implied that the regulation of passage through the yeast cell cycle, at least at the G_2/M boundary involves protein phosphorylation (Simanis & Nurse, 1986; Russell & Nurse, 1987*a*,*b*). There are numerous examples of altered function of cytoskeletal proteins after phosphorylation and it is easy to envisage how the changes documented here could be regulated in this way. Identification, isolation and characterization of fission yeast cytoskeletal proteins will further our understanding of both morphological and cell cycle control in *S. pombe*.

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References

ADACHI, Y., TODA, T., NIWA, O. & YANAGIDA, M. (1986). Differential expression of essential and non-essential α-tubulin genes in *Schizosaccharomyces pombe*. *Molec. cell. Biol.* **6**, 2168–2178.

ADAMS, A. E. M. & PRINGLE, J. R. (1984). Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic mutant *Saccharomyces cerevisiae*. *J. Cell Biol.* **98**, 934–945.

AIST, J. R. & BERNS, M. W. (1981). Mechanics of chromosome separation during mitosis in *Fusarium* (Fungi Imperfecti): New evidence from ultrastructural and laser microbeam experiments. *J. Cell Biol.* **91**, 446–458.

BEACH, D., DURKACZ, B. & NURSE, P. (1982). Functionally homologous cell cycle control genes in budding and fission yeast. *Nature, Lond.* **300**, 706–709.

DRAETTA, G., BRIZUELA, L., POTASHKIN, J. & BEACH, D. (1987). Identification of p34 and p13, human homologs of the cell cycle regulators of fission yeast encoded by $cdc2^+$ and $suc1^+$. *Cell* **50**, 319–325.

FANTES, P. A. (1981). Isolation of cell size mutants of a fission yeast by a new selective method: Characterisation of mutants and implications for division control mechanisms. *J. Bacteriol.* **146**, 746–754.

FANTES, P. A. (1984). Cell cycle control in *Schizosaccharomyces pombe*. In *The Microbial Cell Cycle* (ed. P. Nurse & E. Streiblova), pp. 109–125. Boca Raton, Florida: CRC Press.

FRASER, R. S. S. & NURSE, P. (1979). Altered patterns of ribonucleic acid synthesis during the cell cycle: A mechanism compensating for variation in gene concentration. *J. Cell Sci.* **35**, 25–40.

GALLWITZ, D. & SEIDEL, R. (1980). Molecular cloning of the actin gene from yeast *Saccharomyces cerevisiae*. *Nucl. Acids Res.* **8**, 1043–1059.

HAGAN, I. M. & HYAMS, J. S. (1986). Spatial control of microtubule initiation in *Schizosaccharomyces pombe*. *J. Cell Biol.* **103**, 415a.

HAYLES, J. & NURSE, P. (1986). Cell cycle regulation in yeast. *J. Cell Sci. Suppl. 4*, 155–170.

HEREWARD, F. V. (1974). Cytoplasmic microtubules in a yeast. *Planta* **117**, 355–360.

HIRANO, T., FUNDAHASHI, S., UEMURA, T. & HANAGIDA, M. (1986). Isolation and characterisation of *Schizosaccharomyces pombe cut* mutants that block nuclear division but not cytokinesis. *EMBO J.* **5**, 2973–2979.

HIROKOA, Y., TODA, T. & YANAGIDA, M. (1984). The *NDA3* gene of fission yeast encodes β-tubulin. A cold sensitive *nda3* mutation reversibly blocks spindle formation and chromosome movement in mitosis. *Cell* **39**, 349–358.

HOCH, H. C. & STAPLES, R. C. (1985). The microtubule cytoskeleton in hyphae of *Uromyces phaseoli* germlings: Its relationship to the region of nucleation and to the F-actin cytoskeleton. *Protoplasma* **124**, 112–122.

HUYSMANS, E., DAMS, E., VANDERBERGE, A. & DE WATCHERR. (1983). The nucleotide sequences of the 5S rRNAs of four mushrooms and their use in studying the phylogenetic position of basidiomycetes among the eukaryotes. *Nucl. Acids Res.* **11**, 2871–2880.

HYAMS, J. S., MARKS, J. & HAGAN, I. M. (1985). The cytoskeleton of *Schizosaccharomyces pombe*. *J. cell. Biochem. Suppl.* **9C**, 108.

KILMARTIN, J. V. & ADAMS, A. E. M. (1984). Structural rearrangements of actin and tubulin during the cell cycle of the yeast *Saccharomyces*. *J. Cell Biol.* **98**, 922–933.

KILMARTIN, J. V., WRIGHT, B. & MILSTEIN, C. (1982). Rat monoclonal antitubulin antibodies derived by using a new nonsecreting rat cell line. *J. Cell Biol.* **93**, 576–582.

KING, S. M. & HYAMS, J. S. (1982). Interdependence of cell cycle events in *Schizosaccharomyces pombe*.

Terminal phenotypes of *cdc* mutants arrested during DNA synthesis and cell division. *Protoplasma* **110**, 54–62.

LEE, M. G. & NURSE, P. (1987). Complementation used to clone a human homologue of the fission yeast cell cycle control gene *cdc 2*. *Nature, Lond.* **327**, 31–36.

MARKS, J., HAGAN, I. M. & HYAMS, J. S. (1986). Growth polarity and cytokinesis in fission yeast: the role of the cytoskeleton. In *The Cytoskeleton: Cell Function and Organization* (ed. C. W. Lloyd, J. S. Hyams & R. M. Warn). *J. Cell Sci. Suppl. 5*, 229–241.

MARKS, J., HAGAN, I. M. & HYAMS, J. S. (1987). Spatial association of F-actin with growth polarity and septation in the fission yeast *Schizosaccharomyces pombe*. In *Spatial Organisation of Eukaryotic Microbes* (ed. R. K. Poole & A. P. Trinci). *Symp. Soc. micro. Biol.* (in press).

MARKS, J. & HYAMS, J. S. (1985). Localization of F-actin through the cell division cycle of *Schizosaccharomyces pombe*. *Eur. J. Cell Biol.* **39**, 27–32.

MCCULLY, E. K. & ROBINOW, C. F. (1971). Mitosis in the fission yeast *Schizosaccharomyces pombe*: A comparative study with light and electron microscopy. *J. Cell Sci.* **9**, 475–507.

MCKERRACHER, L. J. & HEATH, I. B. (1985). Microtubules around migrating nuclei in conventionally-fixed and freeze-substituted cells. *Protoplasma* **125**, 162–172.

MCINTOSH, J. R. (1984). Mechanisms of mitosis. *Trends Biochem. Sci.* **9**, 196–198.

MITCHISON, J. M. (1970). Physiological and cytological methods for *Schizosaccharomyces pombe*. In *Methods in Cell Physiology*, vol. 4 (ed. D. M. Prescott), pp. 131–165. London, New York: Academic Press.

MITCHISON, J. M. (1971). *The Biology of the Cell Cycle*. Cambridge University Press.

MITCHISON, J. M. & NURSE, P. (1985). Growth in cell length in the fission yeast *Schizosaccharomyces pombe*. *J. Cell Sci.* **75**, 357–376.

NASMYTH, K. & NURSE, P. (1981). Cell division cycle mutants altered in DNA replication and mitosis in the fission yeast *Schizosaccharomyces pombe*. *Molec. gen. Genet.* **182**, 119–124.

NEFF, N. F., THOMAS, J. H., GRISOFI, P. & BOTSTEIN, D. (1983). Isolation of the β-tubulin gene from yeast and demonstration of its essential function in vivo. *Cell* **33**, 211–219.

NURSE, P. (1975). Genetic control of cell size at cell division in the fission yeast *Schizosaccharomyces pombe*. *Nature, Lond.* **256**, 547–551.

NURSE, P. (1981). Genetic control of the yeast cell cycle: a reppraisal of 'start'. In *The Fungal Nucleus*, British Mycological Society Symposium 5 (ed. K. Gull & S. G. Oliver), pp. 332–345. Cambridge: Cambridge University Press.

NURSE, P. (1985). Cell cycle control genes in yeast. *Trends Genet.* **2**, 51–55.

NURSE, P., THURIAUX, P. & NASMYTH, K. (1976). Genetic control of the cell division cycle of the fission yeast *Schizosaccharomyces pombe*. *Molec. gen. Genet.* **146**, 167–178.

OAKLEY, B. R. & MORRIS, N. R. (1981). Nuclear movement is β-tubulin dependent in *Aspergillus nidulans*. *Cell* **19**, 255–262.

PRINGLE, J. R., COLEMAN, K., ADAMS, A., LILLIE, S., HAARER, B., JACOBS, C., ROBINSON, J. & EVANS, C. (1984). Cellular morphogenesis in the yeast cell cycle. In *Molecular biology of the cytoskeleton* (ed. G. G. Borisy, D. W. Cleveland & D. B. Murphy), pp. 193–209. New York: Cold Spring Harbor Laboratory.

PRINGLE, J. R. & HARTWELL, L. (1981). The *Saccharomyces cerevisiae* cell cycle. In *The molecular biology of the yeast Saccharomyces* (ed. J. Strathen, E. Jones & J. Broach), pp. 97–142. New York: Cold Spring Harbor Laboratory.

ROBINOW, C. F. (1981). A view through the microscope. In *Current Developments in Yeast Research* (ed. G. G. Stewart & I. Russell), pp. 623–634. London, Canada: Pergamon.

RUNEBERG, P., RAUDASKOSKI, M. & VIRTANEN, I. (1986). Cytoskeletal elements in the hyphae of the homobasidiomycete *Schizophylum commune* visualised with indirect immunofluorescence and NBD-phallacidin. *Eur. J. Cell Biol.* **41**, 25–32.

RUSSELL, P. R. & NURSE, P. (1986). *cdc 25*$^+$ functions as an inducer of mitotic control of fission yeast. *Cell* **45**, 145–153.

RUSSELL, P. R. & NURSE, P. (1987*a*). Negative regulation of mitosis by *wee1*$^+$, a gene encoding a protein kinase homolog. *Cell* **49**, 559–567.

RUSSELL, P. R. & NURSE, P. (1987*b*). The mitotic inducer *nim1*$^+$ functions in a regulatory network of protein kinase homologs controlling the initiation of mitosis. *Cell* **49**, 569–576.

SCHATZ, P. J., PILLUS, L., SOLOMON, F. & BOTSTEIN, D. (1986*a*). Two functional α-tubulin genes from the yeast *Saccharomyces cerevisiae* encode divergent proteins. *Molec. cell. Biol.* **6**, 3711–3721.

SCHATZ, P. J., SOLOMON, F. & BOTSTEIN, D. (1986*b*). Genetically essential and non-essential α-tubulin genes specify functionally interchangeable proteins. *Molec. cell. Biol.* **6**, 3722–3733.

SIMANIS, V. & NURSE, P. (1986). The cell cycle control gene *cdc2*$^+$ of fission yeast encodes a protein kinase potentially regulated by phosphorylation. *Cell* **45**, 261–268.

SIMANIS, V., RUSSELL, P. & NURSE, P. (1986). Cell cycle control of the fission yeast *Schizosaccharomyces pombe*. In *Colloquium Mosbach. Cell Cycle and Oncogenes* (ed. D. Gallwitz & W. Taner), pp. 13–23. Heidelberg: Springer-Verlag.

STREIBLOVA, E. & GIRBART, M. (1980). Microfilaments and cytoplasmic microtubules in cell division cycle mutants of *Schizosaccharomyces pombe*. *Can. J. Microbiol.* **26**, 250–253.

TANAKA, K. & KANBE, T. (1986). Mitosis in fission yeast *Schizosaccharomyces pombe* as revealed by freeze-substitution electron microscopy. *J. Cell Sci.* **80**, 253–268.

THURIAUX, P., NURSE, P. & CARTER, B. (1978). Mutants altered in the control co-ordinating cell division with cell

growth in the fission yeast *Schizosaccharomyces pombe*. *Molec. gen. Genet.* **161**, 215–220.

THYBERG, J. (1984). The microtubular cytoskeleton and the initiation of DNA synthesis. *Expl Cell Res.* **155**, 1–8.

TODA, T., ADACHI, Y., HIRAKOA, Y. & YANAGIDA, M. (1984). Identification of the pleitropic cell division cycle gene *NDA 2* as one of two different α-tubulin genes in *Schizosaccharomyces pombe*. *Cell* **37**, 233–242.

TODA, T., UMESONO, K., HIRAOKA, A. & YANAGIDA, M. (1983). Cold sensitive nuclear division arrest mutants of the fission yeast *Schizosaccharomyces pombe*. *J. molec. Biol.* **168**, 251–270.

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

UEMURA, T. & YANAGIDA, M. (1986). Mitotic spindle pulls but fails to separate chromosomes in type II DNA topoisomerase mutants: uncoordinated mitosis. *EMBO J.* **5**, 1003–1010.

UMESONO, K., TODA, T., HAYASHI, S. & YANAGIDA, M. (1983). Two cell division genes *NDA 2* and *NDA 3* of the fission yeast *Schizosaccharomyces pombe* control microtubular organization and sensitivity to antimitotic benzimidazole compounds. *J. molec. Biol.* **168**, 271–284.

VANDRE, D. D. & BORISY, G. G. (1986). The interphase/metaphase transformation of the microtubule network in mammalian cells. In *Cell Motility: Mechanism and Regulation* (ed. H. Ishikawa, S. Hatano & H. Sato), pp. 389–401. New York: Alan Liss Inc.

WALKER, G. M. (1982). Cell cycle specificity of certain antimicrotubular drugs in *Schizosaccharomyces pombe*. *J. gen. Microbiol.* **128**, 61–71.

WATTS, F. Z., MILLER, D. M. & ORR, E. (1985). Identification of myosin heavy chain in *Saccharomyces cerevisiae*. *Nature, Lond.* **316**, 83–85.

WILLIAMSON, D. H. & FENNELL, D. J. (1975). The use of fluorescent DNA binding agent for detecting and separating yeast mitochondrial DNA. *Meth. Cell Biol.* **12**, 335–351.

YANAGIDA, M. (1987). Yeast tubulin genes. *Micro. Sci.* **4**, 115–118.

YANAGIDA, M., HIRAKOA, Y., UEMURA, T., MIYAKE, S. & HIRANO, T. (1985). Control mechanisms of chromosome movement in mitosis of fission yeast. In *Yeast Cell Biology, UCLA Symposium*, vol. 33 (ed. J. Hicks), pp. 279–301. New York: Alan Liss.

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