The STRIPAK component SipC is involved in morphology and cell-fate determination in the nematode-trapping fungus Duddingtonia flagrans

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Abstract

The striatin-interacting phosphatase and kinase (STRIPAK) complex is a highly conserved eukaryotic signaling hub involved in the regulation of many cellular processes. In filamentous fungi, STRIPAK controls multicellular development, hyphal fusion, septation, and pathogenicity. In this study, we analyzed the role of the STRIPAK complex in the nematode-trapping fungus Duddingtonia flagrans which forms three-dimensional, adhesive trapping networks to capture Caenorhabditis elegans. Trap networks consist of several hyphal loops which are morphologically and functionally different from vegetative hyphae. We show that lack of the STRIPAK component SipC (STRIP1/2/HAM-2/PRO22) results in incomplete loop formation and column-like trap structures with elongated compartments. The misshapen or incomplete traps lost their trap identity and continued growth as vegetative hyphae. The same effect was observed in the presence of the actin cytoskeleton drug cytochalasin A. These results could suggest a link between actin and STRIPAK complex functions.

Keywords: striatin-interacting phosphatase and kinase (STRIPAK) complex; nematode-trapping fungi; Duddingtonia flagrans; fungal development; trap formation; septation

Introduction

Polar growth, polarity establishment, and maintenance of polarity of cells are common themes in biology and largely depend on cytoskeletal elements and functions. A bona fde example of polar growth is filamentous fungi with their extremely elongated hyphae (Riquelme et al. 2018). Most fungal species grow apically by continuous cell wall and cell membrane extension. Secretion vesicles containing, e.g., cell wall biosynthesis enzymes are transported along microtubules (MTs) toward the tip, accumulate transiently in a so-called vesicle supply center or Spitzenkörper from where they are transported toward the apical membrane. After fusion with the membrane, they deliver their protein content (Riquelme et al. 2007). For the last travel distance, the actin cytoskeleton is required (Bergs et al. 2016; Schultzhaus et al. 2016). The MT and the actin cytoskeletons are coordinated through the action of so-called cell-end marker proteins at the cortex (Mata and Nurse 1997; Takeshita et al. 2013, 2014, 2017). Any variation from the tube-like morphology of hyphae is probably preceded and accompanied by cytoskeletal rearrangements.

One intriguing fungal hyphal morphology is the ring-like structure of traps of nematode-trapping fungi (NTF) (Jiang et al. 2017). These fungi can switch from a saprotrophic to a predatory lifestyle under low-nutrient conditions and in the presence of nematodes. More than 200 NTF have been identified and many are found in the Orbiliaceae (Ascomycota). A wide range of morphologically different traps are produced by different species, ranging from adhesive knobs and columns, adhesive trap rings and networks, and constricting and non-constricting rings (Su et al. 2017). One of the best-studied NTF at the molecular level is Arthrobotrys oligospora, where also the signal exchange between fungus and nematodes and the control of trap formation were studied (Niu and Zhang 2011; Hsueh et al. 2013, 2017; Yu et al. 2021). In the case of Duddingtonia flagrans, a close relative of A. oligospora, ring-like, adhesive traps are formed, in which Caenorhabditis elegans or other nematodes are captured (Youssar et al. 2019). The ring-like morphology of the traps is rather unique and requires a redirection of the polarity axis, cell–cell recognition, and hyphal fusion. This morphological differentiation goes along with physiological differentiation, the production of adhesives, and the preparation for the penetration of caught nematodes. Another fascinating aspect is the determination of the size of the traps. They should have a defined diameter to effectively catch nematodes. If they are too large, nematodes will slip through, and if the diameter is too small, nematodes will not be able to enter the traps. Size determination probably also requires a functional cytoskeleton. How these fascinating morphological changes are initiated and which signaling modules are required are largely unknown.

Although some trapping-deficient mutants have been identified in A. oligospora, the molecular basis and cell biology of trap
morphogenesis are poorly understood (Kuo et al. 2020; Chen et al. 2021). Recently, a random mutagenesis analysis identified 15 mutants with deficiencies in trap morphogenesis. However, there are still difficulties in gene mapping because of heterokaryotic transformants and the lack of genetic crosses of NTF (Huang et al. 2021). In D. flagrans, it was shown that a cell dialog between the tip trap cell and the basal cell is required for ring closure (Youssar et al. 2019).

The early stages of trap formation in D. flagrans resemble the spiral growth of ascogonia or crozier formation of ascomycetes during sexual development (Read and Beckett 1996). An ascogenous hypha elongates and bends over to form a hook-shaped structure. In Sordaria macrospora, a genetic screen has identified several “pro” mutants with defects in sexual development, and some PRO proteins are components of the strati-in-interacting phosphatase and kinase (STRIPAK) complex (Stein et al. 2020; Teichert et al. 2020). The STRIPAK complex is a highly conserved eukaryotic signaling module responsible for the regulation of numerous cellular and developmental processes. It was initially identified in mammals, where it was linked to human diseases like Alzheimer’s, diabetes, and cardiac diseases (Goudreault et al. 2009; Hwang and Pallas 2014). Recently, the structure of the human STRIPAK core was solved by cryo-EM (Jeong et al. 2020). The serine–threonine protein phosphatase 2 (PP2A) core enzyme consists of a scaffold subunit A (PP2A), the catalytic subunit C (PP2AC), and a regulatory subunit of the B” (stratiin) family. In addition, multiple subunits are associated with the core subunits of the phosphatase, including the strati-in-interacting protein 1 or 2 (STRIP1 or STRIP2), the monopolar spindle one-binder (Mob) protein Mob3, cerebral cavernous malformation 3, the sarcolemmal membrane-associated protein (SLMAP), and the coiled-coil protein suppressor of IκB kinase-ε. Furthermore, various germinal center kinases (GCKs) are associated with the STRIPAK complex for enhanced regulation.

The STRIPAK complex is involved in the regulation of key cellular processes including signaling, cytoskeletal organization, cell cycle control, cell migration, cell polarity, apoptosis, Golgi assembly, cell morphology, and vesicular trafficking (Hwang and Pallas 2014; Kück et al. 2019). In Drosophila melanogaster, it was shown that the STRIPAK complex is a negative regulator of the Hippo signaling pathway through its phosphatase activity (Ribeiro et al. 2010). RNAI screening identified the STRIP1/2 orthologs FAM40A and FAM40B to affect cell shape in Drosophila S2 cells (Rohn et al. 2011). In an RNAI screening in PC3 prostate cancer cells, FAM40A-depleted cells were flattened with high levels of cortical F-actin compared to the control, whereas FAM40B-depleted cells were elongated with long thin protrusions (Bai et al. 2011). In Saccharomyces cerevisiae, the STRIPAK homolog Factor arrest protein complex (Far complex) is involved in pheromone-induced cell cycle arrest and affects signaling through the target of rapamycin complex 2 (Kemp and Sprague 2003; Pracheil et al. 2012). In Schizosaccharomyces pombe, the STRIPAK homologous Septation initiation network (SIN) Inhibitory PP2A complex (SIP) negatively regulates SIN and is important for coordinating mitosis and cytokinesis (Singh et al. 2011; Simanis 2015).

Functional analyses in filamentous fungi revealed that different components are required for cell-cell fusion, sexual fruiting body development, septation, secondary metabolite production, symbiotic interactions, and pathogenicity (Xiang et al. 2002; Pöggeler and Kück 2004; Green et al. 2016; Zhang et al. 2018; Elramli et al. 2019). But only recent proteomic and phosphoproteomic studies in S. macrospora revealed an extended signaling network that is modulated by the STRIPAK complex (Märker et al. 2020).

Here, we studied the STRIPAK complex in the NTF D. flagrans, investigated the role of the STRIPAK complex component SipC/STRIP1/2 during septation and the unique hyphal ring formation during trap morphogenesis, and found that SipC is involved in cell-fate determination.

Material and methods

Strains and culture conditions

D. flagrans strains were cultivated at 28°C on potato dextrose agar (PDA; Carl Roth). All fungal strains used in the study are listed in Table 1.

To induce trap formation, the fungal strains were cultivated on thin low-nutrient agar (LNA) slides by adding around 1 × 10^4 D. flagrans spores and around 100–500 C. elegans individuals. Co-incubation was carried out at 28°C in darkness for 12–48 h. C. elegans strains were cultivated at 20°C on nematode-growth medium seeded with Escherichia coli strain OP50 as food source.

Targeted gene deletion of sipC

sipC was deleted by homologous recombination. One kb flanks homologous to the 5’ and 3’ regions of sipC were amplified by PCR with primers with 25 bp overhangs homologous to the hygromycin-B resistance cassette as well as to the pET1.2 plasmid backbone (Thermo Fisher), digested with EcoRV (Tables 2 and 3). The hygromycin-B resistance cassette hph was amplified using plasmid pFCS32 as template, and all fragments were assembled into pET1.2 using the NEBuilder HiFi DNA Assembly Cloning Kit [New England Biolabs (NEB), Frankfurt]. The fragments were amplified using Q5 polymerase (NEB) and the manufacturer’s recommended protocol. Standard protocols were used for E. coli transformation and plasmid isolation. The gene deletion construct was introduced into D. flagrans protoplasts as described in Youssar et al. (2019). For selection of transformants, PDA plates supplemented with 100 µg/ml hygromycin-B were used. To introduce the full-length target gene into the deletion strains, the whole gene including its 1 kb upstream and 0.5 kb downstream regulatory regions was amplified by PCR. To select for positive

| Table 1. D. flagrans strains used in this study |
| Strain | Reference | Strain | Reference |
|-------|-----------|-------|-----------|
| WT CBS 349.94 | CBS-KNAW | sVW03 sipC::hph [ΔsipC]; trpC(p)::hph::trpC(t); gpdA(p)::neo::trpC(t) | This study |
| sVW05 ΔsipC; sipC(p)::sipC::sipC(t); gpdA(p)::neo::trpC(t) | This study | sVW10 h2b(p)::h2b::GFP::h2b(t); gpdA(p)::neo::trpC(t) | This study |
| sVW15 ΔsipC; tubA(p)::mCherry::tubA::tubA(t); gpdA(p)::neo::trpC(t) | This study | sVW16 ΔsipC; tubA(p)::lifact::GFP::gluC(t); gpdA(p)::neo::trpC(t) | This study |
| sVW18 tubA(p)::mCherry::tubA::tubA(t); trpC(p)::hph::trpC(t) | This study | sVW22 ΔsipC; h2b(p)::h2b::GFP::h2b(t); gpdA(p)::neo::trpC(t) | This study |
| sVW24 sVW10 + ΔsipC(p)::h2b::mCherry::h2b(t); trpC(p)::hph::trpC(t) | This study | sVWZ tubA(p)::lifact::GFP::gluC(t); trpC(p)::hph::trpC(t) | This study |
| sVW X p12(p)::p12::mCherry::gluC(t); trpC(p)::hph::trpC(t) | This study | sVW Y ΔsipC; p12(p)::p12::mCherry::gluC(t); gpdA(p)::neo::trpC(t) | This study |
transforms, the geneticin resistance cassette [trpC(p)::neo::trpC(t)] was used. Both fragments were assembled into the pJE12 plasmid backbone using the NEBuilder HIFI DNA Assembly Cloning Kit. The complementation plasmid was introduced into the respective mutant strain via protoplast transformation. For selection of transformants, PDA plates supplemented with 150 μg/ml geneticin sulfate (G418) were used.

**Verification of homologous recombination**

To extract genomic DNA from *D. flagrans*, around 1x 10^8 spores were inoculated in potato dextrose broth and incubated in a 6-cm diameter Petri dish at 28°C for 48 h. Afterward, the mycelium was collected, and DNA was extracted using the protocol described in Zhou et al. (2018). Southern blot analysis was done with digoxigenin (DIG)-labeled probes. Genomic DNA was digested with the appropriate restriction enzyme overnight, separated in a TAE agarose gel and transferred to a nylon membrane. Probes were synthesized using the PCR DIG Probe Synthesis Kit (Roche) according to the manufacturer’s protocol. After probe hybridization, the membrane was hybridized with anti-DIG-AP Fab fragments (Roche) and development was performed using CDP-Star solution (Roche).

**Localization of proteins with GFP and mCherry**

**mCherry-TubA** TubA was tagged with mCherry at the N-terminus expressed from the natural promoter. Using Gibson assembly, PCR-amplified fragments of the hygromycin resistance cassette *hph*, 1 kb tubA promoter, GFP, and tubA open reading frame plus 1 kb downstream regulatory region were cloned into a pJE12 plasmid backbone. To express the construct in *D. flagrans* mutant strains, the tubA(p)::mCherry::tubA(t) fragment was cloned into pJM16 containing the G418 resistance cassette. Plasmids and oligonucleotides are listed in Tables 1 and 2.

**Lifeact-GFP** Lifeact was tagged with GFP at the C-terminus expressed from the *tubA* promoter, a 5' and 2 kb regulatory region were cloned into pJM16 containing the G418 resistance cassette.

**Table 2 Plasmids used in this study**

| Name | Description | Reference |
|------|-------------|-----------|
| pVW28 | sipC::hph (AspC) | This study |
| pVW04 | h2b(p)::h2b::GFP; h2b(t); gpdA(p)::neo::trpC(t) | This study |
| pVW37 | sipC(p)::sipC::trpC(t); gpdA(p)::neo::trpC(t) | This study |
| pVW41 | lifeact::GFP | This study |
| pVW42 | tubA(p)::lifeact::GFP; trpC(p)::hph::trpC(t) | This study |
| pVW56 | p12(p)::h2b::mCherry::gpdC(t); trpC(p)::hph::trpC(t) | This study |
| pVW57 | tubA(p)::mCherry::tubA(t); gpdA(p)::neo::trpC(t) | This study |
| pVW70 | sipC(p)::h2b::mCherry::h2b(t); trpC(p)::hph::trpC(t) | This study |
| pVW77 | tubA(p)::mCherry::tubA(t); gpdA(p)::neo::trpC(t) | This study |
| pVW78 | tubA(p)::lifeact::GFP; gpdA(p)::neo::trpC(t) | This study |
| pVW114 | p12(p)::p12::mCherry::gluC(t); trpC(p)::hph::trpC(t) | This study |
| pVW115 | p12(p)::p12::mCherry::gluC(t); gpdA(p)::neo::trpC(t) | This study |
| pVH02 | p12::mCherry::tubA::trpC(t) | J. Menzner, KIT |
| pJM16 | plasmid backbone containing gpdA(p)::neo::trpC(t) | N. Wernet, KIT |
| pNH20 | plasmid backbone containing tubA(p)::neo::trpC(t) | N. Wernet, KIT |

**Table 3 Oligonucleotides used in this study**

| Name | Sequence (from 5' to 3') | Description |
|------|--------------------------|-------------|
| pro22_ko_LB_rev | GATGGCTCGAGTTTTTCAGCAAGATCGATGCCTAACTGATATGGTAG | AspI |
| pro22_ko_RB_rev | GATGGCTCGAGTTTTTCAGCAAGATCGATGCCTAACTGATATGGTAG | AspI |
| pro22_term_rev | GATGGCTCGAGTTTTTCAGCAAGATCGATGCCTAACTGATATGGTAG | AspI |
| pro22_term_prom_fwd | GATGGCTCGAGTTTTTCAGCAAGATCGATGCCTAACTGATATGGTAG | AspI |
| pro22_term_prom_rev | GATGGCTCGAGTTTTTCAGCAAGATCGATGCCTAACTGATATGGTAG | AspI |
| pro22_term_prom_fwd | GATGGCTCGAGTTTTTCAGCAAGATCGATGCCTAACTGATATGGTAG | AspI |
| pro22_term_prom_rev | GATGGCTCGAGTTTTTCAGCAAGATCGATGCCTAACTGATATGGTAG | AspI |
| pVW114 | p12::p12::mCherry::gluC(t); trpC(p)::hph::trpC(t) | This study |
| pVW115 | p12::p12::mCherry::gluC(t); gpdA(p)::neo::trpC(t) | This study |
| pVH02 | p12::mCherry::tubA::trpC(t) | J. Menzner, KIT |
| pJM16 | plasmid backbone containing gpdA(p)::neo::trpC(t) | N. Wernet, KIT |
sequence coding for lifeact was included in the forward primer to amplify GFP and subcloned into a pJET1.2 plasmid backbone. Using Gibson assembly, the PCR-amplified Lifeact-GFP fragment was cloned into pPH20 containing the tubA promoter and a hygromycin-B resistance cassette. To express the construct in D. flagrans mutant strains, the tubA(p):lifeact:gfp:glucC(t) fragment was cloned into pMJ16 containing the G418 resistance cassette.

**Transcription reporter assay**

To localize the expression of sipC in D. flagrans during different developmental stages, the sipC promoter was fused to the fluorescent reporter gene construct h2b-mCherry. The promoter fragment was amplified by PCR and assembled into the plasmid backbone pVW56 containing the h2b: mCherry reporter and the hygromycin-B resistance cassette by Gibson assembly. The plasmid was transformed into D. flagrans sVW10 expressing h2b(p)::h2b::GFP.

**Quantification of compartment length, conidiation, and trap morphogenesis**

To quantify compartment length of vegetative mycelium, around 1000 spores of the respective D. flagrans strain were inoculated on LNA at 28°C for 24 h. The cell wall was stained with Calcofluor White and images were taken at 100× magnification. For each strain, the length of ten compartments of five germinated spores was quantified and performed in three replicates.

To quantify the amount of conidia produced by the different D. flagrans strains, 8-mm diameter hyphal discs were punched from the edges of a 7-day-old cultured PDA plate, transferred into 500 μl dH2O, and vortexed for 5 min. Conidia were quantified using a Helber counting chamber. Each biological replicate was quantified four times. To quantify chlamydospore quantities and when the promoter is active (Yu et al. 2020). While each data point is depicted as dot, the averages are shown as triangles (Lord et al. 2020).

**Results**

**Conservation of the STRIPAK signaling complex in Duddingtonia flagrans**

To unravel a role of the STRIPAK complex in trap formation, we identified orthologs of S. macrospora PRO11, PP2AA, PP2Ac1, PRO22, SmMOB3, PRO45, SmKIN3, SmKIN24, and SCI1 in D. flagrans (Figure 1). They all share conserved domains with homologs from other fungi, suggesting that D. flagrans harbors a functional STRIPAK complex. In A. nidulans, the STRIPAK complex consists of the striatin scaffold protein StrA and six striatin (StrA) interacting proteins (Sip), and the heptameric complex is involved in the regulation of light-dependent development, the production of secondary metabolites, and the stress response (Elramli et al. 2019). We followed the nomenclature of A. nidulans for the D. flagrans STRIPAK proteins and named the proteins striatin StrA and StrA-interacting proteins (SipA-F). StrA [DFL_002622; 764 amino acids (aa)] contains a conserved striatin domain consisting of a cavelolin-binding domain and a calmodulin-binding domain. Additionally, it contains WD40 repeats at the C-terminus. SipA [DFL_000993; 426 aa] is an ortholog of Mob3 (Phocein) containing the conserved Mob1-Phocein domain. The ortholog of STRIP1/2 SipC (DFL_007682; 1010 aa) harbors the putative N1221 family domain and a domain of unknown function (DUF3402). The SLMAP protein SipD (DFL_001177; 744 aa) harbors a forhead-associated (FHA) domain. The ortholog of the catalytic subunit of the phosphatase PFP2Ac SipE (DFL_003255; 313 aa) contains the catalytic domain of protein phosphatase 2A. The ortholog of the phosphatase regulatory subunit PFP2AA SipF (DFL_008478; 619 aa) contains HEAT repeats (Huntingtin, elongation factor 3, protein phosphatase 2A, and the yeast kinase TOR1). Two putative STRIPAK-associated GCks DFL_002427 and DFL_007266 were identified. No ortholog for SipB was found.

**STRIPAK sipC expression**

As a first step to characterize the functions of the STRIPAK complex in D. flagrans, we analyzed the sipC gene. The open reading frame comprises 3528 nucleotides and is disrupted by nine introns [confirmed by RNAseq data (Youssar et al. 2019)]. To test if the expression levels change in different hyphae, a microscopic reporter assay was used. To this end, we fused the sipC-promoter to the fluorescent reporter gene mCherry which was further linked to the histone h2b gene (h2b), resulting in red fluorescent nuclei when the promoter is active (Yu et al. 2021). As a control, the fluorescent reporter gene h2b-GFP was expressed with the h2b-promoter. The overall expression of sipC was weak. No fluorescence
Hyphal branches became visible. Higher levels of expression were observed in older parts of the mycelium (16 h incubation). During trap formation, no significant changes in expression were detected, and the fluorescence of the reporter construct resembled the intensity of later-stage mycelium. These results suggest a function after germ-tube formation during hyphal differentiation.

**sipC is required for fast vegetative growth and septation**

Since sipC was expressed during septum formation, we next generated a sipC-deletion strain by replacing the gene with a hygromycin-B resistance cassette by homologous recombination. Twenty-nine hygromycin-B resistant transformants were obtained and screened by PCR and Southern blot analysis. One transformant showed the expected integration and was used for further studies (Figure 3A).

Deletion of sipC resulted in slow-growing colonies. While the WT completely covered the medium on a 9-cm Petri dish in 7 days when center inoculated, the ΔsipC mutant needed around 21 days (Figure 3B). The formation of aerial mycelium is a common feature of *D. flagrans* on PDA medium. In the ΔsipC-deletion strain, it was restricted to the first 1–2 cm of the initial inoculation site. Additionally, the ΔsipC mutant showed a 1-cm ring-like growth pattern on PDA. The mutant phenotype could be fully re-complemented with an ectopic copy of the sipC gene, including 1 kb upstream and 0.5 kb downstream regulatory elements.

Since we observed the start of expression of sipC during septum formation, we quantified the number of septa in the ΔsipC-deletion strain after inoculating spores on a low-nutrient medium and incubation for 24 h (Figure 3C). Generally, growth of the ΔsipC strain was slower in comparison to the WT and the respective re-complementation strain. While hyphal compartments in WT and the re-complemented mutant strain had a length of 48.5 ± 5 μm [mean standard deviation (SD)] and 42.6 ± 0.3 μm, respectively (*n* = 150), the compartment size was doubled in the ΔsipC-deletion strain with a length of 102 ± 5 μm. The differences were still present after an additional 24 h of incubation, resulting in decreased numbers of septa compared to WT. In the ΔsipC-deletion strain, septa were mainly found at branching points. Interestingly, whereas the *Apr22* mutant in *S. macrospora* and *Δham2* mutant in *N. crassa* failed to undergo cell–cell fusion (Xiang et al. 2002; Bloemendal et al. 2010), in the *D. flagrans* ΔsipC-deletion strain, the process appeared unaffected (Figure 3D). The frequency of hyphal fusion in the ΔsipC-deletion strain was difficult to compare to WT, since growth speed was heavily reduced.

**SipC is required for asexual development and determines chlamydospore morphology**

Deletion of sipC heavily influenced the number of asexual conidia production, resulting in an 80% reduction of conidia of 7-day-old cultures compared to WT (Figure 4A). Likewise, the amount of chlamydospores produced after 72 h on LNA was reduced by 88% compared to WT. In addition, the morphology of chlamydospores was drastically altered, with 71 ± 3% highly elongated and expanded spores in the mutant (*n* = 3) (Figure 4, C–E). All chlamydospores of the WT had a round shape with an average diameter of 22.7 ± 0.5 μm compared to the chlamydospores of the ΔsipC-deletion strain which had a length of 33.9 ± 0.7 μm. Yet, these spores were still able to germinate. Chlamydospores develop from differentiated vegetative hyphae, while conidia are formed at the tip of conidiophores. Chlamydospores are formed in older mycelium by
condensing the cytoplasm within a compartment into a thick-walled spore. This formation is rather difficult in the \( D\)\(_{\text{sipC}}\)-deletion strain since the compartment size is much larger than in WT.

\textbf{SipC determines the fate of trap cells}

To investigate trap morphogenesis of the \( D\)\(_{\text{sipC}}\)-deletion strain, \textit{C. elegans} was co-incubated with fungal spores on LNA slides for at least 48 h. While trap formation in WT and the re-complemented strain showed ring-like trap networks, this was heavily disturbed in the \( D\)\(_{\text{sipC}}\)-deletion mutant (Figure 5A). Trap loops were often incomplete and resulted in column-like structures. These column-like traps were either identified and distinguished from normal vegetative hyphae by comparing the hyphal diameter or by an increased fluorescence after CFW staining (Figure 5B). An already formed trap can be a new starting point for another trap resulting in more complex trapping networks. Compared to WT, this phenomenon was restricted in the \( D\)\(_{\text{sipC}}\)-deletion strain (Figure 5C).

Trap morphology was restored in the re-complemented strain (Figure 5D). The number of nuclei in trap cells in WT and the \( D\)\(_{\text{sipC}}\)-deletion mutant strain showed no apparent difference (Figure 5E).

Traps of the \( D\)\(_{\text{sipC}}\)-deletion strain were divided into four groups according to the morphology of the trap, and the occurrence was quantified (Figure 6A). Of all counted traps, 77\% showed a column-like structure (I), 13\% a ring-like structure without cell fusion (II), 8\% a 90 degrees bending (III), and 1\% a ring with cell fusion (IV) appearance (total trap number in three biological replicates were \( n = 224\), \( n = 156\), and \( n = 276\), respectively; Figure 6B). In contrast, mostly traps of group II and IV occurred in WT and the re-complemented strain. Despite the morphological differences, traps of the \( D\)\(_{\text{sipC}}\)-deletion strain were still adhesive, and the strain was able to immobilize and digest nematodes (Figure 7A).
Figure 3 SipC is required for asexual development and determines chlamydospore morphogenesis. (A) Quantification of the number of conidia and chlamydospores in WT, the ΔsipC mutant, and the re-complemented strain. (B) Microscopic pictures of conidia of the WT and the ΔsipC-mutant strain. (C) Microscopic pictures of chlamydospores of the WT and the ΔsipC-mutant strain. (D) Overview of chlamydospores of the WT and the ΔsipC-mutant strain. (E) Quantification of chlamydospore morphology in the ΔsipC strain and quantification of chlamydospore length of the WT, the ΔsipC mutant, and the re-complemented strain. Each biological replicate is color-coded (orange, blue, gray). While each data point is depicted as dot, the averages are shown as triangles. Error bars show the SD.
Traps in WT usually consist of two to four compartments before fusing to the neighboring basal hypha. In contrast, compartments of the traps in the ΔsipC deletion strain were highly elongated as in vegetative mycelium. Interestingly, the elongated trap cells often switched to a vegetative growth mode after one, or a maximum of three trap compartments, indicated by a thinner diameter of the hyphae. The change of cell fate from trap cells to normal vegetative hyphae was confirmed by expressing the ortholog of the trap-specific serine protease P12 (DFL_008096) tagged with mCherry in the WT and the ΔsipC mutant background. Serine proteases have been identified as crucial virulence factors upon exposure to nematodes, and the *A. oligospora* P12 was highly upregulated during nematode exposure (Yang et al. 2011; Zhen et al. 2018). P12-mCherry localized in dynamic spots inside the traps of the WT and the ΔsipC mutant (Figure 7B). The change of cell fate from trap cells to normal vegetative hyphae in traps of the ΔsipC-deletion strain was indicated by the absence of fluorescent accumulations during trap morphogenesis. This change also coincided with a change in hyphal diameter (Figure 7C). Additionally, we observed dynamic spots of P12-mCherry with weaker intensities in neighboring compartments. Therefore, we visualized the promoter activity of *p12* as described above by fusing the *p12* promoter to the fluorescent reporter construct of H2B-mCherry and transformed the plasmid into a strain expressing H2B-GFP under the control of the *h2b* promoter. Promoter activity was also present in neighboring cells but decreased in more distant vegetative hyphae (Figure 7D).
LifeAct with GFP. In filamentous fungi, the minus ends of MTs locate at spindle pole bodies which serve as MT-organizing centers (MTOC). Additionally, areas close to the septum can act as septal MTOC (sMTOC) (Zekert et al. 2010; Gao et al. 2019). In the tip compartments, we observed dynamic MTs reaching the hyphal tip (Figure 8A). In trap cells, the most visible MTs nucleated from septal MTOCs (Figure 8A). Interestingly, major bending of the trap cell was only visible after the first septum at the base (Figure 8, B and C). Although MTs were dynamic, long MTs were observed at the inner side of the trap, reaching from the septum to the tip. In the ΔsipC mutant, MT localization appeared similar (Figure 8D). To study the role of MTs during trap morphogenesis, we added cytoskeleton-destabilizing drugs during trap development. The presence of benomyl (5 μg/ml) led to hyperbranching of the mycelium (Figure 8E). Hyphal growth during trap development stopped after the addition of benomyl (Figure 8F). Depolymerization of MTs was confirmed by imaging mCherry-labeled MTs after the addition of benomyl (Figure 8G).

Next, we visualized actin using the actin-binding peptide LifeAct-GFP (Riedl et al. 2008). Mostly, actin patches at the hyphal tip and subapical regions as well as the actomyosin ring during septum formation were visualized (Figure 9, A and B). Rarely, dynamic actin cables at the apex of hyphae were observed. Expression of LifeAct-GFP in the ΔsipC mutant revealed that septum formation was delayed during trap morphogenesis, resulting in elongated cells with decreasing hyphal diameter (Figure 9, C and D). The ΔsipC-deletion strain did not show any difference in the sensitivity toward cytochalasin A as compared to WT (data not shown).

To study the role of actin during trap morphogenesis, we added the F-actin-destabilizing drug cytochalasin A (5 μg/ml) during trap development, which led to swelling of hyphal tips of vegetative mycelium (Figure 9D). This effect was less obvious in growing traps; however, they continued to grow as vegetative hyphae at the end of the reaction (Figure 9E; Supplementary Movie S1). These results suggest a putative function of SipC in regulating cytoskeleton dynamics. However, we did not see apparent differences in the actin organization in the ΔsipC-deletion strain as compared to WT.

### Discussion

The STRIPAK complex is involved in many cellular processes in eukaryotes. In filamentous fungi, it is important for hyphal fusion, sexual development, and hyphal growth (Kück et al. 2019). In this study, we show that the D. flagrans STRIP1/2 ortholog SipC plays a role in septation, conidiation, and trap morphogenesis...
and is important for cell-fate determination. Although the early steps of trap formation appeared unaffected in a sipC-mutant strain, trap cells apparently lost their memory and re-differentiated into normal vegetative hyphae. The same phenotype was observed after disturbance of the actin cytoskeleton. Disturbance of the MT cytoskeleton had a completely different effect and blocked further growth of traps. There are numerous examples for the role of the STRIPAK complex in cytoskeletal functions.

Figure 6 SipA controls the expression of trap-specific genes. (A) Trapping of C. elegans by traps of the ΔsipC mutant. The cell wall of the fungus is stained by CFW. A C. elegans strain expressing a C-terminal GFP fusion protein of the histone HIS-72 was used to distinguish between digested (surrounded by a dashed line) and trapped but alive (arrow) nematodes. (B) C-terminal mCherry tagging of the serine-protease P12 (colored in yellow) in the WT and the ΔsipC mutant. The arrow shows a septum and indicates the switch from a trap cell to vegetative growth. (C) The relative fluorescent intensity (RFI) along a ΔsipC trap was measured and plotted (x-axis displays the distance in μm; y-axis displays the measured RFI). Arrows indicate the septa of the trap. (D) Visualization of the expression of p12 during trapping of C. elegans using a H2B-mCherry reporter construct. The expression of H2B-GFP under the h2b promoter was used as control. Arrows indicate transition points of reduced p12-mCherry expression in vegetative hyphae.
It was reported that the Strip-dependent regulation of the GCKs MST3 and MST4 modulates actomyosin contraction and therefore cell motility in cancer cells (Madsen et al. 2015). Our results raise the question of possible links among the STRIPAK signaling complex, the actin cytoskeleton, and cell-fate determination in the traps of *D. flagrans*.

In filamentous fungi, F-actin forms actin cables, rings, and patches (Berepiki et al. 2011). Actin cables are nucleated by formins and localize at the apex of hyphae, where they are involved in polar growth (Riquelme et al. 2018). Filamentous fungi usually only encode one formin and deletion of the gene in *A. nidulans* or *N. crassa* is lethal (Sharpless and Harris 2002; Justa-Schuch et al. 2010). Interestingly, the STRIPAK subunit PRO45 was found as an interaction partner of formin in *S. macrospora*, although the exact molecular function of the interaction has not yet been solved (Stein et al. 2020). It is conceivable that PRO45 influences the activity of formin. Additionally, a class II myosin was found as an interaction partner of the STRIPAK complex component PRO45 in *S. macrospora* (Nordzieke et al. 2015). The fact that *D. flagrans* sipC mutants grow very slow may reflect the role of SipC on the actin cytoskeleton in the apical dome. The effect is clearly different from the effect of cytochalasin, which destroys the actin cytoskeleton. In addition to an effect on polar growth, sipC mutation had a strong effect on septation. Comparable to the effect on hyphal growth, septation is still possible without SipC, but the timing appears to be different. Although this has no obvious effect on vegetative hyphae, the process appears to be crucial for trap-cell memory. A similar effect was observed during sexual development of the *S. macrospora* mutant Δpro22, where aseptated ascogonia showed a developmental arrest at the protoperithecial stage (Bloemendal et al. 2010). This defect resulted in elongated ascogonial coils.

In addition, to a direct effect of SipC on the actomyosin system, there is good evidence in *S. pombe* that the SIN can be regulated by the STRIPAK complex. It was shown that SIP-mediated dephosphorylation of the Ste20 kinase Cdc7p initiates SIN assembly (Singh et al. 2011). Misregulation of SIP inhibits the assembly of SIN. In *A. nidulans*, the Cdc7p ortholog SepH is required for initiation of septation prior to actin ring assembly. While downregulation of sepH results in a loss of septation, overexpression results in hyper-septation (Bruno et al. 2001). A similar phenotype was observed in *S. macrospora* in a Smkin3 mutant. Smkin3 is the ortholog of *A. nidulans* SepL, and the downstream target of SepH (Radchenko et al. 2018). In this study, the deletion of the STRIPAK-complex component sipC resulted in a drastic reduction of septa and trap compartments of very different sizes.

Taken together, there are multiple links how SipC may affect the actin cytoskeleton and septation. Compartment size appears to be very important for cell-fate determination during trap formation.

### Data availability

Strains and plasmids are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables. Supplementary material is available at figshare: https://doi.org/10.25386/genetics.16617721.

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**Figure 7** Characterization of the microtubule cytoskeleton. (A) Visualization of an N-terminal mCherry fusion protein of the alpha-tubulin TubA in a vegetative hypha and trap of *D. flagrans* WT (colored in yellow). The cell wall was stained by CFW (colored in magenta). Asterisks indicate the localization of septal MTOCs. Three-dimensional reconstruction. (B) Microtubules in a forming trap, where still no septum was formed at the base (asterisk). (C) MTs in a bending trap. The asterisk indicates a septal MTOC and the presence of a septum. The arrow points to microtubule bending at the inner site of the trap. (D) Localization of mCherry-TubA in a trap of the ΔsipC mutant. (E) Hyperbranching of vegetative hyphae after 1 h treatment with the microtubule-depolymerizing drug benomyl (5 μg/ml). (F) The addition of benomyl (5 μg/ml) stopped trap morphogenesis in *D. flagrans* WT. Forming traps are labeled with an asterisk. (G) Disassembly of MTs after 5 min treatment with benomyl (5 μg/ml).
Figure 8 Characterization of the actin cytoskeleton. (A) Actin patches at a hyphal tip and an actin-ring during septum development visualized with Lifeact-GFP (colored in green). Three-dimensional reconstruction. (B) Actin during trap formation and in a mature trap. (C) Actin trap morphogenesis in the ΔsipC mutant strain. The white arrow indicates the septum at the base. The cell wall was stained by CFW. (D) Septum formation in a column-like trap in the ΔsipC mutant strain. The arrow indicates septum development by localization of Lifeact-GFP at a developing septum. The asterisk indicates a mature septum without GFP signal. (E) The addition of the actin-depolymerizing drug cytochalasin A (5 μg/ml) leads to swollen hyphal tips. As solvent control, hyphae were treated with 0.5% DMSO. (F) Loss of trap identity after the addition of cytochalasin A (5 μg/ml). After some time, traps continued to grow as vegetative hyphae (see also Supplementary Movie S1).
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**Conflicts of interests**

The authors declare that there is no conflict of interest.

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