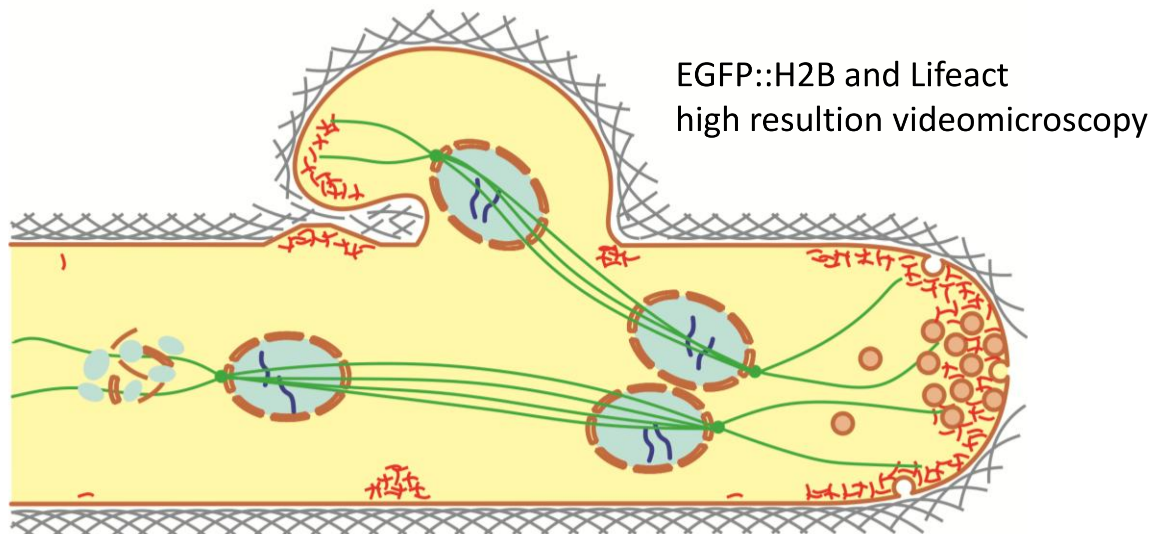
Graphical abstract

Mitosis in monokaryon and in
dikaryon with clamp formation



Nuclear movement throuth mitosis
Actin filaments during nuclear division and septation
Vesicle staining shows Spitzenkörper

Highlights

- Mitosis in haploid, monokaryotic cells revealed condensation of elongated nuclei.
- Dikaryotic hyphae with synchronously dividing nuclei thereby keep nuclear identity.
- Division of one nucleus is re-directed by actin networks towards the clamp cell.
- Septation is preceded by actin patches and vesicles to form the actin ring.

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The making of a mushroom: Mitosis, nuclear migration and the actin network

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Abstract

Basidiomycetes feature a prolonged dikaryotic life stage. A dispute over open *versus* closed mitosis could be solved using *in vivo* fluorescence videomicroscopy of histone 2B::EGFP and Lifeact labeled *Schizophyllum commune*. It revealed nuclei to condense to approximately one fifth in diameter during mitotic prophase. In addition, the specifics of clamp cell formation typical of many basidiomycetes included an actin network at the future site of nuclear division, which allowed for cessation of nuclear movement and re-localization of one nucleus towards the emerging clamp cell, while the other divided along the hyphal axis. Subsequent fusion of the clamp cell to form the clamp connection restored the close association of the two nuclei in a very fast process after clamp fusion. Septation was preceded by actin patches and vesicles involved in formation of the actin ring.

Keywords

Basidiomycetes, *Schizophyllum commune*, mitosis, actin, nuclear division, septation.

Introduction

Within the fungal kingdom, the phylum Dikarya is named after its feature of having extended stages in the life cycle where, after mating, the nuclei of both parents do not fuse until directly before meiosis. Instead, two nuclei are present in every hyphal compartment, each surrounded by their nuclear envelope (Girbardt, 1958; Raper, 1966). Within the Ascomycota, the different nuclei are present in several copies per vegetative hyphal compartment, studied extensively by live cell imaging (Delgado-Alvarez et al., 2014; Freitag et al., 2004; Riquelme et al., 2014; Takeshita et al., 2014). The Basidiomycota show a true dikaryon, with one copy of each of the two parental nuclei per cell. Nuclear division in living cells of the yeast life stage of the basidiomycetes *Ustilago maydis* (Straube et al., 2005; Theisen et al., 2008), during formation of clamp-like structures in *U. maydis* (Scherer et al., 2006) and in *Cryptococcus neoformans* (Kozubowski et al., 2013) has revealed new features of the nuclear envelope dynamics during mitosis. This "open" mitosis is different from closed mitosis in ascomycetes and clearly warrants a re-investigation of filamentous basidiomycete mitosis with respect to cellular functions. However, so far the visualization of nuclear division in living hyphae during real-time is still missing from filamentous basidiomycetes.

The filamentous basidiomycete *Schizophyllum commune* has been used to extensively study mating, prerequisite for the formation of the dikaryotic life-stage (Freihorst et al., 2016; Raper, 1966; Raudaskoski, 2015; Raudaskoski and Kothe, 2010). This includes pairing of the nuclei, synchronous nuclear division, and clamp cell formation. Directly associated with the plane of metaphase chromosomes in nuclear division, the formation of septa is initiated. After two septa are formed, the tip cell is dikaryotic while one nucleus stays trapped in the clamp and the other in the subapical hyphal cell, which is resolved to a dikaryon through clamp fusion with the subapical cell. The formation of clamp cells is typical for basidiomycetes (see Raper, 1966), although some basidiomycetes have lost that feature. However, it easily explains how the control of dikaryotic growth can be maintained indefinitely without mixing up the nuclear identities. It thus is an important feature, and its control can be

used to identify traits that are important, but easily overlooked in other members of the Dikarya.

Here, we present the first life cell video-microscopy imaging of dividing nuclei of both hyphal types, monokaryon and dikaryon of a mushroom-forming basidiomycete, stained with eGFP fused to histone 2B. This core histone is not released during cell cycle, and histone 2B showed a high level of expression (Ohm et al., 2010). In addition, it features an intron in the 5' non-translated region to promote high levels of gene expression in *S. commune* (Lugones et al., 1999). To stain actin during nuclear movement within the living hyphae, we used Lifeact (Riedl et al., 2008). Living hyphal cells were screened for the four different populations of actin that had been described: patches, cables, rings and subapical actin webs (Schultzhaus et al., 2016). Mitosis in *S. commune* was recorded, and a dynamic role of filamentous actin not only during polar tip growth and septation, but also during nuclear division and at clamp cell formation could be deduced.

Material and methods

Strains, growth and cloning

Compatible *Schizophyllum commune* A26 ($B_{\alpha4\beta1}$; ura9) and A43 ($B_{\alpha3\beta6}$; ura7) were grown on complete medium CYM (Schwalb and Miles, 1967).

To obtain strains with histone H2B::EGFP, histone H2B gene (PID2605148) with a 826 bp long promoter region was used and linked to *egf.* Two different length terminator regions from *cdc42* and a phleomycin resistance cassette were added (for detailed cloning procedure, see suppl. Fig. S1). Both plasmids were sequenced and used for transformation of *S. commune* A26.

For Lifeact imaging, the plasmid pEGFP_N1-Lifeact-EcoRI-BHI (by courtesy from Dr. Roland Wedlich-Soldner to Marjatta Raudaskoski) was used for cloning. In the plasmid, the 17 amino acid Lifeact peptide is linked to EGFP (linker sequence Gly-Asp-Pro-Pro-Val-Ala-Thr). The promotor was replaced with 889 bp from *S. commune* β-tubulin (Russo et al., 1992) amplified with primers BTub*Vsp*IF3 Iand BTub*Eco*RI (suppl. Tab. S1) to yield plasmid p6K, into which

the 1125 bp terminator region of *cdc42* was cloned using *Not*I. The PCR with primers BTub*Vsp*IF3 and Scdc42P2 (suppl. Tab. S1) produced a fragment with β-tubulin promoter, linker, *egf* gene and 500 bp of the *cdc42* terminator. The fragment was cloned into TOPO vector, the phleomycin cassette was added into the *Apa*I site resulting in the plasmid p15-1 (suppl. Fig. S2).

Transformation

Protoplast purification and transformation were performed as described (Weber et al., 2005). Transformants were selected on phleomycin (20-40 µg ml^{-1}) in CYM medium. Strong EGFP expressing transformants (screened by epifluorescence microscopy) were mated with *S. commune* A43 for dikaryotic hyphae. If indicated,compatible progeny were mated.

Fluorescence microscopy

Fluorescence microscopy was performed on mycelium grown on a 0.5 % agarose on cellophane membranes overlaying CYM medium. The thin agarose layer was carefully separated from the cellophane membrane and floated to a microscope slide, after which the cover glass was added. Alternatively, the mycelium was grown directly on a thin layer of diluted complete medium spread on a purified cover glass. Hyphae were examined with epifluorescence microscopy using Leitz Orthoplan Large Field Research Microscope equipped with a 4-lambda Reflected Light Fluorescence Illuminator and a100W mercury lamp or high resolution confocal laser scanning fluorescence microscopy (Zeiss, Jena, Germany). Images were photographed with a digital microscope camera Leica DFC420C or Digital Images and digital photographs had brightness and contrast optimized with Corel PhotoPaint and CorelDRAW X7. Zeiss LSM780 confocal microscope
with objective alpha Plan-APOCHROMAT 100x/1.46 Oil DIC (UV) VIS-IR was used to collect images of septum formation for 3D images with Imaris 8.1.2 software. To stain membranes, 2.5 µM FM4-64 (Life Technolgies) were added to microscopy slides (Delgado-Alvarez et al., 2010).

Results

Visualization of nuclei in mitosis

In unmated, monokaryotic hyphae expressing EGFP labeled histone, the single nuclei were seen as elongated structures in every hyphal compartment. In apical cells, the nuclei were located in the middle of the cell (Fig. 1, suppl. Movie S1). The nucleolus was visible by lower fluorescence (Fig. 1 a, c, d). Condensed chromatin visible by stronger fluorescence gathered at the apical part of the nucleus prior to nuclear division, while the fluorescence in the rest of the nucleus remained weak and finally became separated from the bright part expelling the contents of the nucleus without chromatin (Fig. 1 d-f). As a consequence of chromatin condensation, the diameter of dividing nuclei was less than 20 % of an interphase nucleus (Fig. 1g, h). After nuclear division, the daughter nuclei separated (Fig. 1 i - k), with a speed of 5 µm min^{-1} (Fig. 1 l). During separation, the size of the daughter nuclei increased, and diffuse fluorescence of chromatin again filled the nucleus.

When a strain carrying H2B::EGFP was mated to a compatible wildtype strain, both nuclei were fluorescent, showing import of the labeled histone to either nucleus. In the resulting dikaryon, both nuclei moved connectedly towards the growing hyphal tip at a rate proportional to tip growth (suppl. Movie S2). Occasionally, the nuclei showed elongated ends in either direction. The formation of the clamp cell started as an outgrowth at the side of the apical cell, associated with a change in the nuclear forward movement. First, the paired nuclei became more closely associated (Fig. 2) resulting in a position at the same place in the hypha below the outgrowth for the developing clamp cell (Fig. 2 a, b), followed by backwards movement towards the extending clamp cell (Fig. 2 c for kymograph from suppl. Movie S2), which caused stretching in both nuclei (Fig. 2 c, d). One of the prophase nuclei in the pair extended towards the growing clamp cell, again with chromatin condensation at one side of the elongated nucleus (0 to 3 min into nuclear division; Fig. 2 e-i). The dividing nuclei condensed to about one fifth of the size of the prophase nuclei (Fig. 2 j-l). The condensed chromatin assumed a spindle shape at 7 minutes (see Fig. 2 m). At anaphase, the spindle

elongated (Fig. 2 n), and two daughter nuclei became visible within two minutes (Fig. 2 o-q). Both the nucleus in the clamp cell and the one in the hyphal compartment passed through the anaphase into telophase (Fig. 2 r-s). The following movement of the two daughter nuclei was very rapid, as had been seen for the monokaryotic nuclear division. During telophase, the size of the nuclei increased again to interphase nucleus size and the nucleolus reappeared (Fig. 2 t). The clamp cell fusion releasing the trapped nucleus took up to 15 min (Fig. 2 u).

Actin in tip growth and septum formation

In monokaryotic hyphae, actin fluorescence was visualized at hyphal tips and at the sites of septum formation. Mainly, the filamentous actin was associated with patches, which formed aggregates during specific developmental stages displaying strong fluorescence in the center and diffuse fluorescence at the edges. Clear, thin, separate actin filaments were only observed during the immediate early development of contractile actin rings associated with the formation of septa. Actin cables were occasionally distinguished in apical cells in the tip region.

The apical tip region in mono- and dikaryotic hyphae showed abundant cortical actin patches in the tip region, extending for a distance of 5 µm from the apex (suppl. Fig. S3). Membrane staining revealed a round structure less than 1 µm in diameter encompassing the cortical actin patches at the very tip, which was interpreted to represent the Spitzenkörper. In movies, the actin patches are moving within the 5 µm tip region (suppl. Movie S3). In addition to the fluorescence at the tip, the only actin fluorescence along the apical cell were spots, with cables or networks missing from the apical cells. A green crescent at the hyphal tip and actin spots connected to each other were visualized in slowly growing hyphae (e.g., on diluted media). A newly formed growing tip next to the one ceasing growth was often formed after disturbance by, e.g., illumination. Cortical actin patches also covered the tips of newly induced branches.

The first sign of septum formation (suppl. Movie S4) as monitored by video-microscopy was the occurrence of actin filaments at the point of nuclear division (Fig. 3), forming a radial halo of very thin filaments, likely forming the first phase of a contractile actin ring for septation. Imaging the contractile actin rings of monokaryotic and dikaryotic hyphae in 3-D view revealed a thin actin ring and big opening at the onset with a small opening at later stages (suppl. Movie S4).

Actin during clamp cell development and concomitant nuclear division

In mated, dikaryotic hyphae, mitosis occurred with the formation of clamp connections. The first sign of clamp cell development was actin (Fig. 4 a) assembling at the plasma membrane to one side of the hypha in the middle of the tip cell (suppl. Fig. S4). From cortical actin patches, fluorescence spread (as filaments and networks) into the outgrowth for the clamp cell and into the hypha below this outgrowth, occupying an area of more than 10 µm in length (Fig. 4 b-g). The formation of the actin aggregates was associated with the cessation of the forward nuclear movement at the point of clamp development.

When the shape of the outgrowth started to resemble a clamp cell, the fluorescence was strongest in the apical region of the outgrowth, finally concentrating at the clamp cell tip (see Fig. 4 h-j). At this stage of clamp cell development, one nucleus of the pair was drawn into the clamp connection where it divided, during which the actin fluorescence concentrated at the tip of the clamp cell. After division, cortical actin patches trapped the telophase daughter nucleus in the clamp cell. The septa formed at the base of the clamp cell and in the hypha initiated with cortical actin rings (see Fig. 4 k). After septum formation, last remnants of the acting ring fluorescence occurred in the middle of the ring, perhaps representing the formation of the septum dolipore, few cortical actin patches were seen in the tip of the clamp cell and in the subapical cell at the future clamp fusion site (Fig. 4 l). The newly formed septum and the future fusion site of the clamp cell to the subapical cell was well visualized by FMN4-64 staining (Fig. 4 m) suggesting ample membrane and vesicle trafficking.

Discussion

Growth and mitosis in living hyphae

Here, living hyphae could be visualized through mitosis, both for unmated mono- and dikaryotic hyphae of *S. commune*. The nuclear movement in living dikaryotic hyphae and the conjugate division of nuclei have been previously observed by phase contrast microscopy in the filamentous basidiomycete *Trametes versicolor* (Girbardt, 1973). The observations on the nuclear movement and division in monokaryotic and dikaryotic hyphae by *in vivo* fluorescence microimaging at very high resolution allowed for a better view on processes accompanying mitosis and clamp formation in *S. commune* (compare also Raudaskoski, 2015, and citations therein). The role of the cytoskeleton can be envisioned with features separating the mitotic process in basidiomycete filamentous fungi from those evolved in multinucleated filamentous ascomycetes for the same processes.

Like with the ascomycete *Neurospora crassa*, the structure of the hyphal apex contained a structure remnant of the Spitzenkörper, the vesicle supply center. The exocyst and polarisome compononents (Riquelme et al., 2014) at the growing tip responsible for secretion and maintaining the acting cytoskeleton, respectively, have not yet been identified for *S. commune*. The most distant cortical actin patches from the tip could belong to the subapical ring known to be involved in endocytosis of surplus membrane material (Schultzhaus et al., 2016; Schultzhaus and Shaw, 2015).

In *N. crassa* as well as *Aspergillus nidulans*, microtubules are shown to pass the Spitzenkörper, and some of the exocyst proteins in *N. crassa* bind to the microtubules (Riquelme et al., 2014; Torralba et al., 1998). It is thought that in the hyphal apex the vesicles are released from microtubules to microfilaments for actin transport towards the apical plasma membrane (Harris et al., 2005; Riquelme, 2013; Takeshita, 2016). In *A. nidulans* apex, polarity marker proteins link microtubules *via* TeaA with its cognate receptor TeaR anchored to the apical plasma membrane (Takeshita, 2016). In the *S. commune* hyphal apex, no microtubules extending to the very tip have been observed (Raudaskoski, 1998; Raudaskoski et al., 1991, 1994; Rupes et al., 1995). Homologs for both polarity marker

proteins, TeaA and TeaR (Takesshita et al., 2014), are present in the *S. commune* genome. Their function at the *S. commune* hyphal apex remains to be clarified. The absence of microtubules from the very tip region in *S. commune* might be interpreted to be due to a different tip organization in this filamentous basidiomycete, relying more on actin than on the microtubule cytoskeleton for the last step of vesicle delivery to the growing tip.

A "condensed closed mitosis" may resolve the dispute

An elongated form of the nucleus with condensed chromatin at one end has been observed. If a part of the nucleus was left behind prior to nuclear division, this would lead to release of contents of nucleoplasm into the cytoplasm. Similar processes have been noted earlier with the basidiomycete *T. versicolor* (Girbardt, 1973). *Ustilago maydis* shows a part of the nuclear envelope being left behind in the mother cell with disassembly of nucleoporins during mitosis, and re-assembly of the nuclear envelope at telophase (Straube et al., 2005; Theisen et al., 2008) interpreted as an “open mitosis”. In investigations including in planta observations for *U. maydis*, a structure with retracting parts of the nucleus have been shown which closely resemble the findings here (Scherer et al., 2006). In the basidiomycetous yeast *Cryptococcus neoformans,* the nuclear area is reduced by 66 %, before the nucleus migrates into the bud with simultaneous nuclear envelope/nuclear pore complexes disassembly in the mother cell during mitosis (Kozubowski et al., 2013).

A new term of "condensed closed mitosis" might better describe the features of nuclear division in filamentous basidiomycetes. This would mean that during spindle formation and chromatin separation an envelope surrounds the condensed chromosomes, which seems essential for keeping the chromosomes of the dividing nuclear pair in a dikaryon separate during the synchronous nuclear division. Especially since the two nuclei are dividing in very close proximity, this aspect is of special importance in filamentous basidiomycetes with no clamp cell formation in dikaryotic hyphae. In these fungi, the synchronous division of the nuclear pair takes place in the hypha with the two nuclei parallel or overlapping each other (Salo et al., 1989). After mitosis, only one septum is formed. Hence, the nuclear envelope

seems essential to separate the two different genomes without mixing of single chromosomes. If part of the nucleoplasma was shed during the process of condensation, the observed occurence of nucleopore complexes in the cytoplasm could well be accomodated. A condensed closed mitosis may thus have evolved for the nuclear division in basidiomycetes, as opposed to the closed mitosis seen with ascomycetes and the open mitosis with plant and animal cells.

Clamp cell formation and septation are connected to nuclear division

Ascomycetes show that the site of future septation is marked by nuclear division (Harris, 2001; Seiler and Justa-Schuch, 2010), although a tight visualization of nuclear division and septum formation is obscured in these multinucleate cells. *S. commune*, like all basidiomycetes, shows a close connection of septal development to nuclear division including the site of future septum formation (Raudaskoski, 2015). For the ascomycete *N. crassa*, a mesh of bundles of thin actin filaments that gradually orientated to a contractile actin ring were reported (Delgado-Alvarez et al., 2010, 2014; Mourino-Peres and Riquelme, 2013). In *S. commune,* the contractile ring is associated with cell wall material deposition *via* vesicles in the forming septa.

With dikaryotic hyphae at the beginning of clamp cell formation, a strong fluorescent area in association with the clamp cell growth was observed. Diffuse and granular structures also have been observed at the same site by immunofluorescence microcopy with actin antibodies (Runeberg et al.,1986; Salo et al., 1989). This actin structure is interpreted to stop the forward nuclear movement and direct the nuclei to the site of nuclear division in the hypha and clamp cell. In living hyphae, the leading nucleus is diverted into the clamp cell for nuclear division. In the mushroom forming model basidiomycete *Coprinopsis cinerea,* alternating placement of either nuclear type has been proposed (Iwasa et al., 1998). This regular assortment has not been supported with *S. commune*, as nuclei passing each other during cell elongation can be seen regularly with *in vivo* imaging. An irregular arrangement of nuclei has also been reported when one of the two nuclei carried a GFP-tagged pheromone

receptor (Erdmann et al., 2012). Actin patches in front of the telophase nucleus in the clamp cell are associated with vesicle supply during clamp cell growth and clamp fusion. The peg growth at the hyphal site of clamp fusion (Schubert et al., 2006) needs tip growth as well and thus explains similar structures at this site of future clamp fusion in the hyphal compartment.

Nuclear positioning and cell cycle progression

The nuclear positioning towards the cell apex has been discussed with relation to signals derived from the tip in ascomycetes (Etxebeste and Espeso, 2016), which in *S. commune* has been linked to cell cycle progression (Raudaskoski and Kaukonen, 1978). The present study suggests that in dikaryotic hyphae the actin cytoskeleton, together with astral microtubules emanating from the spindle pole bodies of each nucleus (Runeberg et al., 1986), play a central role in locating the nuclei for division in the hypha and in the growing clamp cell. In the next step after the reduction of nuclear size, the two units of the spindle pole body separate and travel to the opposite ends of the condensed nucleus in preparation of spindle formation (Girbardt, 1973; Runeberg et al., 1986).

Phosphorylation is involved in progression through the cell cycle at spindle pole bodies for *A. nidulans* (Engle et al., 1988) and *N. crassa* (Heilig et al., 2013). Spindle pole bodies and associated phosphoproteins also play an important role in the septation intiation network (SIN) of *S. pombe* or mitotic exit network (MEN) in *S. cerevisiae* and require induction by Ras-related GTPases Spg1 and Tem1, respectively (Jones et al., 2011; Simanis, 2015). A homolog to *spg1* in *S. commune* (Raudaskoski et al., 2011) is highly expressed during vegetative growth (Ohm et al., 2010). A role for small GTPase family proteins in regulation of nuclear division and cytokinesis in filamentous basidiomycetes is likely since the constitutively active *ras1* mutant, as well as deletion of the Ras1 specific GTPase activating protein, *gap1*, reduced growth, exhibited a strong phenotype in clamp cell formation, and lacked sexual reproduction (Knabe et al., 2013; Schubert et al., 2006). Another small GTPase known to be involved in actin regulation, Cdc42, induced continuous nuclear division

and swelling of hypha at septation sites when ectopically over-expressed indicating a role in nuclear division as well as in septation (Weber et al., 2005).

The current study could show a distinct, albeit related to ascomycetes', cytoskeleton and nuclear division machinery in basidiomycetes. The specific features of mono-/dikaryotic *versus* multinucleate growth of filamentous fungi, and the formation of clamp connections in basidiomycetes, could be addressed using detailed *in vivo* studies of mitosis and cell division in basidiomycetes.

Author contributions

E-M.J. and M.R. performed the investigation and visualization, M.R. and E.K. provided resources and expertise, and acquired funding. E.-M.J., M.R. and E.K. wrote and reviewed the paper.

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Legends to the Figures

Fig. 1. Nuclear structure and nuclear division in living monokaryotic apical cells expressing H2B::EGFP visualized by epifluorescence (a-j) or light microscopy (k). Growth direction from left to right, and nucleolus indicated by white arrows. Interphase nuclei (a-c), chromatin condensation at the beginning of mitosis (d), separation of a part of the nuclear content from the condensed chromatin (e-f), nuclear division with tightly packed (g) and more open chromatin (h), telophase nuclei separated by 12 µm (i) and 27 µm (j) in the hypha visualized by light microscopy in (k). Telophase movement of nuclei is also shown in supplementary Movie S1. Note the nucleus changes shape from round to elongate. Bars 10 µm and 20 µm in (j). Kymograph for mitosis in monokaryotic *S. commune* l-11 growing from left to right, see suppl. movie S1 for comparison (l). The separation of the two nuclei after mitosis is shown with respect to time (y-axis) and distance (x-axis).

Fig. 2. Nuclear division at clamp formation in dikaryotic hyphae visualized with the expression of H2B::EGFP in epifluorescence illumination (a, c, f-q, r, t) , with bright field microscopy (b, d, e, s) and overlay images (f, q). Growth direction from left to right is indicated by white arrow in (a), stars in (e - l) and tags in (m - p) mark the clamp outgrowth and the dividing nucleus in the clamp outgrowth, respectively. A nuclear pair (a) below the outgrowth for clamp cell development (b), with the nucleolus visible as a transparent area in the front nucleus in (a) are shown. The forward movement of nuclei slows down at this site. From the front nucleus an extension stretches toward the clamp cell outgrowth (c, d; arrows for outgrowth and nucleoli). A bright field image shows the developing clamp of a leading hypha (e), in which the synchronous division was followed by one minute intervals. The overlay image (T0, f) shows both nuclei below the developing clamp; a part of the front nucleus extends into the clamp (compare to c). During the first two minutes, the chromatin of the nuclei condensed and the front nucleus extended into the clamp cell outgrowth (g - h). After three minutes, the front nucleus (between white arrows) had moved into the neck of the clamp cell (i), the size of both nuclei decreased and chromatin became more condensed (j -

l). During the following three minute interval, chromatin condensation progressed until the nuclear size reached about 20 % of the prophase nuclear in diameter (m). At seven minutes, both nuclei assumed a spindle shape. From seven to 10 minutes, the division proceeded to anaphase (n) and then to telophase by the nucleus in hypha (location of telophase nuclei marked by white arrows; o, p). The nucleus within the clamp cell lagged behind and completed mitosis after 11 minutes, probably due to the disturbing effects of long epifluorescence illumination. In the overlay image (q), some chromatin still extends between the telophase nuclei from clamp cell towards the hypha (white arrows). A hypha after completed synchronous division of the nuclear pair shows two nuclei in the tip cell, one nucleus (tag) in the clamp cell and one in the compartment behind the clamp cell (r), also shown with bright field imaging (s). The nuclear pair of the apical cell increased in size and re-formed nucleoli (arrows, t). Bars 10 µm, except for (r, s) with 20 µm. Kymograph for mitosis and clamp formation in dikaryotic *S. commune* I-11 x 12-43 growing from left to right, see suppl. movie S2 for comparison (u). The separation of the two nuclei in the main hypha after mitosis is shown with respect to time (y-axis) and distance (x-axis). The nucleus that goes into the clamp cell is seen in the middle. It goes through mitosis with one nucleus stayin trapped in the clamp, while its second daughter nucleus also moves toward the tip.

Fig. 3: Septation. Monokaryotic (a-c) and dikaryotic (d-f) living hyphae were visualized by Lifeact-EGFP with epifluorescence (a, d) confocal (b and e) microscopy, visualized in 3D views (c and f). Pictures taken from video-microscopy to judge time-line of events (compare suppl. Movie S3). Bars 5 µm.

Fig. 4: Actin cytoskeleton during clamp cell development in living hyphae (compare suppl. Movie S4). Lifeact-EGFP micrographs (a, b, d, e, f, h, i, k, l), light microscopy (c, g, j), and FM4-64 staining of membrane vesicles (m). Cortical actin patches at the plasma membrane at the beginning of clamp cell development (a), increased actin formation in the hypha (b) below the developing clamp cell (c). Cortical actin aggregation (d-e) below the plasma

membrane at the beginning of clamp cell development at higher magnification. The intensity of actin fluorescence increased in the hypha below the developing clamp cell (f-g), where forward movement of nuclei slowed down (compare Fig. 2a, b). Actin distribution in the clamp cell and hypha at the time the nucleus moves into the clamp cell (h - i) and strong Lifeact signal in the tip of the clamp cell at the stage of the dividing nucleus (j). Actin fluorescence during formation of the septum at the base of the clamp cell and in the hypha after telophase was reached (k). Weak actin patches were visible in the clamp cell tip, and bright actin spots at the growing peg in the subacpical cell (white arrow, l), as well as at dolipore formation in the hyphal septum. FMN4-64 stained vesicles at the tip of the clamp cell, in the subapical cell below the clamp cell tip and along the septa (m; growth direction from left to right). Bars 10 µm except for (d, e) with 5 µm.

Figure 1 combined

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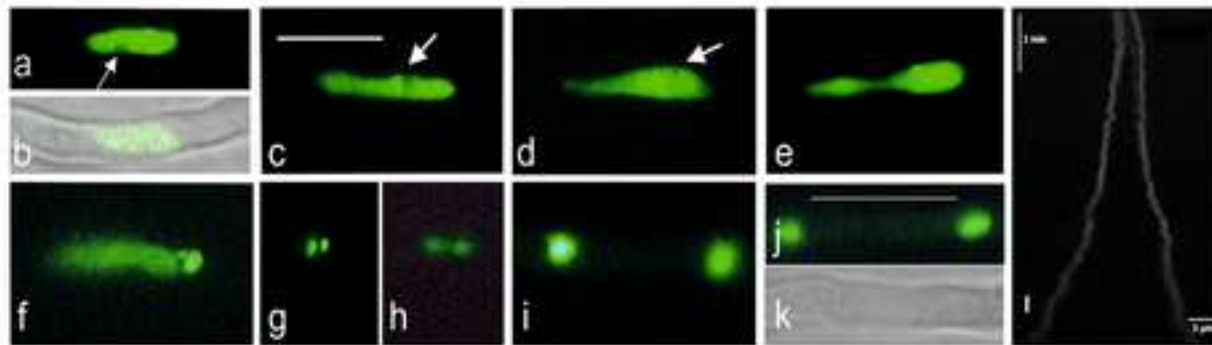

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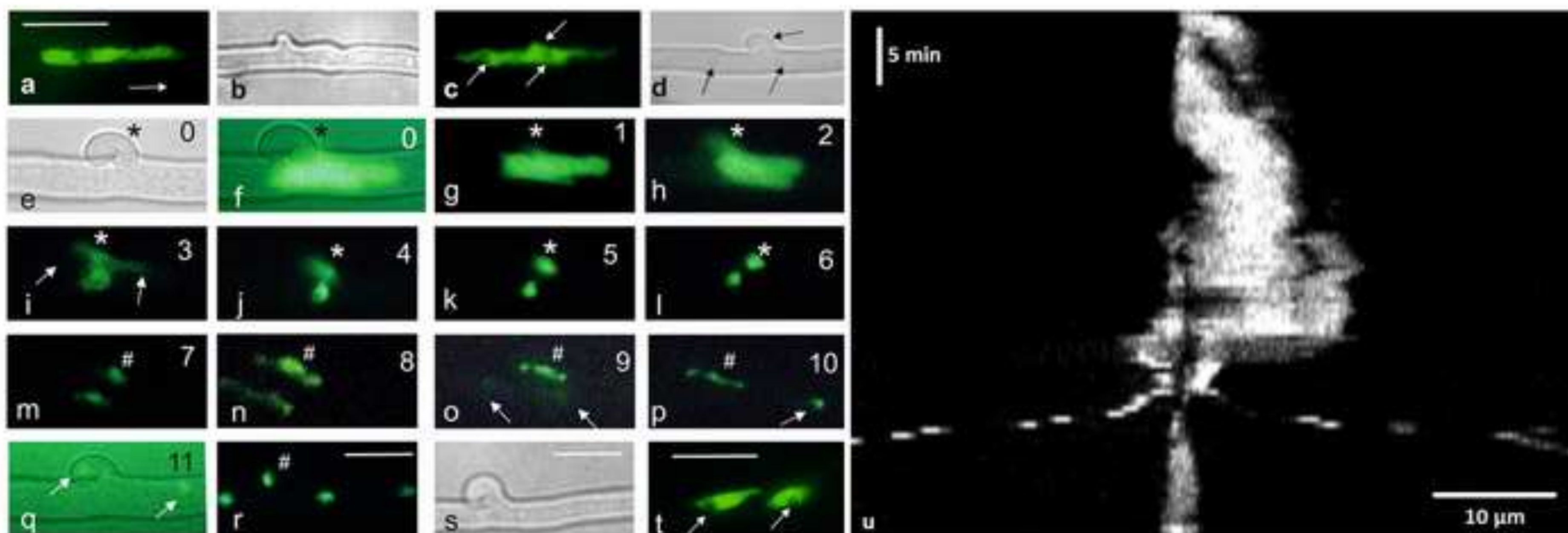

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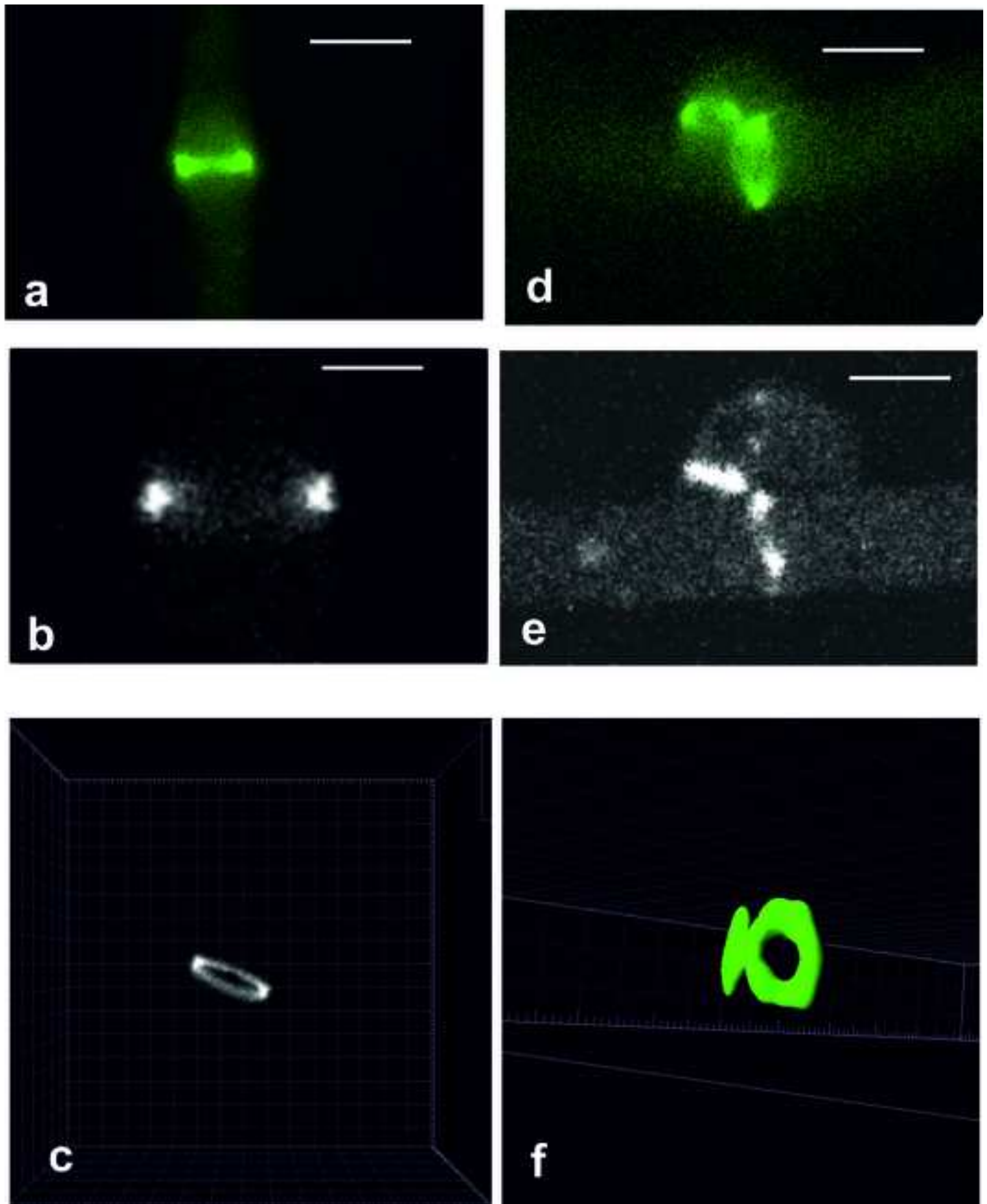

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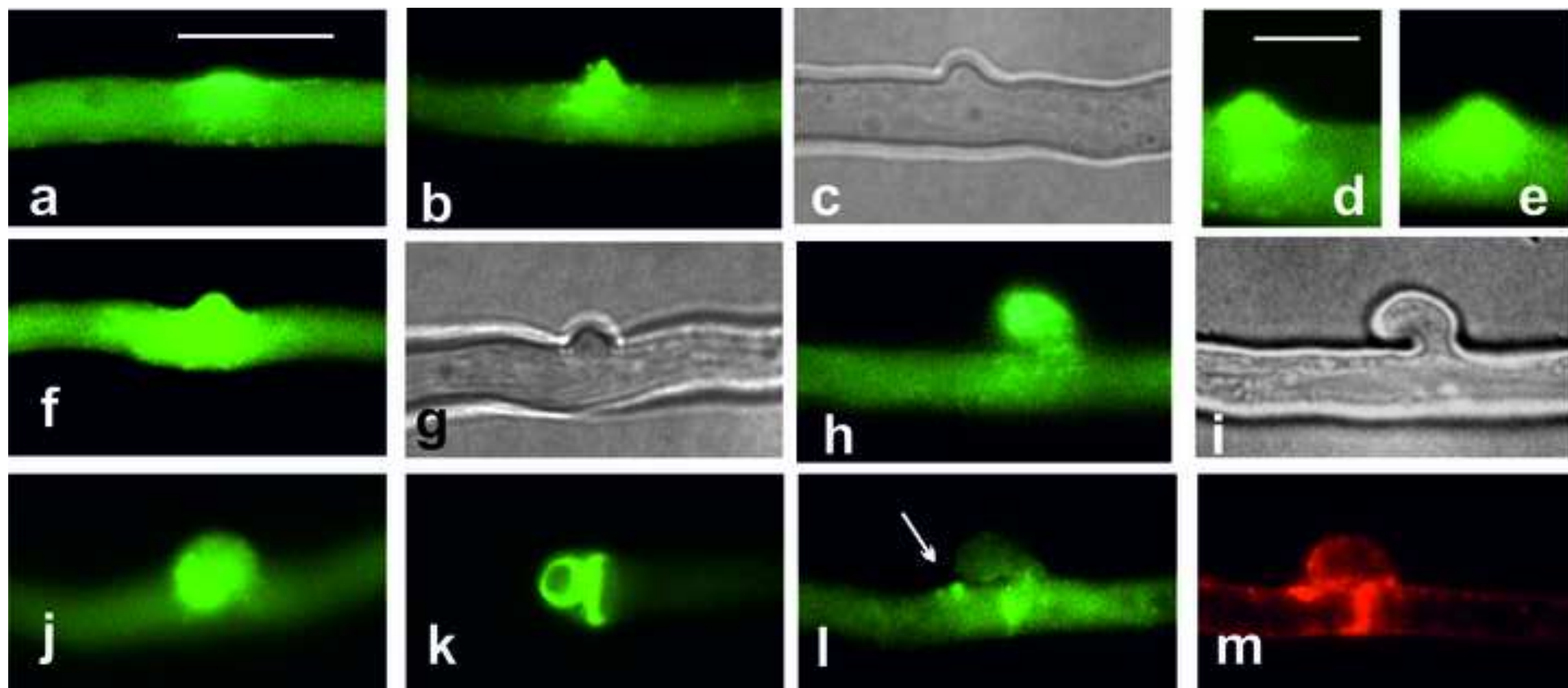

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Movie S1

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