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CHANGES IN CYTOSKELETON MORPHOLOGY DURING THE LIFE CYCLE OF *SCHIZOSACCHAROMYCES JAPONICUS* VAR. *VERSATILIS*

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A b s t r a c t

The morphology of the cytoskeleton (microtubules and actin) in the cells during the life cycle and in the protoplasts of the yeast *Schizosaccharomyces japonicus* var. *versatilis* were studied. Three types of microtubules were observed: (i) interphase microtubules (MTs) consisting of microtubule bundles arranged in parallel to the longitudinal cell axis; (ii) spindle microtubules involved in karyokinesis and (iii) astral microtubules emanating from the poles of the spindle at the end of mitotic anaphase and after meiosis I and II. The astral MTs were responsible for migration of the nucleus into the conjugation projection during mating, and facilitated contact between the haploid nuclei. Actin was visualised as actin patches situated at the sites of cell wall synthesis, i.e., at growth poles and at the equatorial plane, which also showed the presence of an actin ring during cytokinesis. Actin patches accumulated at the tip of the conjugation projection during the process of mating and, in the fresh zygote, they were assembled at the conjugation tube being involved in the wall reconstruction. During sporulation they appeared under the developing spore wall. Actin cables extended along the longitudinal axis. In mating cells, they converged at the conjugation projection. Both MTs and actin cables depolymerised during protoplasting. Freshly prepared protoplasts showed no visible microtubules. During protoplast regeneration, MTs gradually re-polymerised; this process was initiated by development of astral MTs arising from MTOCs (microtubule-organising centres) and was followed by the appearance of parallel bundles of MTs that did not seem embedded in the protoplast surface. In freshly prepared protoplasts and during cell wall regeneration, actin was present in the form of actin patches evenly distributed under the surface.

K e y w o r d s

Actin, cell cycle, cytoskeleton, microtubules, protoplasts, *Schizosaccharomyces*.

A b b r e v i a t i o n s u s e d

MTs, microtubules; SPB, spindle pole body; MTOC, microtubule-organising centre; DAPI, 4'-6-diamidino-2-phenylindole

INTRODUCTION

Microtubules and microfilaments are basic components of the cytoskeleton in all eukaryotic cells. In yeasts, MTs are present as cytoplasmic and nuclear (spindle) MTs (*1, 2, 3*). They emanate from microtubule-organising centres

(MTOCs), one type of which is called the spindle pole body (SPB) and is embedded into the nuclear membrane (*4, 3*). Microfilaments in yeast cells appear in two polymerised forms, i.e., cytoplasmic cables and cortical patches (*5, 6*). Cytoplasmic cables are longitudinal bundles of actin filaments, while patches are present as coils of filaments surrounding membrane invaginations (*7, 8*). In many fungal species, cortical patches cluster at the growing tip and cytokinetic ring, while cables extend from the tip towards the main body of the cell (*9, 10, 4*). These cytoskeletal components are thought to play an important role in morphogenic processes including cell division, chromosome segregation, organelle movement, secretion, endocytosis, conjugation and sporulation of yeast cells.

Yeast cells have proved to be very convenient systems for studying the role of cytoskeletal components in cell morphogenesis since the function and inter-relationship of gene products can be investigated with the use of a broad variety of biochemical, biological and sophisticated genetic techniques. The real time imaging technology using green fluorescence protein-tubulin fusions (*11, 12, 13*) provides a tool for a detailed investigation of the dynamics and function of MTs and other protein-based structures.

Although much information has been obtained on the structure and function of cytoskeletal components in yeasts, many questions still remain to be answered, namely, the role of astral MTs and other cytoplasmic MTs, development of MTs from MTOCs, relationship of actin patches to cell wall synthesis, involvement of microtubules and actin in sporulation, etc. *Schizosaccharomyces japonicus* var. *versatilis* differ slightly from *Schizosaccharomyces pombe*. The cells of this species are comparatively large, their nuclei are visible even in living cells and they show a high frequency of mating and sporulation. These properties have been used with advantage for studies on the role of microtubules during conjugation (*14*) and the role of actin in cytokinesis (*15*).

In this paper, an emphasis was placed on the investigation of the dynamics of astral microtubule formation during sexual activation of cells and relationships between astral MTs and nuclear migration prior to fusion and during meiotic divisions. Attention was paid to changes in actin distribution related to changes in cell shape during pheromonal activation and subsequent fusion of cells, the development of a zygote and the spore wall.

MATERIALS AND METHODS

Organisms and culture conditions

A homothallic strain of *Schizosaccharomyces japonicus* var. *versatilis,* CCY 44-3-1, was used in the experiments. The strain was maintained on malt extract agar. Cells were grown in liquid, malt extract broth medium (MEB; Fluka) containing 2% glucose at 28 °C.

Protoplasts preparation

Protoplasts were prepared from cells at the logarithmic phase of growth by treatment with NovoZym 234 (Novo Industri A/B, Nagsvaerd, Denmark; 1 mg/mL) in non-buffered 0.7 M mannitol for 30 min at 28°C. After washing, the protoplasts were suspended in MEB containing 0.7 M mannitol as an osmotic stabiliser.

Protoplasts at meiosis were prepared by the same procedure from a culture of agglutinated cells, containing more than 60% of zygotes, collected at the beginning of the stationary phase of growth.

Fluorescence microscopy

Both cells and protoplasts were fixed with formaldehyde in M-buffer and permeabilised with Triton X-100, as described previously (*14,16*). Cell walls were permeabilised with NovoZym 234 (see above). To visualise microtubules, the specimens were labelled with primary TAT 1 antitubulin monoclonal antibody *(17)* and secondary SwAM-FITC antibody (Institute of Sera and Vaccines, Prague, CR). The cells were transferred to a Vectashield mounting medium (Vector, USA) with DAPI (Sigma) at a concentration of 2 μg/ml in order to stain DNA in nuclei and mitochondria (18).

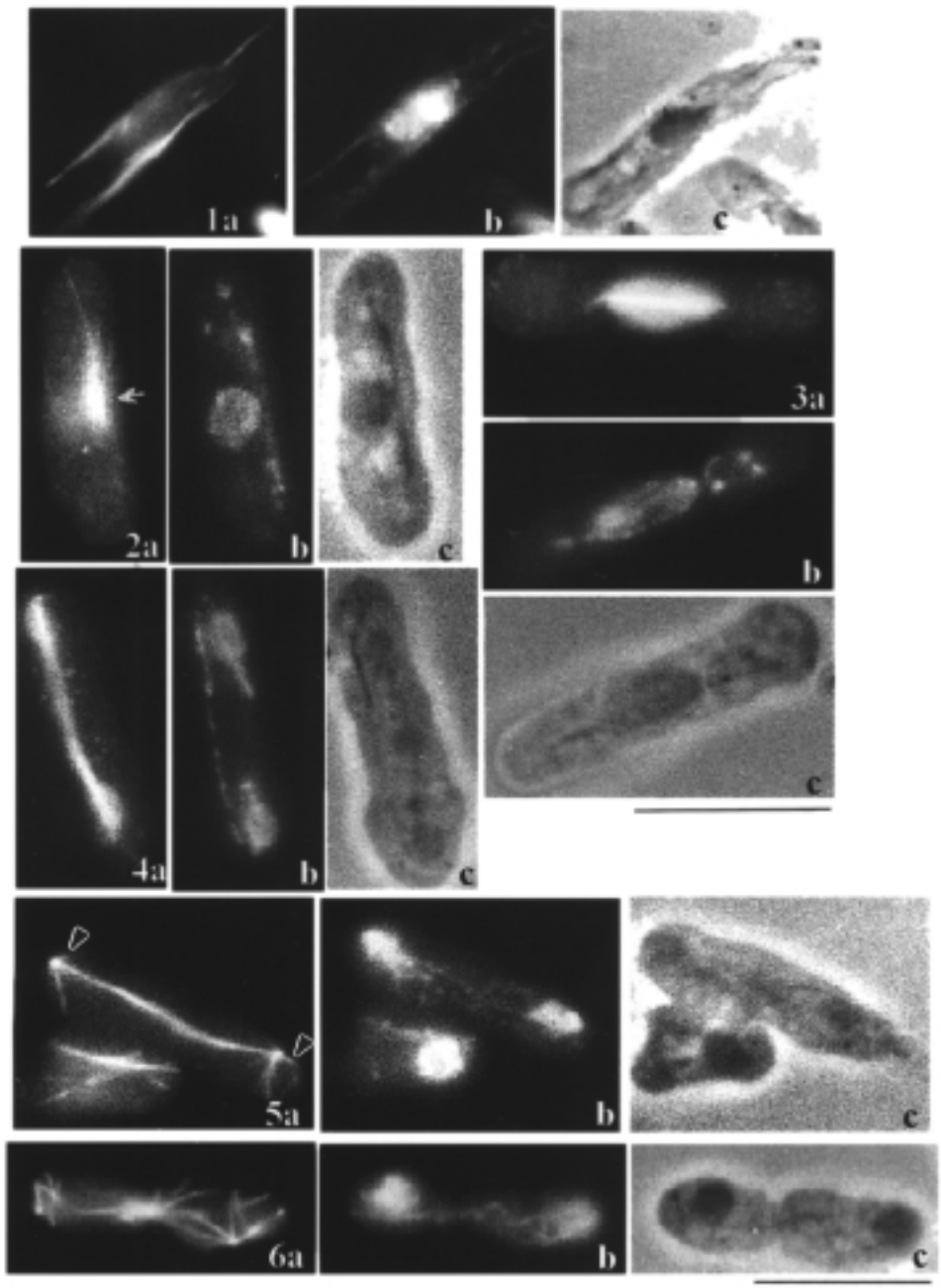
Actin was detected in cells by means of rhodamine-phalloidin (Molecular Probes Inc, USA), using the techniques described by *Pringle et al*. (*18*).

The specimens were viewed in a Zeiss Fluoval fluorescence microscope.

RESULTS

Microtubules during the cell cycle

Vegetative, growing cells usually contained nuclei situated centrally. Most of the MTs were arranged in two parallel bundles that, in elongated cells, converged at one of the cell poles (*Fig. 1*). These MTs disintegrated before mitosis and a short spindle appeared at the periphery of the nucleus, frequently in the vicinity of one bundle of resting cytoplasmic MTs *(Fig. 2*, arrow). Later, the spindle grew and extended along the nucleus and, at this stage, the nucleus became fluorescent (*Fig. 3*). During mitosis, the elongated spindle was situated along the longitudinal axis of the cell and its both ends showed the presence of short microtubules (*Fig. 4*). At the end of anaphase, an array of astral MTs emanating from both ends of the spindle appeared (*Fig. 5* arrowheads). Then the spindle MTs disappeared and new cytoplasmic MTs were observed (*Fig. 6*). Subsequently, astral microtubules emanating from MTOCs were seen in the region of a developing septum (*Fig. 7*). As the septum grew and closed up, the number of organising centres was reduced. Because some dislocation of MTs was possible, it was not clear whether there were more than one MTOC at the border of two daughter cells (*Fig. 8*). When the septum was closed, astral MTs disintegrated and cytoplasmic MTs were again stretched along the cell axis.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Microtubules during mating process

At the end of the logarithmic phase, the culture consisted of small agglutinated cells. In the cells that produced mating projections, the parallel bundles of cytoplasmic MTs disappeared. SPB sent out an array of astral MTs, which were directed towards both the developing conjugation projection and the cell cytoplasm (*Fig. 9*). The elongated, drop-shaped nucleus moved to the projection tip with SPB situated ahead of the nucleus, and astral MTs radiating backwards to the cytoplasm (*Fig. 10,11*). The cells fused at the tips of conjugation projections, giving rise to a conjugation tube (*Fig. 12*). The drop-shaped nuclei (*Fig. 13*) subsequently fused at their SPB regions. The SPBs apparently fused too, MTs arrays were seen emanating from one point of the diploid nucleus of the zygote. During prophase of meiosis I, the nucleus elongated to attain a horse-tail appearance (*Fig. 14*). Assisted by MTs arrays, the nucleus migrated from one end of the zygote to the other (*14*). At the end of the prophase, the nucleus resumed its original, oval shape. Cytoplasmic MTs disappeared and the spindle developed. After nuclear division ended, short astral MTs were again observed to emanate from SPB and to remain on the pole of the subsequent spindle during meiosis II (*Fig. 15*). Spindles during nuclear division can be seen in *Fig. 16*. Cytoplasmic MTs reappeared after postmeiotic mitosis as V-shaped, fluorescent structures, each being present near one of the 8 haploid nuclei (*Fig. 17)*. These structures were later found under the inner surface of spores.

Fig. 1

Vegetativly growing cells of *S. versatilis.* Microtubules are arranged in 2 parallel bundles, which converge at one of the cell poles in elongated cells, (a – indirect immunofluorescence of tubulin). Nuclei are situated centrally, mitochondria are visible as bundles colocalised with MTs (b – DAPI staining; c, phase contrast).

Fig. 2

A spindle developing from one bundle of parallel MTs attached to the nuclear membrane (arrow); (a) b and c as in Fig. 1.

Fig. 3

Spindle grows and moves to the centre of the nucleus. After labelling with TAT–1 antibody, the nuclei stain as well (a). DAPI staining of nuclei and mitochondria (b), phase contrast (c).

Fig. 4

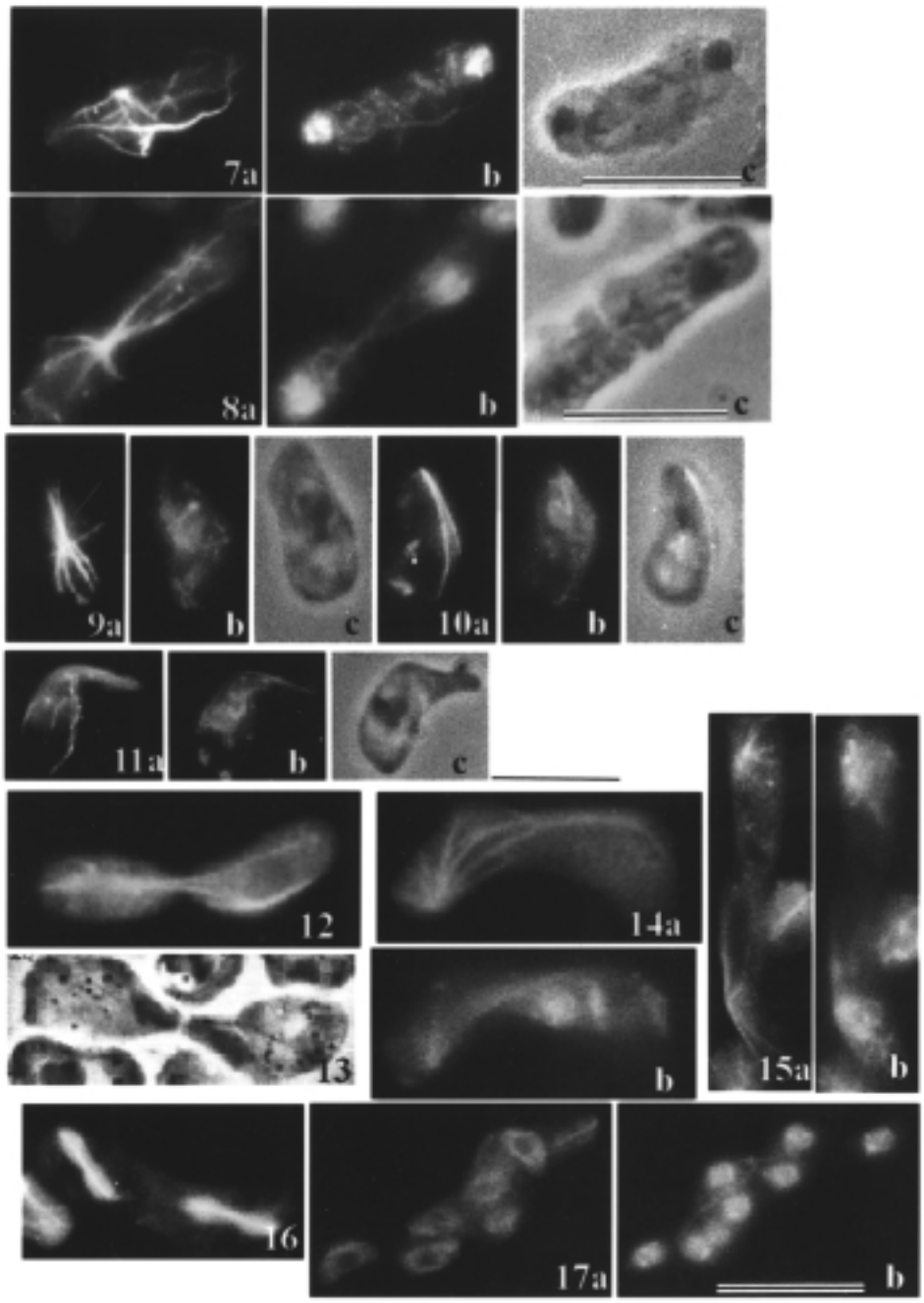
During separation of the nuclei, a long spindle is apparent and the nuclei still remain stained (a). DAPI staining of nuclei and mitochondria (b), phase contrast (c).

Fig. 5

After complete nuclear separation at the end of anaphase, the organisation centres give rise to astral MTs (arrowheads) (a). DAPI staining of nuclei and mitochondria (b), phase contrast (c).

Fig. 6

Astral MTs can be seen in the cytoplasm after disruption of the spindle (a). DAPI staining of nuclei and mitochondria (b), phase contrast (c). (Bars = 10 μm).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Actin cytoskeleton

In growing, vegetative cells, actin was present in the form of patches accumulated in growing ends and in the region of growing septum. Actin cables were observed to stretch along the longitudinal axis of the cell. During septum formation, an actin ring was seen in the proximity of the growing septum wall (*Fig. 18*).

On pheromonal activation of the cell (*Fig. 19*), actin patches accumulated in the tip of the conjugation projection and actin cables also converged there. *Fig. 20* shows two activated cells at the moment of fusion. After cell fusion, actin cables were no longer seen and actin patches were located under the zygote wall. Immediately after fusion, actin patches were accumulated in the conjugation tube taking part in the cell wall recconstruction. During prophase of meiosis I, actin

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Fig. 7
At the site of the developing septum, the astral microtubules emanate from organising centres, which show ring-like arrangements (post anaphase MTs arrays) (a). DAPI staining of nuclei and mitochondria (b), phase contrast (c).

Fig. 8
Arrangement of MTs at the moment of septum closing (a). DAPI staining of nuclei and mitochondria (b), phase contrast (c).

Figs. 9–11
Microtubules in a pheromone-activated cell. Astral microtubules are directed towards both the developing conjugation projection and the cell cytoplasm (fig.9a). The SPB, together with the nucleus, travel to the tip of the conjugation projection (figs. 10,11a,b).Microtubules DAPI staining of nuclei and mitochondria (b), phase contrast (c).

Fig. 12
MTs of conjugating cells. The cells fuse in the tips of conjugation projections.

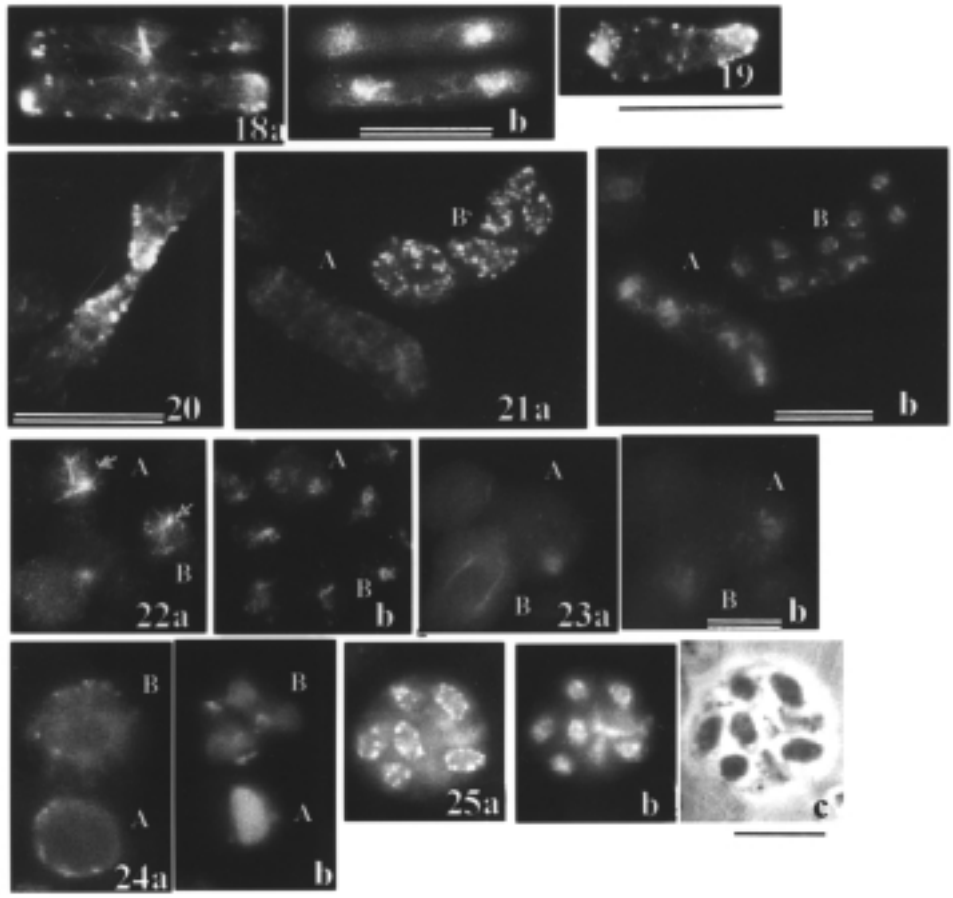
Fig. 13
Phase-contrast microscopy of conjugating cells. The nucleus shape is altered from spherical to drop-like. Nuclei fuse at the SPB region.

Fig. 14
Astral MTs in a zygote during meiotic division I (a). At this stage the nucleus elongates and single chromosomes become apparent; this is observed as a horse-tail structure (b).

Fig. 15
Zygote shortly after completion of meiotic division I. Astral MTs arise from SPBs located in the nuclear envelope (a). DAPI staining of nuclei and mitochondria (b).

Fig. 16
Microtubules during meiotic division II. Two spindles are visible. Nuclei are stained similarly to those at mitosis.

Fig. 17
Sporulating zygote. V-shaped arrangement of MTs in spores (a), 8 haploid nuclei (b). (Bars = 10 μm).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patches disappeared (*Fig. 21* zygote A) and reappeared in the form of a ring around the nucleus at the time of spore wall formation at the end of meiosis II and at the end of postmeiotic mitosis (*Fig. 21*, zygote B). In mature spores the actin patches were not visible.

Restoration of microtubules and actin during protoplast regeneration

In freshly isolated protoplasts, MTs were absent; the surfaces of nuclei showed occasional fluorescent spots, which may have been microtubular material surrounding SPBs (*Fig. 22, Fig. 23* protoplasts A). MTs repolymerised during 10 to 30 min of protoplast regeneration. They first presented as astral MTs radiating from the nuclear surface (*Fig. 22* protoplast A, protoplast B, arrows) and then, during continuing regeneration, as parallel MT bundles arranged in a pattern similar to that in growing, vegetative cells (*Fig. 23* protoplast B). No diversion from the behaviour of MTs in vegetative cells was observed during nuclear division.

Fig. 18
Actin cytoskeleton is present in the form of actin patches concentrated in the cell poles and in the septum area and actin cables running in the longitudinal axis of the cells (a -Rh-phalloidin staining of actin). DAPI staining of nuclei and mitochondria (b).

Fig. 19
Pheromone activated cell. Actin patches are concentrated in the tip of conjugation projection, actin cables converge toward the conjugation projection.

Fig. 20
Actin in fusing cells. Actin patches are accumulated at the site of a developing conjugation tube.

Fig. 21
Actin in zygotes. Actin cytoskeleton dissappears before prophase of meiosis I and is absent during meiotic divisions (zygote A). Actin patches reappear during the postmeiotic division and probably participate in spore wall synthesis (zygote B) (a). Nuclei (b).

Figs. 22 and 23
MTs in protoplasts. In freshly prepared protoplasts, MTs are absent and SPBs are seen only occasionally (Fig. 23, protoplast A). Distinct repolymerisation of astral MTs (Fig. 22, protoplasts A, B) (arrows). These are transformed into parallel MT bundles, which are typical of interphase cells, within 40 min. (Fig. 23 protoplast B) Microtubules (a). Nuclei (b).

Fig. 24
Meiosis in protoplasts. During meiotic divisions, actin patches are evenly scattered under the whole protoplast surface in a manner similar to that in growing protoplasts. Actin (a) DAPI staining of nuclei (b).

Fig. 25
Protoplast sporulation. After postmeiotic mitosis, actin patches are arranged around nuclei. Actin (a). DAPI staining of nuclei (b), phase contrast (c). (Bars = 10 μm).

Actin in freshly prepared protoplasts was present in the form of patches, distributed evenly under the protoplast surface, which also remained the only actin structures discernible during protoplast regeneration. Actin cables were not restored in the process of regeneration.

Meiosis and sporulation in protoplasts

Protoplasts prepared from zygotes underwent meiotic division and sporulation. Neither the structure nor the function of their microtubules and nuclei differed from those in the zygotes. Actin patches were observed only occasionally under protoplast surfaces during meiotic division (*Fig. 24*) and, after postmeiotic mitosis, were found located around the nuclei in a manner similar to that seen in the zygotes (*Fig. 25*).

DISCUSSION

Several papers reporting details on the behaviour of MTs and actin in *S. pombe* have been published during the period of our investigation (*13, 3, 10, 19*). The results of our experiments with *S. versatilis* are in agreement with the published data and also provide new information on microtubules in terms of dynamic changes taking place during mating, meiosis and sporulation, and on restoration of the system of microtubules in regenerating protoplasts.

Morphology and function of astral microtubules during mating and meiosis

Our observations showed that the induction of mating was accompanied by disruption of the bundles of microtubules arranged in parallel to each other, and by appearance of astral microtubules, which radiated from the SPB located in nuclear surface. These microtubules elongated and changed the position of the nucleus and the SPB gradually approached the tip of the conjugation projection. We suggest that this elongation of microtubules is a mechanism responsible for migration of the nucleus into the conjugation projection and also a mechanism which mediates contact between the two nuclei in a zygote (*14*). Astral microtubules have so far been studied in more detail only as regards their function during anaphase B and two models for their action have been proposed: (i) microtubules provide a track for the SPB to slide along (*20*); (ii), the plus-ends of microtubules interact with motor proteins under the cell cortex and the microtubules thus push forward the nucleus to which their minus-ends are attached at the site of SPB (*21,22*). In cells activated with a pheromone it cannot be excluded that, on the leading ends of drop-shaped nuclei, short MT bundles are present in a manner similar to that observed in *S. cerevisiae* (*23,24*). An interaction of these MTs has been regarded as a mechanisms for drawing nuclei close to each other (*24*). However, this leading band of microtubules was not observed in our experiments. The appearance of astral microtubules radiating

from the poles of divided nuclei at the period following meiotic division or postmeiotic mitosis may have also been related to the movement, or a change in position, of a nucleus getting ready for subsequent division, or to a change in position of the nucleus in a developing spore.

In *Schizosaccharomyces pombe,* apart from an SPB embedded in the nuclear envelope, there are MTOCs at the equatorial plane and at the tip of the conjugation projection (*19*). At the end of anaphase, after the spindle has disintegrated, cytoplasmic MTs that arise from the MTOCs localised at the equatorial plane produce a post-anaphase array (*25, 3*). A similar organisation of MTs was also observed in *S. versatilis.* However, we failed to detect the organising centres for MTs either with an antibody against *sad1* protein (*26*) or an antibody against γ-tubulin (unpublished results). In our specimens, the microtubule organising centres were seen as dots of bright fluorescence after staining with antitubulin antibody.

Assembly of actin structures during mating and meiosis

Similarly to *Petersen et al* (*10*) we observed actin cables converging at the tip of the conjugation projection and also accumulation of actin patches at this site. This is probably related to intensive synthesis of the cell wall during projection growth (*27,28*). Actin patches were present also at the site of a developing conjugation tube, which is characterised by extensive reorganisation of the cell wall. Actin patches were discernible around nuclei at later stages, i.e., after meiosis II and postmeiotic mitosis. They are thought to be involved in synthesis of the spore wall. Either no actin structures were observed in the course of meiotic division or they were scattered throughout the cytoplasm during horsetail movement. It has not been explained what is the role for these scattered patches (*10*).

Changes in cytoskeletal structures in protoplasts

We found that, during protoplasting, microtubules were depolymerised, actin cables disappeared and the polar location of actin patches was destroyed. These two features may be due to a response of the cytoskeleton to an osmotic shock (*29*). During the early stages of regeneration of protoplasts in an osmotically-stable medium, the first structures to repair were microtubules, while actin remained in the form of patches under the protoplast surface for the whole duration of regeneration. This distribution is most probably associated with cell wall regeneration, which takes place all over the protoplast surface. The first step of repolymerisation gave rise to astral MTs radiating from the SPB. It is possible that these MTs were present only in protoplasts that arise from mitotic cells. During the next steps, the protoplasts showed bundles of MTs arranged in parallel to each other, as seen in the interphase cells. These finding suggest that, in

protoplasts, organising centres for microtubules can also function. A more detailed study of the dynamics of microtubular cytoskeleton repair was hindered by the fact that regenerating protoplasts produced clusters and, therefore, individual protoplasts and relevant changes were difficult to identify. However, protoplasts seem to be a convenient model system for investigation and, ultimately, definition of functions carried out by the different types of MTOC in the cell. The study of microtubular structures in meiotic protoplasts did not reveal any new findings, as compared with cells undergoing meiosis. It can only be concluded that the cell wall is not a prerequisite for either meiosis or sporulation.

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ZMĚNY V MORFOLOGII CYTOSKELETU V PRŮBĚHU ŽIVOTNÍHO CYKLU *SCHIZOSACCHAROMYCES JAPONICUS* VAR. *VERSATILIS*

S o u h r n

Byl studován obraz cytoskeletu (mikrotubulů a aktinu) v průběhu životního cyklu *Schizosaccharomyces japonicus* var. *versatilis* a u regenerujících protoplastů těchto kvasinek. V průběhu životního cyklu se objevují 3 typy mikrotubulů (MTs). 1) Interfázové mikrotubuly sestávající většinou z paralelních svazků MTs ležících v podélné ose buňky, 2) vřeténkové MTs angažované v karyokinéze a 3) astrální mikrotubuly, které se objevují na půlech spindlu na konci anafáze mitůzy a meiozy I a II. Tyto MTs realizují pohyb jádra do konjugačního výběžku v průběhu párovacího procesu a umožňují kontakt shaploidních jader. Aktin se vyskytuje v podobě aktinových patches lokalizovaných v místech formace buněčné stěny, tj. na růstových půlech buňky a v ekvatoriální rovině, kde se při cytokinezi vyskytuje rovněž aktinové ring. Při párovacím procesu se aktinové skvrny koncentrují ve vrcholu konjugačního výběžku, u čerstvé zygoty jsou koncentrovány v místě konjugačního kanálu, kde dochází ke zpevňování stěny zygoty. Při sporulaci se objevují pod tvořící se sporovou stěnou. Aktinová vlákna probíhají v podélně ose buňky a směřují k místům tvorby buněčné stěny. V konjugujících buňkách se sbíhají do konjugačního výběžku. V průběhu protoplastování dochází k depolymeraci MTs a aktinových vláken. U čerstvých protoplastů nejsou patrné žádné mikrotubuly. Při regeneraci protoplastů MTs repolymerují a to nejprve tvorbou astrálních MTs z MTOCs (microtubule-organising centres). Později se objevují i paralelní svazky MTs bez zjevného zakotvení v povrchu protoplastu. U čerstvých protoplastů a v průběhu regenerace buněčné stěny je aktin přítomný pouze ve formě aktinových skvrn rovnoměrně rozmístěných pod povrchem.

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