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COMMENTARY

The fission yeast microtubule cytoskeleton

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SUMMARY

The *Schizosaccharomyces pombe* genome sequencing project (http://www.sanger.ac.uk/Projects/S_pombe/) is nearly complete, and this is likely to generate interest in fission yeast as a model system beyond its traditional strongholds in the study of the cell cycle and sexual differentiation. In many fields *S. pombe* will offer a useful complement to the more widely studied *Saccharomyces cerevisiae*, but in some areas the impact of *S. pombe* may well rival or exceed that of this budding yeast in terms of relevance to higher systems. Because of the considerable differences from the *S. cerevisiae* microtubule cytoskeleton, studying microtubules in *S. pombe* is likely to enhance the contribution of model systems to our understanding of the principles and practices of microtubule organisation in eukaryotes in general.

Key words: Microtubule, SPB, *Schizosaccharomyces pombe*, Cell cycle, Mitosis

THE FISSION YEASTS

The order *Schizosaccharomycetales* currently contains six members; two varieties of *S. pombe* (*var. malidevorans* and *var. pombe*), three varieties of *S. japonicus* (*var. versitalis*, *var. longobardus* and *var. japonicus*) and *S. octosporus* (Sipiczki, 1995). Of these *S. pombe var. pombe* is the organism that is commonly referred to as fission yeast. Whilst the spotlight currently rests on this fission yeast, the study of the other members of the order is likely to assume increasing importance as the lessons learnt from morphogenesis in *S. pombe* are applied to the longer range problems presented by hyphal growth and the switching processes that accompany dimorphic transitions in its cousins. Alfa and Hyams (1990) have already shown that the cytoskeleton of *S. japonicus* closely resembles that of *S. pombe* and yet is much more amenable to cytological analysis.

THE *S. pombe* MICROTUBULE CYTOSKELETON

As with all eukaryotes, fission yeast microtubules are polymers of α and β-tubulin. The related molecule, γ-tubulin, is found at microtubule organising centres where it acts as a nucleating template for the polymerisation of microtubules (Pereira and Schiebel, 1997). To date four tubulin genes have been identified in fission yeast: two α (*nda2*$^+$, *atb2*$^+$), one β (*nda3*$^+$) and one γ (*gtb1*$^+$/*tug1*$^+$) (Yanagida, 1987; Horio et al., 1991; Stearns et al., 1991). Genetics, western blotting and DNA hybridisation approaches suggest that no more α- or β-tubulins will be found (Fig. 1). Transcription of one of the two α genes, *nda2*$^+$, is up-regulated in response to the reduction in α-tubulin levels that results from deletion of *atb2*$^+$ (Adachi et al., 1986). α- and β-tubulins are assembled into the functional heterodimeric complex by a conserved pathway (Hirata et al., 1998).

Microtubules were first recorded by McCully and Robinow (1971) who used a chemical fixation to prepare samples for electron microscopy. Subsequent studies using high pressure freezing and freeze substitution (McDonald et al., 1996) have extended these observations to generate a comprehensive view of spindle architecture. Cytoplasmic microtubules have been most successfully documented by immunofluorescence microscopy (Hagan and Hyams, 1988). A combined analysis of data generated by these complementary techniques has been used to form the current view of the microtubule cytoskeleton shown in Fig. 2 (McCully and Robinow, 1971; Hereward, 1974; Streiblova and Girbardt, 1980; King and Hyams, 1982; Hiraoka et al., 1984; Tanaka and Kanbe, 1986; Kanbe et al., 1989; Horio et al., 1991; Ding et al., 1993, 1997; Hagan and Yanagida, 1995; Hagan and Yanagida, 1997). This depiction of the cytoskeleton should be treated with the caution that befits all cytological studies because different fixation procedures give different artifacts. Interpretation is likely to be modified by technical advances such as the application of real time imaging of microtubules with fusions of tubulins and MAPs to green fluorescent protein (GFP) (Nabeshima et al., 1995, 1997; Ding et al., 1998).

During interphase microtubules extend along the long axis of the cell (Fig. 2). Upon commitment to mitosis a small dot of tubulin staining is associated with the nucleus in the presence of an otherwise intact cytoskeleton. The dot lies between two

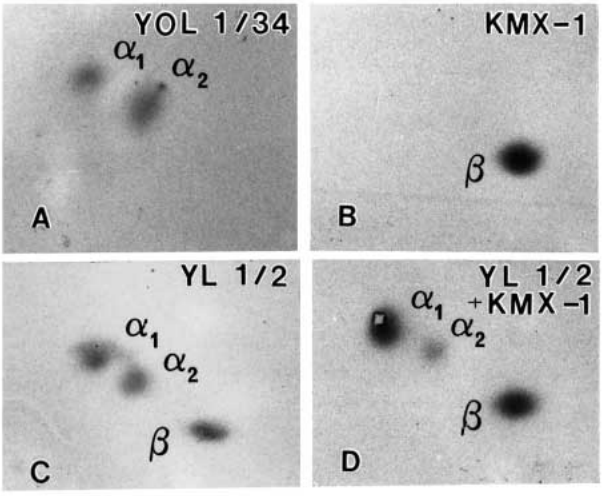

Fig. 1. Anti-tubulin western blot analysis of fission yeast protein extracts following two dimensional electrophoresis. Isoelectrical focusing was performed using the appropriate ampholines to establish a pH gradient from 6-4 across the entire tube gel. Following separation on a 7% second dimension gel proteins were transferred to nitrocellulose and blotted with: (a) the anti-α-tubulin antibody YOL1/34 (Kilmartin et al., 1982), (b) the anti-β-tubulin antibody KMX-1 (Birkett et al., 1985), (c) the anti-tubulin antibody YL1/2 (Kilmartin et al., 1982), and (d) YL1/2 + KMX-1. The two spots recognised by YOL1/34 are in similar positions to two of the spots recognised by YL1/2. Similarly one of the spots recognised by YL/1/2 is recognised by the KMX-1 antibody. The mixing experiment in d confirms that YL1/2 recognises both α- and β-tubulins whilst YOL1/34 recognises the two α-tubulins and KMX-1 the single β-tubulin. This is consistent with the sequence recognition of YL1/2 and the amino acid sequence of *S. pombe* β-tubulin (Wheland et al., 1984; Yanagida 1987). The two α-tubulin isotypes are identified by comparison with the data of Adachi et al. (1986).

microtubule organising centres (MTOCs), called spindle pole bodies (SPBs), and is rapidly replaced by a short bar which extends to produce the prophase spindle as the cytoplasmic microtubules disappear (Hagan and Yanagida, 1995). As this spindle elongates to span the nucleus the chromosomes undergo the migrations typical of eukaryotic mitoses (Funabiki et al., 1993; Ekwall et al., 1995; Saitoh et al., 1997) culminating in the formation of a metaphase plate. Ding et al. (1993) have noted electron dense patches in the metaphase spindle. Because the patches coincide with the ends of two to three microtubules they have suggested that they represent kinetochores. Immunofluorescence images consistently show that the two ends of the metaphase spindle are often much thicker than the middle, presumably because kinetochore fibres contribute to the signal at either end whereas the signal in the middle arises solely from the pole to pole microtubules of this simple linear spindle (Hiraoka et al., 1984; Hagan and Hyams, 1988; Funabiki et al., 1993). Cytoplasmic microtubules which have been termed astral microtubules extend tangentially from the cytoplasmic faces of the SPBs at the ends of the metaphase or early anaphase spindle (Hagan and Hyams, 1996). In all cases in which they have been recorded to date astral arrays are composed of bundles of multiple microtubules (Tanaka and Kanbe, 1986; Ding et al., 1997).

S. pombe is somewhat unusual in initiating anaphase B slightly before anaphase A (Ding et al., 1993). Once the nuclei have been separated towards the cell ends, in an extended anaphase B, the spindle breaks down and microtubules appear in the middle of the cell in a pattern that is called the post-anaphase array (PAA) (Hagan and Hyams, 1988). This was originally interpreted as microtubule nucleation from two discrete microtubule organising centres. Consistently, electron microscopy showed several individual microtubules extending from an amorphous mass in late mitotic cells (Kanbe et al., 1989; Horio et al., 1991). However, a combination of a novel immunofluorescence procedure and confocal microscopy has more recently described a different pattern as the early PAA appeared as a ring of tubulin staining (Pichova et al., 1995). Similar rings have now been seen with microtubule associating GFP fusion proteins suggesting that the microtubules of the PAA extend from a ring (Nabeshima et al., 1995; Beinhauer et al., 1997). Although the PAA is likely to be a ring, the electron micrographs of Kanbe et al. (1989) and Horio et al. (1981) suggest that microtubules are released to form aster like arrays. These latter arrays may arise by a similar motor driven mechanism that organises the poles of mitotic spindle in higher eukaryotes (Merdes and Cleveland, 1997).

The co-localisation of the PAA with the cytokinetic ring suggests that the PAA plays some role in cytokinesis or that components of the cytokinetic ring are being used as an anchor for the PAA to perform some other function. The ability to complete cytokinesis before the PAA forms in *wee1.50* cells has been used to argue for a function independent of cytokinesis itself (Hagan and Hyams, 1988), however, concrete conclusions will only be possible when more appropriate or specific reagents become available (for example, mutations which affect the microtubule but not the actin components). If the PAA does not play a role in cytokinesis, it is quite possible that it is required to re-establish the microtubule cytoskeleton quickly to ensure that nuclear positioning mechanisms enable the nuclei to quickly move away from the septum and so avoid a lethal cut event (Hirano et al., 1986; Samejima et al., 1994; see below). Nucleating microtubules at the site of maximum danger ensures the greatest opportunity to move to the nuclei to the safety of the centre of the daughter cells. Interestingly, the presence of a cytoplasmic MTOC is not causally linked to spindle dissolution because microtubules are not initiated from a discrete organising centre after meiosis I, rather they apparently polymerise at random (Hagan and Yanagida, 1997). Whether this is because no cytokinetic ring forms following the dissolution of this spindle or because there is no need for accurate nuclear positioning following meiosis is not yet clear.

Enhanced microtubule polymerisation from the SPB accompanies the appearance of the PAA (Hagan and Yanagida, 1997). Since these microtubules can extend the full length of the cell and do not distort the nuclear envelope it is highly likely that they are in the cytoplasm. The organisation of these microtubules is different from that of the astral microtubules seen during anaphase. They are not tangential to the SPB, they stain more weakly and are more numerous.

It is not yet clear whether the activation of all cytoplasmic microtubule nucleation centres (the PAA and SPB) at the end of mitosis represents a positive commitment to activate an interphase cytoskeleton, or whether an active interphase microtubule MTOC is a ‘ground state’ which must be turned off to form the mitotic spindle.

The interphase microtubule cytoskeleton

As in higher eukaryotes, the interphase microtubule cytoskeleton is required for the correct execution of multiple processes. The correct distribution of nuclei and mitochondria, the maintenance of cell polarity and the integrity of the Golgi stacks all depend upon microtubules (Hirano et al., 1988; Robinow and Hyams, 1989; Ayscough et al., 1993; Yaffe et al., 1996; Hagan and Yanagida, 1997). This implies that there is likely to be a requirement for a broad range of microtubule motor proteins in fission yeast and that the number of fission yeast kinesin related proteins may well exceed that of budding yeast where kinesin related proteins are concerned primarily with genome movements.

MICROTUBULES AND MORPHOGENESIS

Studies which used microtubule inhibitors or mutations in tubulin genes showed that cells became bent and branched when their microtubules were disrupted. However, it was not clear in these original studies whether the altered cell morphology arose from a direct role for microtubules in morphogenesis, per se, or as a secondary consequence of the excessive mitotic delay resulting from these treatments. Concrete evidence for a direct role for microtubules in fission yeast morphogenesis came much later with the isolation of morphogenetic mutants which have defective interphase microtubule cytoskeletons but do not show an appreciable delay in mitosis (Beinhauer et al., 1997; Mata and Nurse, 1997; Verde et al., 1995; Hirata et al., 1998). The proteins encoded by two of these genes, Tea1 and Mal3, associate with microtubules (Beinhauer et al., 1997; Mata and Nurse, 1997). Moreover, cells can only tolerate elevated Mal3 levels in the presence of microtubule stabilising drugs. Together these data strongly support a key role for microtubules in cell morphogenesis.

Three types of morphogenetic defect arise from defects in microtubule related molecules: branching, curving and bending of the usually linear rod shape at one point (Fig. 3). The different morphologies can best be understood in the context of discussion of the distribution of the Tea1 molecule (Mata and Nurse, 1997).

Strains in which *tea1*$^+$ is mutated or deleted are viable and grow as bent, curved or tripolar cells. Tea1 protein is found at the cell tips and tip association is rapidly lost when interphase microtubules are disrupted. If microtubules are subsequently allowed to re-grow, Tea1 is seen at one end of the re-growing interphase cytoplasmic microtubules. Mata and Nurse therefore proposed that the microtubule dependent localisation of Tea1 to the cell tips is required to maintain linear cell extension. In other words, tip localisation of Tea1 directs actin based growth at the ends of the cell. Because microtubules essentially end randomly beneath the cell tip and Tea1 is transported to the zone beneath the tip by microtubules, Tea1 is evenly distributed beneath the tip. This results in an even extension of all parts of the tip. There is likely to be some fine tuning of Tea1 delivery by the molecule itself. Rather than ending just short of the tip as normal, microtubules curve around the tip when Tea1 is absent. This suggests that a Tea1-related process regulates microtubule polymerisation at the cell tip. Interestingly, a similar bending of microtubules around the growth tip has been reported in budding yeast when the gene encoding dynein is deleted, suggesting that microtubule dynamics and interactions with the cortex are influenced by mutations in fungal microtubule motor proteins (Carminati and Stearns, 1997). It remains to be determined whether there is any connection between Tea1 and motor protein function.

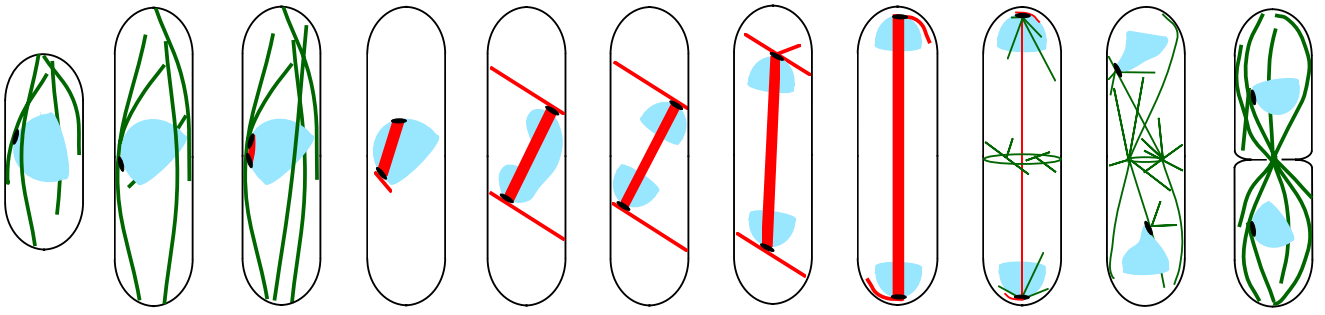

Fig. 2. Changes in microtubule distribution during cell cycle progression in *S. pombe*. Interphase microtubules (green), mitotic microtubules (red), chromatin (blue) and SPBs (black ovals) are all represented. During interphase microtubules are entirely cytoplasmic and form a basket of 6-8 microtubules which extends along the long axis of the cell. These microtubules do not usually all end at the same point. In late G_2 the SPB duplicates. Upon commitment to mitosis the duplicated SPBs nucleate microtubules which interdigitate to form a short bipolar spindle in prophase. The interphase array is still intact at this point indicating either that the depolymerisation of the interphase microtubules takes some time or that the signal to turn off the interphase cytoskeleton comes after that to form the spindle. As the spindle elongates to span the nucleus at metaphase, microtubules extend tangentially from the SPB into the cytoplasm. Whilst a typical spindle has only one astral microtubule bundle per SPB, two or even three are occasionally seen on anaphase B spindles and many astral bundles are seen associated with the spindles of some mutants such as *cdc25.22* and *nuc2.663* at their restrictive temperature. Once the nuclei reach the ends of the cell microtubules extend from the cell equator. These microtubules are nucleated from a ring of microtubule nucleating activity at the cell equator (see text). From electron micrographs it is likely that the tubulin staining filaments of the PAA are single microtubules, whereas those seen at other stages may represent bundles of microtubules. This difference is represented diagrammatically by the altered thickness of the green lines at different stages. Coincident with spindle breakdown, several microtubules are nucleated from the outer face of the SPB and the nuclei are subsequently led back towards the cell centre by their SPBs. When they reach the points that will become the middle of the daughter cells migration stops and they adopt a typical hemispherical morphology once more.

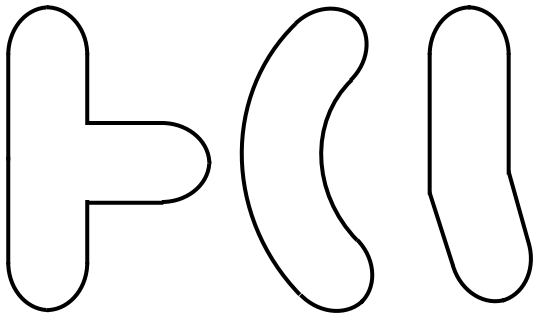

Fig. 3. Cell morphology changes upon disruption of the microtubule function. In contrast to the rod shape assumed by normal *S. pombe* cells, treatment with microtubule destabilising drugs or mutating genes required for microtubule function results in branched, curved and bent cells.

A number of simple predictions arise from the model of Mata and Nurse (1997). First, if Tea1 cannot get to the tips because microtubules are too short, the cell should be unable to maintain linear growth: this prediction is borne out by *tea2* mutants and *mal3* deletants (Beinhauer et al., 1997; Verde et al., 1995; Mata and Nurse, 1997). In these cells a basket of short microtubules surrounds the nucleus in bent and T shaped cells. Secondly, if Tea1 protein is only delivered to one part of a tip rather than randomly underneath the entire tip, the tip will be more likely to grow at the site of delivery, resulting in bending of the cell: again the prediction is borne out. In *ban3.2* and *ban4.81* mutants interphase microtubules apparently form a thick bundle on one side of the cell and the cell bends away from this bundle (Verde et al., 1995) (Fig. 4).

The tea phenotype of cells lacking *tea*1 is less severe at lower temperatures. This suggests that Tea1 is either only required at high temperatures or other factors substitute for Tea1 function at lower temperatures, or Tea1 is part of a larger complex.

Mechanistically, it is not clear at present how Tea1 influences the polarity of the actin cytoskeleton, but clues may come from analyses of the curved mutant *ben4* (Roy and Fantes, 1982). The *ben4.C10* mutation confers both resistance to the microtubule de-polymerising drug and renders the cell very sensitive to actin gene dosage (Fantes, 1989). These data suggest that Ben4 is involved in the organisation or co-ordination of both cytoskeletons.

Tea1 as a marker of microtubule polarity?

The localisation of Tea1 to one end of the microtubules has important implications and deserves discussion in its own right. Microtubules are polar structures with a dynamic plus end and a less dynamic minus end. The localisation of Tea1 to one end of a tubulin staining filament in vivo suggests that it is either recognising a structural difference at one end of the microtubule or using microtubule motor proteins to move to the end. If the former, the GTP cap at the plus end is a prime candidate for the recognition motif (Hyman and Karsenti, 1996). Tea1 would then be behaving in a similar manner to the budding yeast kinetochore complex which binds preferentially to GTP caps (Severin et al., 1997).

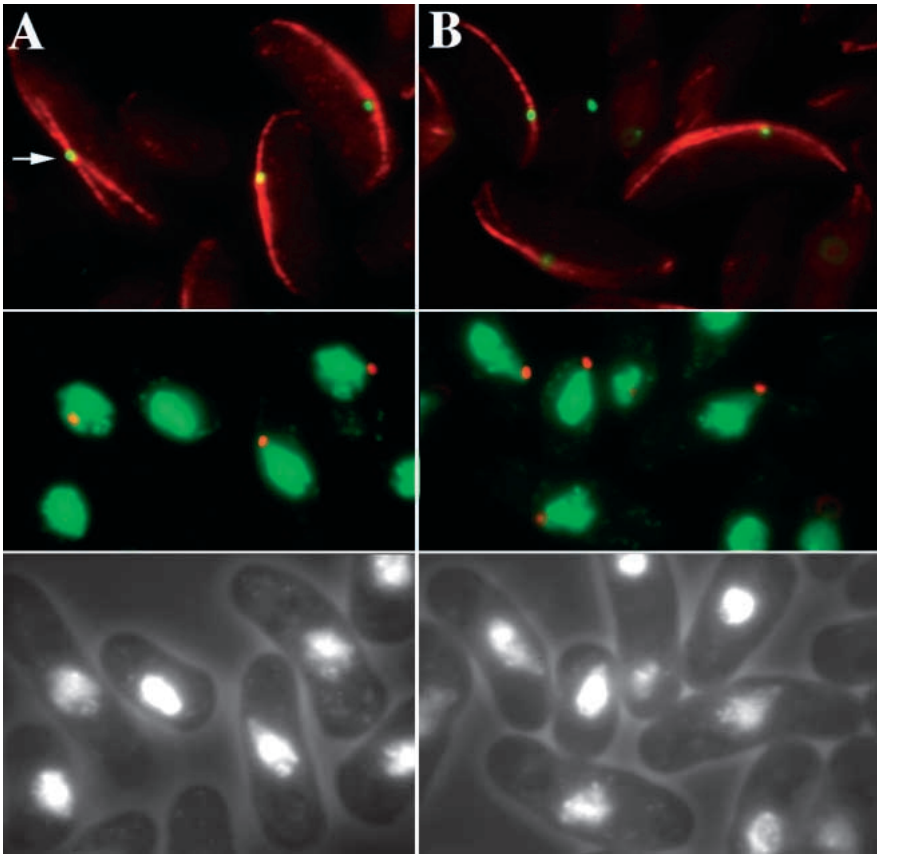

Fig. 4. The SPB is associated with a bundle of microtubules which extend along one side of *ban3.2* cells. The Fig. shows two different fields of *ban3.2* cells which have been incubated at 36°C for 7 hours before being processed for immunofluorescence microscopy. The upper panel shows TAT1 (Woods et al., 1989) anti-tubulin staining of microtubules (red) and Sad1 staining (green) to reveal the SPB; the middle panel DAPI (green) and Sad1 (red) staining and the lower panel shows a DAPI/phase contrast image to show the relative location of the nuclei. The majority of the microtubules in these cells are SPB associated and form a bundle that extends along the outer curve of these cells. Microtubules can often be seen extending from the SPB (e.g. arrow in A). These images are consistent with an inability to release microtubules from the SPB following nucleation or in the ability of cortical motor proteins to pull away the microtubules once they have been nucleated by the SPB (see text).

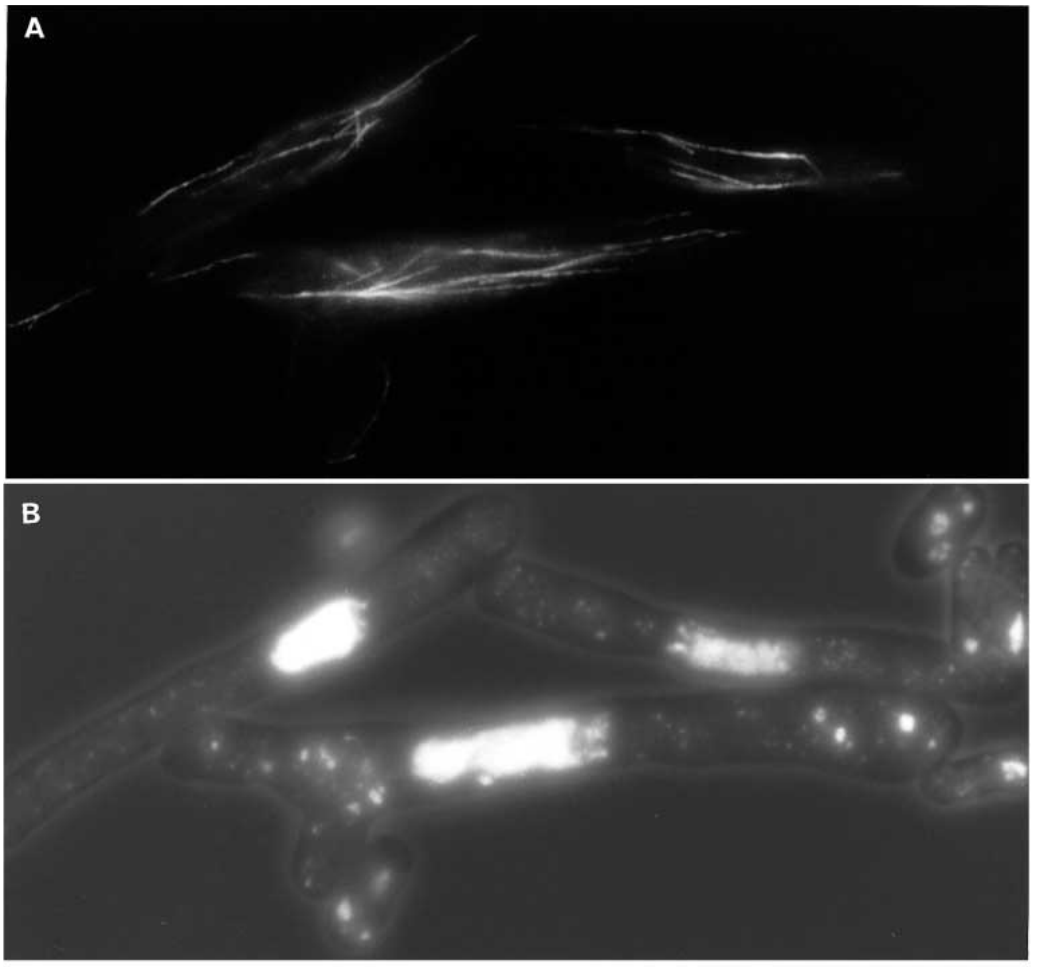

Fig. 5. Cell tips are devoid of microtubules in a strain overexpressing *rum1*$^+$. Strain IHSP447 *(*::*nmt1rum1+ sup3.5 ura4.d18 leu1.32 ade6.704 h+;* Moreno and Nurse 1994) was grown at 33°C and transcription of the *rum1*$^+$ gene was induced for 22 hours. Cells were processed for immunofluorescence microscopy and stained with TAT1 anti-tubulin (top) and DAPI (bottom – in combination with a phase contrast image). Unlike wild-type cells in which the microtubules extend as far at the cell tips, in cells overexpressing the *rum1*$^+$ gene the microtubules terminate a long way from the tip which makes microtubule nucleation from the tip an unlikely possibility. Also note the aster of microtubules extending from the enlarged nucleus.

Some complications to such simple interpretations are raised by the limited electron microscopic images of cytoplasmic microtubules. In several cases the cytoplasmic microtubules recorded were not individual microtubules, but bundles (Streiblova and Girbardt, 1980; Tanaka and Kanbe, 1986). Clearly further analysis needs to be done on microtubules re-growing after cold shock to determine whether they are single microtubules or bundles. If they are bundles, all of the microtubules within a bundle must be in the same orientation if Tea1 is only found at the tip, unless Tea1 is only able to associate with one of the microtubules in each bundle. (Motor movement along microtubules can be processive along a single protofilament and so would not be in conflict with association with just one microtubule in a bundle.)

On balance, given that Tea1 needs to be delivered to the tip, it seems most likely that Tea1 will be at the dynamic plus end of the microtubule rather than at the less dynamic minus end which is less likely to reach the tip before the microtubule disappears.

Where do interphase cytoplasmic microtubules come from?

Mitotic microtubules are nucleated by the SPB. It has been generally assumed that because asters are not seen extending from the interphase SPB, the SPB is inactive in interphase and that microtubules are nucleated elsewhere. An attractive possibility was raised by data from the study of a filamentous fungus *Uromyces phaseoli* which has an interphase array similar to that of *S. pombe* (Hoch and Staples, 1985). Following depolymerisation by drug treatment, interphase microtubules grew back from the apical tip upon drug wash out. However, similar approaches in fission yeast indicate that microtubules grow back from around the nucleus (Mata and Nurse, 1997). The fact that a lot of the microtubules focus around a spot which is recognised by SPB markers (J. Petersen, J. Heitz and I. M. Hagan, unpublished; J. Cope and J. S. Hyams, personal communication) suggests that the SPB is nucleating many of these microtubules. When cells are forced to undergo multiple rounds of S phase due to the overexpression of the maturation promoting factor (MPF) inhibitor Rum1 the cells become extremely long and microtubules rarely reach the cell tips (Fig. 5) (Moreno and Nurse, 1994). Similarly microtubules are not found near the tips of *mal3* deletants or *tea2* mutants. Finally, strong evidence supporting interphase microtubule nucleation from the SPB is provided by the finding that there are ends of cytoplasmic microtubules at the cytoplasmic face of the SPB during interphase (Ding et al., 1997).

Microtubule nucleation and release from the SPB?

If the SPB does nucleate interphase cytoplasmic microtubules, why are the vast majority not SPB associated? One possible explanation is that the microtubules are released after

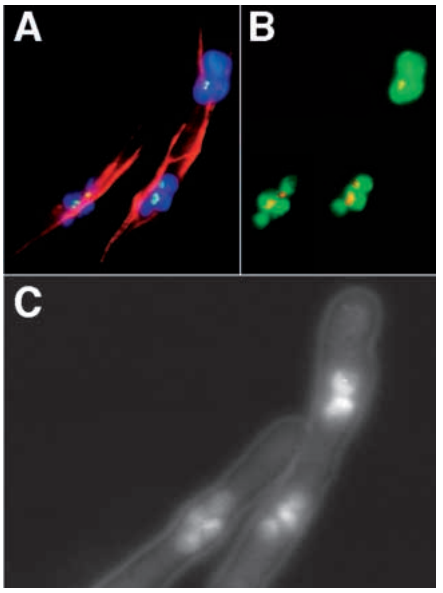

Fig. 6. The role of the SPB in positioning the nucleus is revealed in septation mutants in which multiple nuclei accumulate in single cells. The Fig. shows two *cdc 7.A20* cells that have been growing at the restrictive temperature of 36°C for 6 hours. All panels show different images of the same cells. In A microtubules are shown in red, nuclei in blue and SPBs in green, whilst in B the nuclei are green and the SPBs red. (C) DAPI/phase contrast images of the same cells to show the relative positions of the nuclei and the cell perimeter. The cell on the left has undergone two nuclear divisions without an intervening cytokinesis whilst three divisions have occurred in the right-hand cell. The central clustering of all four nuclei in the left-hand cell is typical of interphase cells with four nuclei. In all cases the SPBs are most central in the cluster which is consistent with them being at or very close to the part of the nucleus which plays a key role in directing the clustering. The two groups of clusters in the left-hand cell is typical of cells after the third nuclear division when the normal central positioning seen in the right-hand cell appears to breakdown and be replaced with a phasing of the nuclei along the cell. For further details see Hagan and Yanagida (1997).

nucleation. Release of nucleated microtubules by MTOCs has been reported in several other systems (for example: Kitanishi-Yumura and Fukui, 1987; Belmont et al., 1990; Keating et al., 1997) and the microtubule severing protein katanin is MTOC associated in higher eukaryotes suggesting that it may sever microtubules at the pole (McNally et al., 1996). If nucleation release does occur, one might expect to see asters emanating from the region around the SPB as the microtubules extend from this point. This would, in fact, only be the case if the microtubules were static upon release. Were they to be rapidly pulled away from the SPB by microtubule motor proteins lining the cortex, an aster would never form. If this were the case, abolishing this motor function might result in one large bundle of microtubules through the action of the bundling activity which normally bundles limited numbers of cytoplasmic microtubules. A large bundle would similarly be produced if release of the microtubules from the SPB was defective because, again, the bundling activity would group the many microtubules into one mass. The behaviour of two mutants, *ban3.2* and *ban4.81*, support these models. Both mutations result in the majority of microtubules forming a single thick bundle of SPB associated microtubules (Verde et al., 1995) (Fig. 4). Comparison with other fungal systems may be warranted here as the cell curvature seen in these mutants is also found in many mutants in *Neurospora crassa* which are defective in different components of the microtubule motor complex dynein (Plamann et al., 1994). Moreover, Num1p which is likely to interact with dynactin/dynein is localised to the cortex in budding yeast (Farkasovsky and Kuntzel, 1995) and the movement of microtubules along the cortex is severely impaired in dynein mutants in budding yeast (Carminati and Stearns, 1997). Thus it is possible that a similar relationship between the dynamics of cytoplasmic microtubules and the cortex exists in fission yeast.

SPBs, microtubules and the central position of the nucleus

Time-lapse microscopy of fission yeast shows that the nucleus undergoes a continual rocking motion rotating first one way and then back again (Hagan et al., 1990). The use of an SPB marker in a number of different genetic backgrounds suggests that the rocking is due to the SPB moving in a stochastic fashion backwards and forwards along cytoplasmic microtubules (Hagan and Yanagida, 1997). It is assumed that the SPB responds to positioning signals that direct its location. It is likely that SPB bound motor proteins are responding to some signals which indicate the central position of the cell in much the same way that the forces produced by opposing motor proteins is postulated to direct the location of the kinetochore to the metaphase plate (Skibbens et al., 1993). The positioning mechanism locates the interphase nucleus to the centre of the cell and the post-mitotic nuclei to the two regions that will become the middle of the daughter cells. The intricacies of these movements are graphically illustrated in mutants which are unable to execute cytokinesis and so accumulate multiple nuclei in a single cytoplasm. The multiple SPBs on these multiple nuclei all cluster in discrete locations indicating that they are all responding to the same positioning signals which shows that similar controls must be acting in wild-type cells (Fig. 6). The molecular basis of nuclear positioning is currently unclear although it is disrupted in some *ban* mutants (Fig. 4; Verde et al., 1995).

MITOSIS

Duplication of the SPB and spindle formation

Unlike the SPB in *S. cerevisiae* which is embedded in the nuclear envelope throughout the cell cycle, the main body of the fission yeast SPB is on the outside of the interphase nuclear envelope (Ding et al., 1997). However, there is a raft of material on the nucleoplasmic side of the nuclear envelope directly beneath and connected to the main body of the SPB (Ding et al., 1997). This structure contains γ-tubulin and presumably the determinants responsible for binding the heterochromatin to the SPB. The centromeres are bound to the SPB throughout interphase and detach upon commitment to mitosis (Funabiki et al., 1993). This association ensures that the chromatin will follow the SPB as it is this organelle that is centrally located throughout interphase.

The size of the single interphase SPB increases before it

divides to produce two smaller SPBs (Ding et al., 1997). Upon commitment to mitosis the nuclear membrane underneath the fission yeast SPB pulls away and dense osmophillic material accumulates in the space between the fracturing membrane and the SPBs. The two SPBs drop into the gap and connect with the γ-tubulin-containing material in the nucleoplasm. The SPB remains within in the nuclear membrane until anaphase when the size of the hole in the envelope reduces as anaphase progresses, until the SPB is once again on the outside of a sealed nuclear envelope at the end of mitosis.

Unlike our in depth knowledge of budding yeast SPB structure composition and duplication (Hagan et al., 1998) very little is known about the molecular composition of the fission yeast SPB. Several molecules have been localised to the SPB by fluorescence microscopy and additional data suggest that some are bona fide SPB components (Horio et al., 1991; Hagan and Yanagida, 1995; Nabeshima et al., 1995; Moser et al., 1997; Sohrmann et al., 1998; Bridge et al., 1998) and the Plo1 protein kinase is a good candidate for a regulator of pole separation during mitosis (Ohkura et al., 1995).

Astral microtubules and anaphase movement

Astral microtubules first appear during prometaphase as the spindle elongates to span the nucleus, but they are most prominent during anaphase B. Tea1 is found at only one end of these microtubule bundles which would suggest that there is some aspect of polarity to these microtubules, probably that they all have the same orientation (Mata and Nurse, 1997). Studies in other fungi show that astral microtubules can provide all the force necessary for genome segregation (Aist and Berns, 1981). The timing of appearance, polarity and the dependence of spindle bending upon the orientation of astral bundles (Hagan and Hyams, 1996) suggest a similar role for astral microtubules in *S. pombe*. Two models have therefore been proposed for astral microtubule function in anaphase B in *S. pombe*. In the first, astral microtubules provide a track for the SPB to slide along (Hagan and Hyams, 1996). A second possibility is that astral microtubules may interact with motor proteins lining the cortex in much the same way as demonstrated for budding yeast (Carminati and Stearns 1997; Shaw et al., 1997).

Mitotic spindle checkpoint controls

In wild-type fission yeast the anaphase spindle generally elongates until the nuclei reach the ends of the cell; however, if cells are genetically manipulated to divide at an increased cell size the spindle breaks down before the nuclei reach the tips (Hagan et al., 1990). This shows that the signal for spindle dissolution is not triggered by contact of the SPBs with the cell ends. As cell size increases the duration of anaphase B is extended such that a 60 μm cell can have a 30 μm spindle which greatly exceeds the length of a wild-type cell grown under similar conditions (14 μm). Significantly, when cells are made to execute mitosis at a reduced cell size the duration of anaphase B remains unaltered, despite the fact that the nuclei reach the ends of the cell before they normally would showing that anaphase B can be extended but not abbreviated.

The identification of fission yeast homologues of the budding yeast mitotic checkpoint control genes and interacting proteins may provide greater insight into the molecular basis for the regulation of mitotic periods (He et al., 1997; Kim et al., 1998). Intriguingly, one such molecule, encoded by the *cdc16*$^{+}$ gene (Minet et al., 1981; Fankhauser et al., 1993), is a fission yeast homologue of the BUB2 gene product which is required to delay mitosis in response to mitotic defects (Hoyt et al., 1991). Both molecules exhibit significant similarity to GAP proteins which activate small GTP proteins of the ras family (Schmidt et al., 1997). Curiously, whilst Cdc16 mimics Bub2p in being required to maintain high p34^{cdc2} kinase activity when microtubules have been de-polymerised, it was identified as a recessive mutation which resulted in deregulated septation (Minet et al., 1981). A large body of genetic data is consistent with an involvement of Cdc16 in a regulatory cascade that regulates cytokinesis through the activity of a small G protein of the ras superfamily called Spg1 (Schmidt et al., 1997). Spg1 activates a pathway which involves the protein kinase Cdc7 (Fankhauser and Simanis, 1994). Recessive mutations in *cdc7* or *spg1* result in an inability to septate, whilst gain of function mutants produce multiple septa. Spg1 binds Cdc7 when the G protein is activated (Sohrmann et al., 1998). Intriguingly, Spg1 is associated with the SPB throughout the cell cycle and is activated on both SPBs upon commitment to mitosis. As cells progress through anaphase B Cdc7 and active Spg1 are only found on one of the two SPBs. Genetic manipulations which force cytokinesis at inappropriate stages of the cell cycle result in Spg1 activation on both SPBs irrespective of cell cycle state which suggests that the monopolar activation of Spg1 in wild-type cells is functionally significant. Thus, Cdc7 localisation to one of the two SPBs is likely to be a key event in the regulation of cytokinesis.

This localisation pattern was unpredicted and changes the way in which one must view the co-ordination of mitosis and cytokinesis. Significantly, there are Spg1 and Cdc7 homologues in budding yeast (Tem1p and Cdc15p, respectively; Shirayama et al., 1994; Schweitzer and Philippsen, 1991). These molecules are not required for septation but for the correct exit from mitosis and dissolution of the mitotic spindle. Cdc15p can partially substitute for Cdc7p function (Fankhauser and Simanis, 1994). It is possible that the two pathways are the same in the different organisms but the end point is different because of underlying differences between these evolutionarily diverse yeast. Nuclear positioning in fission yeast ensures that it is safe to dissolve the spindle even if the synchrony between cytokinesis and spindle dissolution is slightly out of phase. In contrast, in budding yeast the nuclei would re-locate to the bud neck upon spindle dissolution and thus be randomly cleaved by the cytokinesis machinery if the spindle dissolved too early. Therefore, both processes must be held in check in budding yeast.

Why localise regulators to the SPB?

In addition to the regulators of the cytokinesis machinery other regulatory molecules associate with the fission yeast SPB. MPF is found at the SPB until early anaphase B (Alfa et al., 1990), which is around the time that Cdc7 staining switches to being monopolar. The Dis1 protein, which binds to microtubules and the SPB and genetically interacts with the protein phosphatase Dis2, also associates with the SPB (Nabeshima et al., 1995). In other systems protein phosphatases and protein kinases such as IAK1 associate with the pole as do components of the APC cyclosome (Brewis et

al., 1993; Mirabito and Morris, 1993; Golsteyn et al., 1995; Gopalan et al., 1997; Rhogi et al., 1998).

The localisation pattern of Cdc7 to the SPB suggests that localisation itself plays a key role in its regulatory function. Furthermore, key aspects of MPF regulation or cell cycle progression may be co-ordinated at the SPB as mutation of the SPB component Cut12 can bypass a requirement for normal activators of MPF (Bridge et al., 1998). The concentration of multiple key cell cycle regulatory switches in one place would ensure the co-ordination of different pathways and would limit the number of molecules of each component that are required. It is unclear why active Spg1 is only found on one pole but parallels exist in higher systems (Lange and Gull, 1995) and a monopolar distribution would certainly reduce errors that could arise were the different poles to emit contradictory signals.

Regulation of microtubule function

Tubulin is subject to a number of post-translational modifications in many systems. These include tyrosinylation, acetylation, glutamylation and phosphorylation. Some of these are correlated with altered microtubule behaviour.

The tyrosinylation, de-tyrosinylation cycle was sought in fission yeast through the use of antibodies that specifically recognise different isoforms, but no changes were detected (Alfa and Hyams, 1991). There is, however, evidence that phosphorylation regulates microtubule behaviour. Strains in which the *paa1*$^+$ gene, which encodes the regulatory sub-unit of type 2A protein phosphatase, has been deleted contain only spots of tubulin staining around their nuclei suggesting that microtubule polymerisation is virtually inhibited in these cells (Kinoshita et al., 1997). It is not clear whether this reflects altered phosphorylation of tubulin itself, a microtubule associated protein (MAP) or a component of the MTOC, although data from other systems suggest it is more likely to be a MAP. Microtubule morphology is unaffected by overexpression of *ppb1*$^+$ which encodes a protein phosphatase B but nuclear positioning is disrupted (Yoshida et al., 1994). The SPB pulls the nucleus away from the centre indicating that microtubule dynamics or microtubule motor proteins may normally be regulated by PPB1.

It is not clear how mitotic microtubule dynamics are regulated, but it is noteworthy that the microtubules in Sad1 and Cut7 mutants which are defective in spindle formation are no longer than those of metaphase spindles (Hagan and Yanagida, 1990; Hagan and Yanagida, 1995). This suggests that there is a stage dependent regulation of microtubule dynamics in *S. pombe* mitosis because the microtubules of the anaphase spindle are longer than at metaphase (Ding et al., 1993). Consistent with this idea, in some novel spindle formation mutants we see monopolar spindles with much longer microtubules (A. M. Poziemba and I. M. Hagan, unpublished). We assume that the dynamics of these microtubules are in a state reminiscent of anaphase B.

SEXUAL DIFFERENTIATION AND THE MICROTUBULE CYTOSKELETON

Starvation in the presence of mating pheromones results in the conjugation of cells of opposite mating type. The nuclei in the mating partners migrate towards one another along microtubules and their SPBs fuse. A series of nuclear migrations follows during which all of the microtubules extend from the SPB (Chikashige et al., 1994; Hagan and Yanagida, 1995; Svoboda et al., 1995). Microtubule patterns in both fixed and living cells and the effect of anti-microtubule drug treatments upon nuclear migration indicate that microtubule generated forces are responsible for these movements (Svoboda et al., 1995; Ding et al., 1998). Ding et al. (1998) have recently proposed that interactions of microtubules with the cell cortex at the ends of the cell drives SPB migration. In their model proximity of the SPB to an end results in microtubule interactions which produces a force to drive the SPB away towards the opposite end. As the SPB moves away the force from this end reduces and the SPB experiences mounting counteracting forces as microtubule interactions are established with the approaching opposite end. Once these are sufficiently great to overcome the force from the original end the direction switches and the microtubules detach from the original end. The chromatin follows the SPB during these migrations because the telomeres remain attached to the SPB from karyogamy through to the end of this ‘horsetail’ stage of nuclear migrations (Chikashige et al., 1994). It is unclear at present how these meiotic movements relate to the nuclear positioning mechanisms that maintain a central position for the nucleus during interphase of the mitotic cell cycle.

Modification of SPB function is seen in meiosis II when differentiation of the outer face of the SPB results in the formation of the forespore membrane which will generate the spore wall (Hirata and Tanaka, 1982; Tanaka and Hirata, 1982; Hirata and Shimoda, 1994). The SPB component Sad1 is likely to play a key role in this process as its distribution alters from a dot to a cap structure when SPB differentiation is initiated (Hagan and Yanagida, 1995).

PERSPECTIVES

The similarities between the organisation of the microtubule cytoskeleton and eukaryotes in general, such as the dependence of the distribution of mitochondria and the integrity of the Golgi stacks upon microtubules and the complete remodelling of the cytoskeleton that accompanies commitment to mitosis, suggest that much can be learnt from the study of microtubules in fission yeast. The ability to use real time imaging technology to study fluorescent microtubules in vivo (Nabeshima et al., 1995, 1997) will give an unprecedented opportunity to study the cytoskeleton. In particular the use of GFP tubulin fusions to study defective processes in mutant cells in real time will reveal many aspects of protein function which are not revealed by the study of static, fixed, samples (Ding et al., 1998). The current problems encountered with the slow penetration of the benomyl related anti-microtubule drugs may well be overcome by the use of genetically altered strains which are sensitive to the microtubule poisons ansamitosin P-3 and rhizoxin (Takahashi et al., 1990). Finally, the extraordinary migrations executed by the horsetail nucleus in meiosis suggest that mutants with different meiotic defects will reveal much about microtubule function in fission yeast.

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