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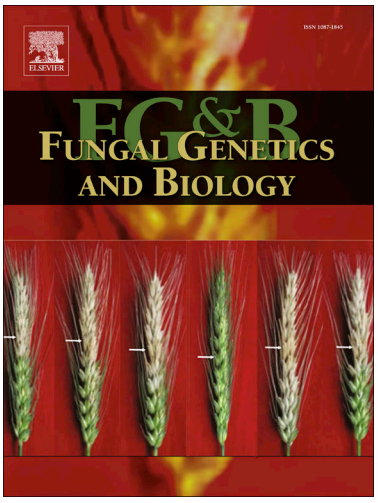

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Investigating the cell and developmental biology of plant infection by the rice blast fungus *Magnaporthe oryzae*

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Abstract

Magnaporthe oryzae is the causal agent of rice blast disease, the most widespread and serious disease of cultivated rice. Live cell imaging and quantitative 4D image analysis have provided new insight into the mechanisms by which the fungus infects host cells and spreads rapidly in plant tissue. In this video review article, we apply live cell imaging approaches to understanding the cell and developmental biology of rice blast disease. To gain entry to host plants, *M. oryzae* develops a specialised infection structure called an appressorium, a unicellular dome-shaped cell which generates enormous turgor, translated into mechanical force to rupture the leaf cuticle. Appressorium development is induced by perception of the hydrophobic leaf surface and nutrient deprivation. Cargo-independent autophagy in the three-celled conidium, controlled by cell cycle regulation, is essential for appressorium morphogenesis. Appressorium maturation involves turgor generation and melanin pigment deposition in the appressorial cell wall. Once a threshold of turgor has been reached, this

triggers re-polarisation which requires regulated generation of reactive oxygen species, to facilitate septin GTPase-dependent cytoskeletal re-organisation and re-polarisation of the appressorium to form a narrow, rigid penetration peg. Infection of host tissue requires a further morphogenetic transition to a pseudohyphal-type of growth within colonised rice cells. At the same time the fungus secretes an arsenal of effector proteins to suppress plant immunity. Many effectors are secreted into host cells directly, which involves a specific secretory pathway and a specialised structure called the biotrophic interfacial complex. Cell-to-cell spread of the fungus then requires development of a specialised structure, the transpressorium, that is used to traverse pit field sites, allowing the fungus to maintain host cell membrane integrity as new living plant cells are invaded. Thereafter, the fungus rapidly moves through plant tissue and host cells begin to die, as the fungus switches to necrotrophic growth and disease symptoms develop. These morphogenetic transitions are reviewed in the context of live cell imaging studies.

Key words

appressorium, septins, actin, effectors, biotrophy, necrotrophy

Introduction

The blast fungus *Magnaporthe oryzae* (synonym of *Pyricularia oryzae*) [1] is able to infect more than 50 different grass species, including staple crops such as rice, millets and barley [2]. It is estimated that rice blast disease causes losses of around 6% of the global rice harvest every year [3] across all rice-growing regions of the world, but epidemics routinely cause up to 30% yield losses [4]. Rice blast therefore represents a severe problem in the 85 countries where rice is grown [5] and to 50% of the world's population who depend on rice as their main source of calories [6].

M. oryzae furthermore has the capacity to jump from one host to another. Wheat blast, for example, first appeared in 1985 in Brazil following a likely host jump from a grass-infecting isolate of the fungus [7]. Increases in global trade since that time, have allowed the disease

to spread, emerging in 2016 in Bangladesh, where it now threatens wheat production [8], with potential to spread to India, the world's second largest wheat producer [9]), and most recently to Zambia [10]. When considered together with the ongoing rice blast disease pressures world-wide, *M. oryzae* represents a very significant threat to global food security. Learning about the basic biology of blast disease is therefore important if new disease-control strategies are to be developed.

The blast fungus has been extensively studied over the past three decades, facilitated by its genetic tractability and development of a suite of molecular genetic tools and genomic resources [11]. Indeed, *M. oryzae* is now a model system that has revealed important concepts associated with fungal-plant interactions [1, 11], such as the molecular basis of appressorium morphogenesis [12, 13] appressorium function [4, 14], secretory processes associated with effector proteins [15], structure-function relationships governing pathogen recognition by host immune receptors [16, 17] and pathogen genome organisation [18, 19].

This video article focuses on a series of investigations that have provided new insight into the manner in which the fungus is able to infect rice plants. We start by reviewing the morphogenetic changes associated with development of an appressorium on the rice leaf surface. We highlight cellular changes accompanying appressorium development and how infection-related morphogenesis is controlled by cell cycle progression. We then describe the manner in which *M. oryzae* re-models its cytoskeleton during appressorium maturation, leading to formation of the rigid penetration peg, which ruptures the leaf cuticle. Next, we outline the biology of plant infection by the rice blast fungus, revealing the extraordinary capacity of this pathogen to proliferate within living host tissue and overwhelm plant defences. The use of quantitative 4D imaging and high-resolution video microscopy has provided unparalleled insights into the biology of this devastating plant pathogen and allows new studies to take place with far greater resolution than was previously possible.

Cell cycle control and regulated autophagy are necessary for appressorium formation

Conidia of *M. oryzae* germinate rapidly on the leaf surface, forming a polarised germ tube within two hours of contact with a hydrophobic surface. The spore sticks tightly to the leaf surface by means of spore tip mucilage, released from an apical compartment upon hydration [20]. The germ tube usually emerges from the apical cell of the conidium, extending for a short distance (15-30 μm see Video 1 from 2:00), while adhering tightly to the underlying surface, before swelling and hooking at its tip to form a specialised infection structure, the appressorium (Figure 1A, B, Video 1 from 2:15 to 3:35) [21]. Appressorium differentiation requires S-phase to have been completed by the nucleus within the germinating conidial cell [22]. A single round of mitosis is then necessary to enable maturation of the appressorium [22, 23]. As the appressorium matures, its cell wall becomes lined with a thick layer of melanin necessary for the development of turgor by the appressorium (Video 1, 4:00-7:50). At the same time, glycerol accumulates to molar concentrations to generate hydrostatic turgor due to rapid influx of water into the cell. The conidium undergoes an autophagy-dependent process leading to cell death, trafficking the contents of all three conidial cells into the appressorium (Video 1, conidial cell death is achieved by 10:00), which is necessary for infection [23]. Conidial collapse has been reported to involve ferroptosis as the ultimate mechanism leading to cell death [24]. To investigate nuclear division, a strain of the fungus expressing a Histone H1: green fluorescent protein (GFP) gene fusion [23] was imaged during appressorium differentiation over a period of 24 hours. After the first round of mitosis (Video 2, 4:00-4:10), one daughter nucleus from the germinating conidial cell migrates into the incipient appressorium, leaving the remaining nuclei to be degraded by autophagy-mediated conidial cell death. (Figure 1C, Figure 2A, B), as shown in Video 2 (conidial nuclei degraded by 13:45) [23, 25]. During appressorium maturation, the nucleus in the appressorium then arrests in G1 before progression through S-phase, which is a necessary pre-requisite to re-polarisation [26, 27]. Mutants impaired in melanin accumulation do not mature or trigger the S-phase checkpoint. A turgor-dependent cell cycle checkpoint therefore regulates appressorium function, leading to cytoskeletal re-organization and penetration peg emergence (Figure 2C)

[22]. After re-polarisation and plant infection, the appressorium remains mitotically active [28], providing nuclei into nascent invasive hyphae [29].

The Pmk1 MAP kinase signalling pathway regulates appressorium morphogenesis

The development of appressoria in response to the hard, hydrophobic leaf surface and absence of exogenous nutrients also requires the Pmk1 MAP kinase signalling pathway, which is critical to invasive growth by *M. oryzae* [30], a function conserved in many diverse pathogenic fungal species [31]. Pmk1 is a homologue of the *Saccharomyces cerevisiae* Fus3 kinase associated with pheromone signalling [32] and the wider pathway links cell surface perception with appressorium morphogenesis [12, 31]. As a consequence, Δ*pmk1* mutants fail to form appressoria, and do not undergo autophagic conidial cell death, as shown in Figure 2D, E. Pmk1 also, however, plays a role subsequent to appressorium-mediated infection. A conditional, analogue-sensitive mutant of Pmk1 (*pmk1*^{AS}) is, for example, unable to move from cell-to-cell at pit field sites, containing plasmodesmata, during rice tissue invasion when inactivated by the specific kinase inhibitor 1 napthyl-PP1 (Figure 2F) [32, 33]. Pmk1 is activated by a MAPKK (Mst7) and a MAPKKK (Mst11) [34], which in turn are regulated by a putative scaffold protein Mst50 [35]. The Pmk1 MAPK cascade controls the activity of a very large number of downstream targets involved in development and pathogenesis. The transcription factors Hox7 and Znf1, for example, are essential for appressorium formation while Mst12 is required for penetration and invasive growth [36-38]. Consequently, the Pmk1 MAPK pathway is fundamental to development of appressoria, although how it is activated by cell surface signals, in particular, is still not well understood.

Septin-dependent re-polarisation of the appressorium

Once formed, appressoria develop enormous turgor of up to 8.0MPa, by developing a melanin-rich cell wall (Video 1) that is able to retain glycerol and other polyols. [39] A critical threshold of turgor allows the fungus to re-orientate its cytoskeleton, and transition from isotropic expansion to anisotropic, polarised, growth during plant infection. A turgor-sensing

histidine aspartate kinase, Sln1, is necessary for modulation of turgor, and acts as a regulator of the downstream pathways required for appressorium repolarisation [40]. These include, most notably, the aggregation of a hetero-oligomeric complex of septin GTPases which form a toroidal structure at the base of the appressorium. Septins re-organise F-actin to the precise point of plant infection [41]. A *M. oryzae* strain expressing Sep5-GFP under control of the native Sep5 promotor allows visualisation of septin ring dynamics during a period of 0-24 hours, when conidia are incubated on hydrophobic glass coverslips (Video 3; Figure 3A). Septin recruitment to the appressorium pore begins to occur markedly from 7:00 (Video 3) and the ring then becomes apparent by 9:48, showing some constriction by 16:00. The ring is then maintained in appressoria incubated on a non-yielding surface. By contrast, on rice leaf sheath which can be penetrated by appressoria, the ring forms in the same manner but then undergoes further constriction to a diameter of approximately 0.9-1.1μm after 28 hpi when the penetration peg is formed (Figure 3A, Figure 4, Video 3 and Video 4). A recent study using quantitative 4D widefield fluorescence imaging has revealed the spatiotemporal dynamics of F-actin and septin ring recruitment and organisation at the appressorium pore [42]. The septin ring provides cortical rigidification and acts as a diffusion barrier for the action of polarity determinants, endocytic proteins, the exocyst complex, and actin-binding proteins [41, 43]. In addition to F-actin, the microtubule cytoskeleton is re-oriented in the direction of cuticle penetration [42]. Organisation of septins in the appressorium involves very long chain fatty acids (VLCFAs), that act as mediators of septin interactions at membrane interfaces. Inhibiting VLCFA biosynthesis therefore prevents rice infection by *M. oryzae* providing a new potential fungicidal target [44]. Assembly of the septin appressorium pore complex is also controlled by the Nox2/NoxR complex which regulates septin-mediated cytoskeletal dynamics. The actin-binding protein gelsolin, for instance, which regulates actin dynamics by uncapping free barbed ends to promote actin polymerisation, is a likely target for regulation by ROS [45] and an important component of the appressorium pore. We generated a *M. oryzae* strain expressing Gelsolin-GFP under control of the native Gelsolin promotor. Using 3D maximum projection, Z-stack images were captured at 24hpi (Figure 3B, Video 5) [45]. Gelsolin forms a

highly organised toroidal structure situated at the base of the appressorium (Video 5) in a septin-dependent manner [25]. Septin and F-actin ring formation also requires the Pmk1 MAP kinase pathway and the putative downstream transcription factors, such as Mst12 [41] and Hox7 [37], as shown in Figure 3C. The turgor sensing Sln1 kinase is necessary for determining when a threshold of hydrostatic pressure has been reached in the appressorium to enable re-polarisation. As a consequence, Δ*sln1* mutants are unable to organise septins at the site of penetration and a clear ring structure does not form (Figure 3B). Sln1 acts in parallel with the protein kinase C cell integrity pathway to suppress melanin biosynthesis and the cAMP-dependent signalling protein kinase A pathway, thereby modulating turgor generation [40]. In addition, the turgor-dependent S-phase checkpoint is triggered enabling septin mediated plant infection (Figure 5) [22, 40]. Another significant factor in enabling the appressorium to function is its attachment to the leaf surface. Critically, the force of fungal attachment must exceed the force of penetration at the peg, otherwise the cell would simply lift off the surface. It has been shown that spermine synthase (*SPS1*) is important for surface attachment by buffering reactive oxygen species generation in the endoplasmic reticulum, thereby facilitating efficient mucilage secretion that provides the tight seal necessary for the appressorium to function. In the absence of Sps1, appressoria do not adhere tightly to the plant surface, impairing infection [46]

The cell biology of invasive growth by M. oryzae

Once the fungus has penetrated the leaf, it develops primary invasive hyphae (IH) that invaginate the plant plasma membrane within the first epidermal cell colonized. The membrane around the invasive hyphae is known as the extra-invasive hyphal membrane (EIHM), but is entirely a plant-derived membrane which tightly surrounds the fungal cell wall [47, 48]. The fungus secretes a battery of effectors proteins during this time, which are either directed to the apoplast– the space between the fungal cell wall and the EIHM –or the cytoplasm of the host cell. Fungal effectors suppress plant host immunity to facilitate colonisation of plant tissue (Figure 6A, B) [47, 49]. A membrane-rich structure forms at the tip

of the primary IH and the fungus appears to then bud from this point, whereby the membrane-rich structure forms the biotrophic interfacial complex (BIC), a plant derived membrane-rich body located outside the fungal cell wall [15, 50]. Cytoplasmic effectors, such as AVR-Pik and Pwl2, accumulate at the BIC and are also translocated into plant cells. A bright single punctum of AvrPik-GFP is, for example, observed defining the BIC structure, as shown in Figure 6A. By contrast, apoplastic effectors such as Bas4 outline the invasive hyphae contained within the EIHM (Figure 6B) when expressed as a Bas4:mCherry fusion protein, although the BIC is also visible (Video 6). Secretion of effectors to these distinct destinations is directed by different secretory pathways. Apoplastic effectors are secreted in a Golgi-dependent, brefeldin A-sensitive manner to the invasive hyphal tip. By contrast, cytoplasmic effectors are secreted from the BIC-adjacent cell into the BIC in an exocyst-dependent pathway that is brefeldin A-insensitive [15]. It has been shown that the TOR (Target-Of Rapamycin) nutrient-signaling pathway is important in mediating membrane integrity at the plant-fungal biotrophic interface. Deletion of a novel vacuolar protein gene *IMP1*, led to mutants impaired in BIC formation which released apoplastic effectors into the plant host cytoplasm [51]. The deployment of a large battery of effector proteins enables *M. oryzae* to overcome plant immunity and rapidly move from the first invaded cell to neighbouring cells, through pit fields. Interestingly, this involves a specific morphogenetic transition in which the hyphal tip swells when it makes contact with a pit field, followed by severe hyphal constriction to around 360 nm in diameter (equivalent to a pit field) and then emergence of a new invasive hypha in the neighbouring cell. This morphological transition is regulated by the Pmk1 MAP kinase [33, 47] (Figure 6C, and Video 7). When an analogue-sensitive *pmk1*^{AS} mutant is allowed to infect a host cell and then treated with the 1NA-PP1 inhibitor, it becomes trapped within the epidermal cell [15]. The Pmk1 MAPK pathway is therefore required for septin-dependent constriction of invasive hyphae at cell wall crossing points in the same way as it is necessary for appressorium morphogenesis. Each crossing point can be clearly seen as being preceded by pronounced swelling of the invasive hypha (Video 7, 517 min), followed by severe constriction as the hypha moves into the next cell (Video 7, 641-713 min). Often mitosis is seen to occur just after the

time of cell-to-cell movement [29, 52], which can be visualised by observing tissue invasion in a *M. oryzae* strain expressing H1-GFP (see Video 8, from 34 min, in which the arrow shows cell crossing point, with mitosis occurring shortly afterwards by 36 min). Appressorium formation and cell wall crossing therefore have many common features requiring swelling into a yeast-like hemispherical cell, followed by re-polarisation and emergence of a narrow penetration peg or IH peg, respectively. The cell wall crossing structure has been termed the 'transpressorium', because of the resemblance to appressorium formation (see Video 9 in which the terminal swelling and infection peg formation can be seen in the 3D image.) [53]. The regulation of transpressorium formation by Pmk1 and the requirement for septin-mediated invasion [15] are consistent with the morphological conservation.

The suppression of immunity responses by *M. oryzae* enables the fungus to colonise leaf tissue very efficiently. This is illustrated by Video 10 in which a strain expressing a nucleolar marker Fib1-GFP is seen to rapidly colonise plant tissue between 24 and 38h after inoculation. During this period the number of fungal nuclei observed increases from 18 to 85 in the field of view shown, highlighting the speed of cell division that accompanies fungal invasive growth in this 14h period. A three-dimensional reconstruction of invasive hyphae demonstrates the extent of host colonization, as shown in Video 11. Here, fungal invasive hyphae are visualised using fluorescent wheat germ agglutinin, within propidium iodide-labelled plant tissue. Invasive hyphae are large bulbous and branched as they spread within epidermal and mesophyll cells, but more elongated as they move into vascular tissue. The colonisation of tissue is extremely rapid, with plant cells losing viability at the centre of a fungal colony as the fungus continually moves into viable cells, maintaining EIHM integrity as it does so. The central part of the colonies, which becomes visible as a disease lesion, will then produce aerial hyphae that develop into conidiophores bearing sympodial arrays of conidia.

Conclusions

In summary, live cell imaging studies have dramatically expanded our understanding of how rice plants are infected by the blast fungus *M. oryzae* [22, 40, 41, 43, 45]. Generation of functional appressoria provides the gateway for establishing rice blast disease, and it is now clear that this is a highly orchestrated developmental process, requiring several important prerequisites– such as perception of the hard-hydrophobic leaf surface, and response to cutin monomers or components of plant epicuticular waxes [21]. Next, the fungus utilises two key signalling pathways, the cAMP protein kinase A pathway and the highly conserved Pmk1 MAP kinase signalling pathway, regulated by G- protein signalling in the developing germ tube tip. The Pmk1 signalling pathway regulates appressorium formation, maturation, invasive growth and infection of plant tissue [32]. Pmk1, for instance, regulates expression of many genes encoding secreted fungal effectors proteins implicated in the suppression of host immunity, in addition to its better known role in symmetry-breaking and morphogenesis [33]. Appressorium development is, however, also tightly coupled to cell cycle progression [4, 22, 26], which is itself linked to nutrient availability and the critical role of TOR kinase as a growth regulator that is fundamental to the control of autophagy [14, 54, 55]. Once formed, the appressorium undergoes further changes and the role of glycerol and melanin biosynthesis in turgor generation are well established, but still lacking in specific details, particularly regarding gene regulation and the precise enzymatic activities necessary at each step in these pathways. A turgor-sensing mechanism has also now been reported for appressoria, linking turgor control to re-polarisation of the infection cell.

Many questions, however, remain to be answered regarding operation of an appressorium. How does the turgor-sensing complex work in *M. oryzae* and what are its components? How precisely does Sln1 negatively regulate melanin biosynthesis, interact with the Pkc1 cell integrity pathway and negatively regulate glycerol production via the cAMP/PKA pathway? What is the precise trigger for septin aggregation at the appressorium pore and how does this differ from septin recruitment in well-studied processes, such as bud formation in *S. cerevisiae* [56].

Following leaf infection, the biology of tissue colonisation by *M. oryzae* and the highly effective strategies deployed by the fungus to suppress host immunity, both metabolic and effector-driven, are becoming clearer [57-59]. The developmental transitions involved in traversing cell junctions within a rice leaf are just as striking and developmentally complex, as those on the leaf surface. The transpressorium has significant developmental parallels to the appressorium, having evolved to traverse the same type of structural barrier. Many components are conserved, such as the Pmk1 MAP pathway and septins, for example. However, there are clear differences, such as need to move from one viable cell to another, all the time maintaining EIHM integrity [33, 47] and the requirement for host immunity to be suppressed at plasmodesmata, where such responses are often focused [60]. Critical questions include, how does the fungus identify plasmodesmata-rich pit fields as potential crossing points and suppress host immune response at these cell junctions? How are effectors secreted by invasive hyphae and delivered across the plant plasma? And what is the precise role of each effector protein and why is such a large arsenal of effectors necessary to establish blast disease [49].

Rapid advances in live cell imaging have therefore revealed the temporal and spatial dynamic of plant infection in unparalleled resolution, allowing a much more holistic understanding of nature of fungal pathogenesis by fungi such as *M. oryzae* to emerge.

Figure legends

Figure 1. Infection related morphogenesis in the rice blast fungus *Magnaporthe oryzae.*

A. Life cycle of *M. oryzae*. Infection begins when a three-celled conidium lands and attaches to the hydrophobic surface of a rice leaf. The spore germinates producing a long narrow germ tube that differentiates into an appressorium. The single celled appressorium matures, and the three celled conidium collapses and dies in a programmed process requiring autophagy and ferroptosis. The appressorium melanises and generates enormous cellular turgor pressure. This is translated into mechanical force leading to rupture of the rice leaf cuticle. Plant tissue invasion occurs by means of bulbous invasive hyphae that invaginate the rice plasma membrane and spread to neighbouring epidermal cells via pit fields containing plasmodesmata. Disease lesions develop after 72-96hpi, and sporulation occurs under humid conditions. Emergence of new infections occurs once spores are delivered to new host plants by dewdrop splash. **B.** Scanning electron micrograph with false colouring, of a dome-shaped appressorium (grey) on the rice leaf surface (green). The contents of the spore are degraded by autophagy and trafficked to the appressorium resulting in enormous turgor that is translated into a mechanical force to rupture the waxy rice leaf cuticle. **C.** Time-lapse confocal fluorescence images of nuclear division and cell-cycle progression during appressorium development in *M. oryzae*. Images show Guy11 expressing H1-GFP germinated on glass coverslips 2-19hpi. Scale bar =10 μm.

Figure 2. Cell cycle control and autophagy are tightly linked processes to appressorium development and invasive growth.

A. Micrograph to show appressorium formation of Guy11 expressing H1-RFP at 24 hpi on hydrophobic glass coverslips. At this time, just one daughter nucleus remains in the appressorium, as the original three nuclei have been degraded by autophagy-mediated conidial cell death. **B.** Confocal micrograph of the autophagy-deficient null mutant, Δ*atg8*, expressing H1-GFP at 24 hpi on hydrophobic coverslips. **C.** Micrographs showing completion of DNA replication is necessary for plant infection. Rice leaf

sheath observed 48 hpi after inoculation with Guy11 expressing H1-RFP after exposure to 1M of the DNA replication inhibitor hydroxyurea (HU) at 10 hpi. Asterisk indicates appressorium penetration site. **D.** Micrographs showing the effect of Pmk1 inhibition on appressorium development 24 hpi. Spores treated with 5 µM 1NA-PP1 at 1 hpi. Asterisks indicate appressorium penetration sites. **E.** Micrographs to show autophagosome localization in Guy11 and Δ*pmk1* mutant expressing GFP-Atg8 at 24 hpi on glass coverslips. **F.** Pmk1 MAP kinase is required for cell-to-cell movement during invasive growth. Micrographs at 48 hpi of infected CO39 rice leaf sheath tissue treated with 5 µM of NA-PP1 inhibitor at 26 hpi. Arrows indicate attempts to cross to neighbouring cells. Scale bar =10 µm.

Figure 3. Septin dependent network organisation in *M. oryzae* appressoria.

A. Time course of cortical septin ring formation during appressorium morphogenesis in *M. oryzae*. Micrographs of septin ring organisation visualised by expression of Sep5-GFP in the wild type strain Guy11. Conidial suspensions at 5 x 10^4 mL^{-1} were inoculated onto glass coverslips and images were captured at different time intervals during infection-related-development (0-22h). **B.** *M. oryzae* mutants displaying aberrant septin ring aggregation. Micrographs and corresponding linescan graphs to show organisation of Sep5-GFP expressed in appressoria of Guy11, Δ*sln1*, Δ*nox2*, and Δ*noxR* mutants after 20-24hpi. Scale bar= 10µm. **C.** Live cell imaging to show cellular localization of Gelsolin-GFP in the appressorium pore of Δ*mst12*, Δ*pmk1* and Δ*hox7* mutants at 24 hpi on glass coverslips. Arrow indicates an incipient appressorium. Scale bar =10 µm.

Figure 4. A toroidal septin network assembles at the appressorium pore on a plant surface.

A. Micrographs of septin ring organisation visualised by expression of Sep5-GFP in wild type strain Guy11. Conidial suspensions at 5 x 10^4 mL^{-1} were inoculated onto rice leaf sheath and images captured at 16hpi. On a rice leaf surface, the septin ring assembles in the same manner as when appressoria form on hydrophobic glass coverslips, but undergoes further

constriction for penetration peg emergence. **B.** Micrographs of Sep5-GFP expressed in primary invasive as small dynamic puncta, after constriction of the septin ring in appressoria. Images were captured after 28hpi. Scale bar =10 µm.

Figure 5. Completion of S-phase affects localization of septin-associated proteins at the appressorium pore.

Micrographs to show the localization of Sep5-GFP, Chm1-GFP, Gelsolin-GFP and Tea1–GFP during appressorium development at 10 hpi, 24 hpi, and 24 hpi with addition of 200 mM of Hydroxyurea (HU) at 10 hpi. Inhibiting DNA replication with HU disrupts septin-mediated cytoskeletal reorganisation. Scale bar =10 µm.

Figure 6. Visualisation of the expression of cytoplasmic and apoplastic effectors during invasive growth.

A. Micrograph of Guy11 expressing cytoplasmic effector Avrpik-GFP 28h post inoculation. Avrpik accumulates predominantly at the biotrophic interfacial complex (BIC). Scale bar =10 µm. **B.** Micrograph of Guy11 expressing apoplastic effector Bas4-mCherry, accumulating in the apoplast 28hpi. **C.** A time course of invasive hyphal growth. Conidial suspensions of Guy11 were inoculated onto rice leaf sheath and images were captured every 10 minutes from the point of penetration (24hpi) for 16 hours. Scale bar =20 µm.

Video 1. Live cell imaging of appressorium development and maturation.

Conidia were harvested from wild type strain Guy11 and inoculated onto glass coverslips. The movie was captured using a Leica SP8 laser confocal microscope 2-15hpi. The movie is a maximum projection of Z-stack, frames were taken every 5 min and are displayed at 15 frames per sec. Time scale is in hours: mins. Scale bar =10 µm.

Video 2. Live cell imaging of cell-cycle progression during appressorium development.

Conidia were harvested from Guy11 expressing H1-GFP and inoculated onto glass coverslips. The white arrow indicates the point at which the nucleus inside the incipient cell undergoes a single round of mitosis. The movie was captured using a Leica SP8 laser confocal microscope 0-24hpi. The movie is a maximum projection of Z-stack Frames were taken every 5 min and are displayed at 15 frames per sec. Time scale is in hour: min: sec. Scale bar =10 µm.

Video 3. Dynamic assembly of a septin ring in *M. oryzae* appressoria.

Live cell imaging of septin dynamics during appressorium development in *M. oryzae*. Movie shows Guy11 expressing Sep5-GFP during appressorium development on glass coverslips. The movie was captured using a Leica SP8 laser confocal microscope 0-24hpi. The movie is a maximum projection of Z-stack. Frames were taken every 5 min and are displayed at 15 frames per sec. Time scale is in hour: min: sec Scale bar= 10µm.

Video 4. Septin ring formation on a rice leaf surface.

Conidia were harvested from a *M. oryzae* transformant expressing a Sep5-GFP gene fusion and inoculated onto rice leaf sheath. The animation is a Z-stack captured at 28hpi using a Leica SP8 laser confocal microscope and displayed at 1 frame per sec. Scale bar= 5µm.

Video 5. Gelsolin ring formation in *M. oryzae*.

Conidia were harvested from a *M. oryzae* transformant expressing a Gelsolin-GFP gene fusion and inoculated onto glass coverslips. Three-dimensional maximum projection Z-stack images were captured at 24hpi using a Leica SP8 laser confocal microscope and displayed at 15 frames per sec. Scale bar= 10µm.

Video 6. Localisation of apoplastic effector Bas4.

Conidia were harvested from a *M. oryzae* transformant expressing a Bas4-mCherry gene fusion and inoculated onto rice leaf sheath. Three-dimensional maximum projection Z-stack

images were captured at 28hpi using a Leica SP8 laser confocal microscope. Scale bar= 10µm.

Video 7. Tissue colonisation by *M. oryzae*.

Conidia were harvested from wild strain Guy11 and inoculated onto rice leaf sheath. Invasive growth was imaged every 10 minutes for a duration of 16 hours using a Leica SP8 laser confocal microscope. Arrows indicate the crossing points of invasive hyphae into neighbouring cells. The movie is a maximum projected Z-stack, frames were taken every 10 min and are displayed at 7 frames per sec. Time scale is in mins. Scale bar= 20µm.

Video 8. Nuclear division during invasive growth by *M. oryzae*

Rice leaf sheath tissue inoculated with Guy11 expressing H1-GFP showing nuclear division during cell-to-cell movement by the fungus. Arrow indicates cell crossing point where transpressorium forms, which is shortly followed by mitosis and nuclear movement to the adjacent cell where it appears close to junction with the next cell (arrowed). The movie is a maximum projected Z-stack, with images recorded every 10 min, displayed at 15 frames per sec. Scale bar = 10 µm

Video 9. High resolution imaging of the *M. oryzae* transpressorium

Conidia were harvested from a *M. oryzae* transformant expressing a GFP-HDEL gene fusion and inoculated onto rice leaf sheath. 3D visualization of *M. oryzae* within an infected rice cell showed the specialised swollen hyphae termed the 'transpressorium', generated prior to movement into neighbouring cells. The 3D movie is displayed at 15 frames per sec.

Video 10. Large field of view of rice tissue invasion by *M. oryzae*.

Time lapse movie showing the colonisation of rice tissue by *M. oryzae* Guy11 strain expressing the nucleolar marker Fib-GFP. Invasive growth was imaged from 24hpi to 38hpi using a Leica SP8 laser confocal microscope. The movie is a maximum projected Z-stack displayed at 8

frames per sec. Scale bar = 50μm. Still image shows four frames from the video which demonstrate the rapid cycles of nuclear division that occur during invasive growth

Video 11. Three-dimensional rendering of invasive hyphae colonising rice tissue

Fluorescence images highlighting the organisation of WGA-AF488-stained hyphae of *M. oryzae* Guy11 within infected rice tissue stained with propidium iodide. Scale bar = 50μm.

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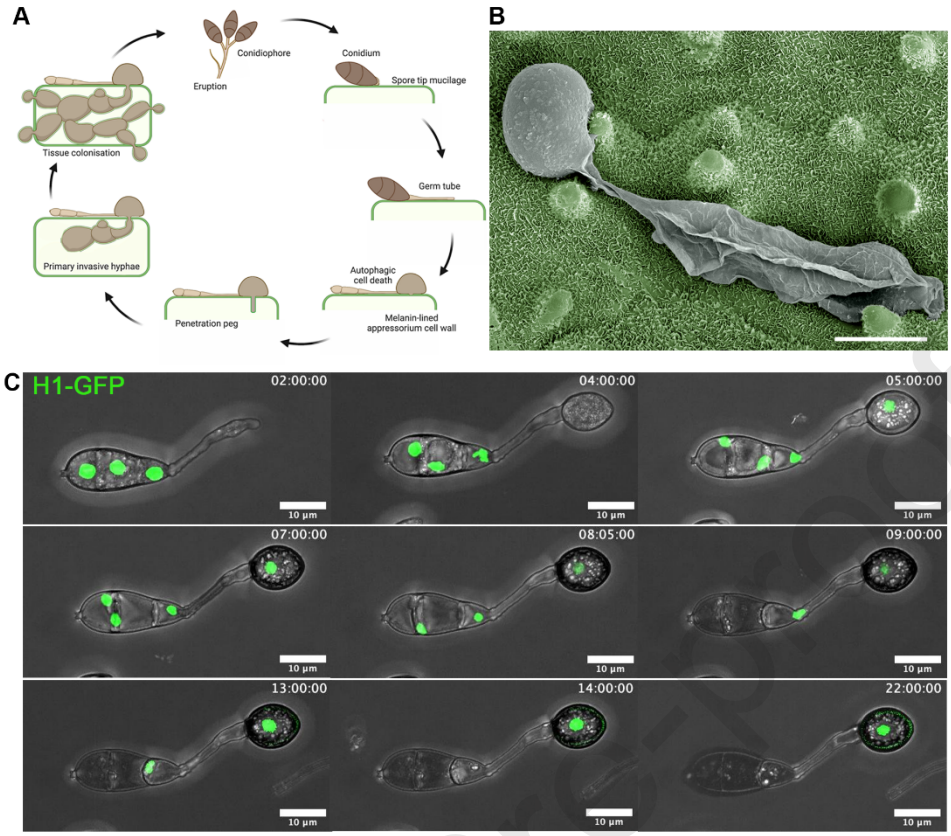
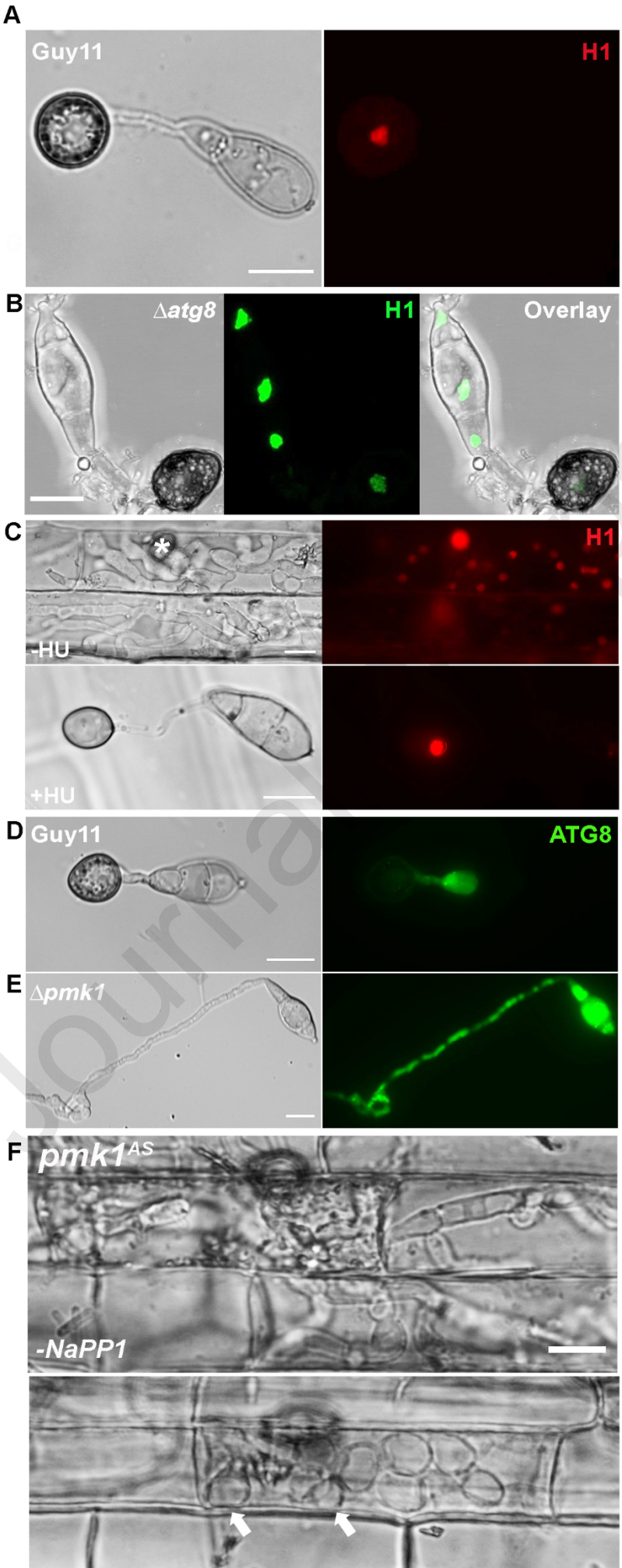


Figure 1



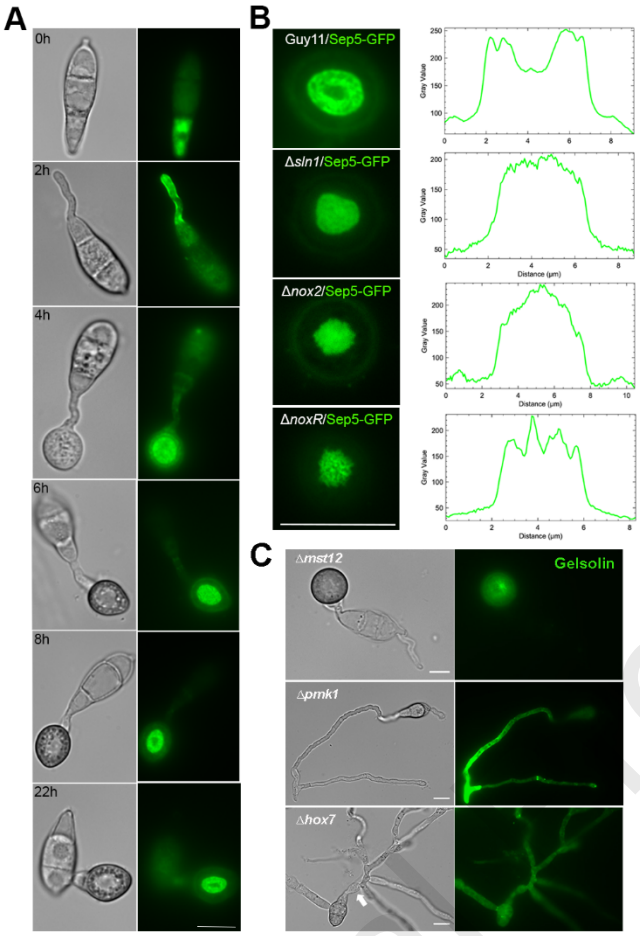


Figure 3

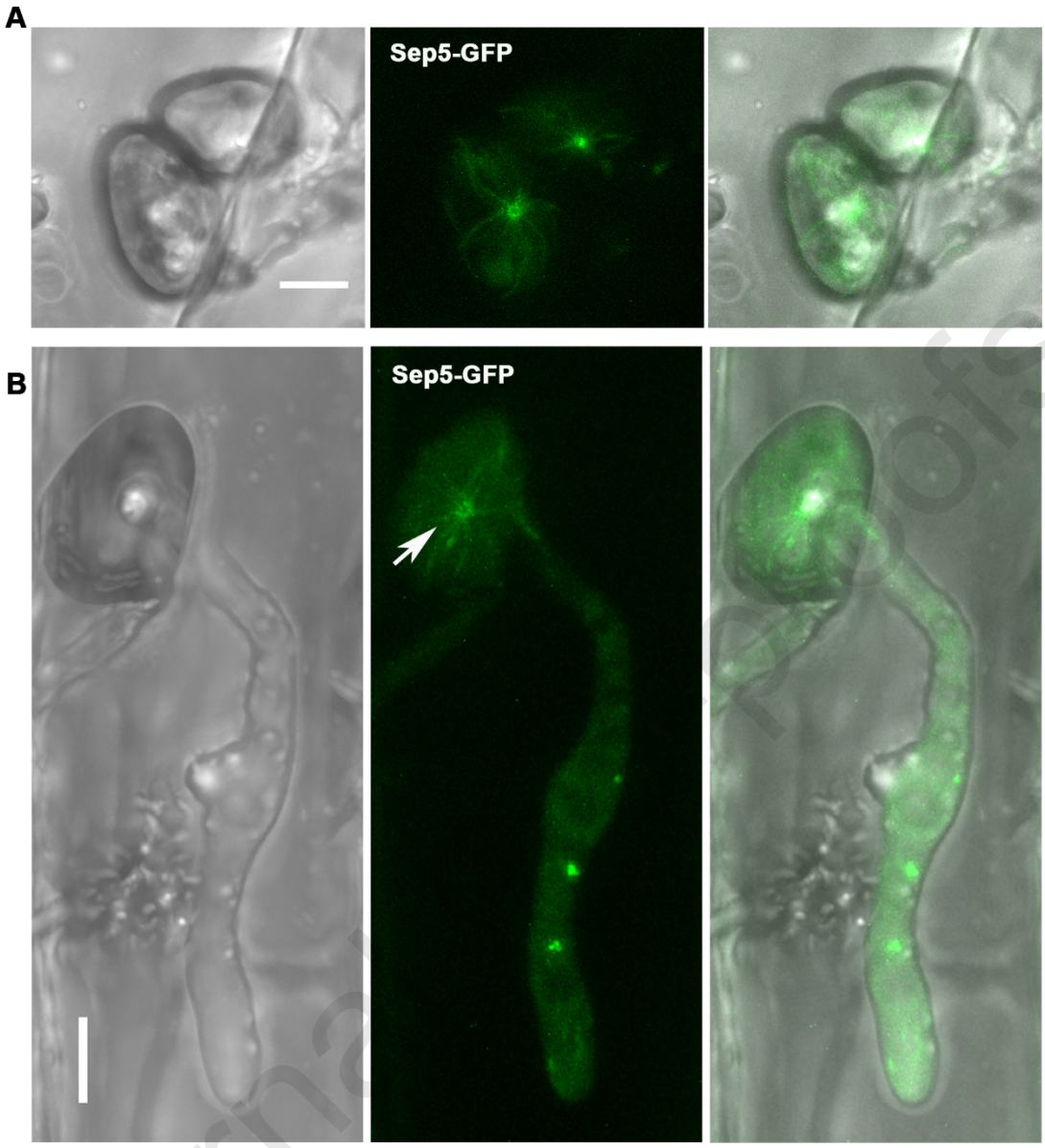


Figure 4

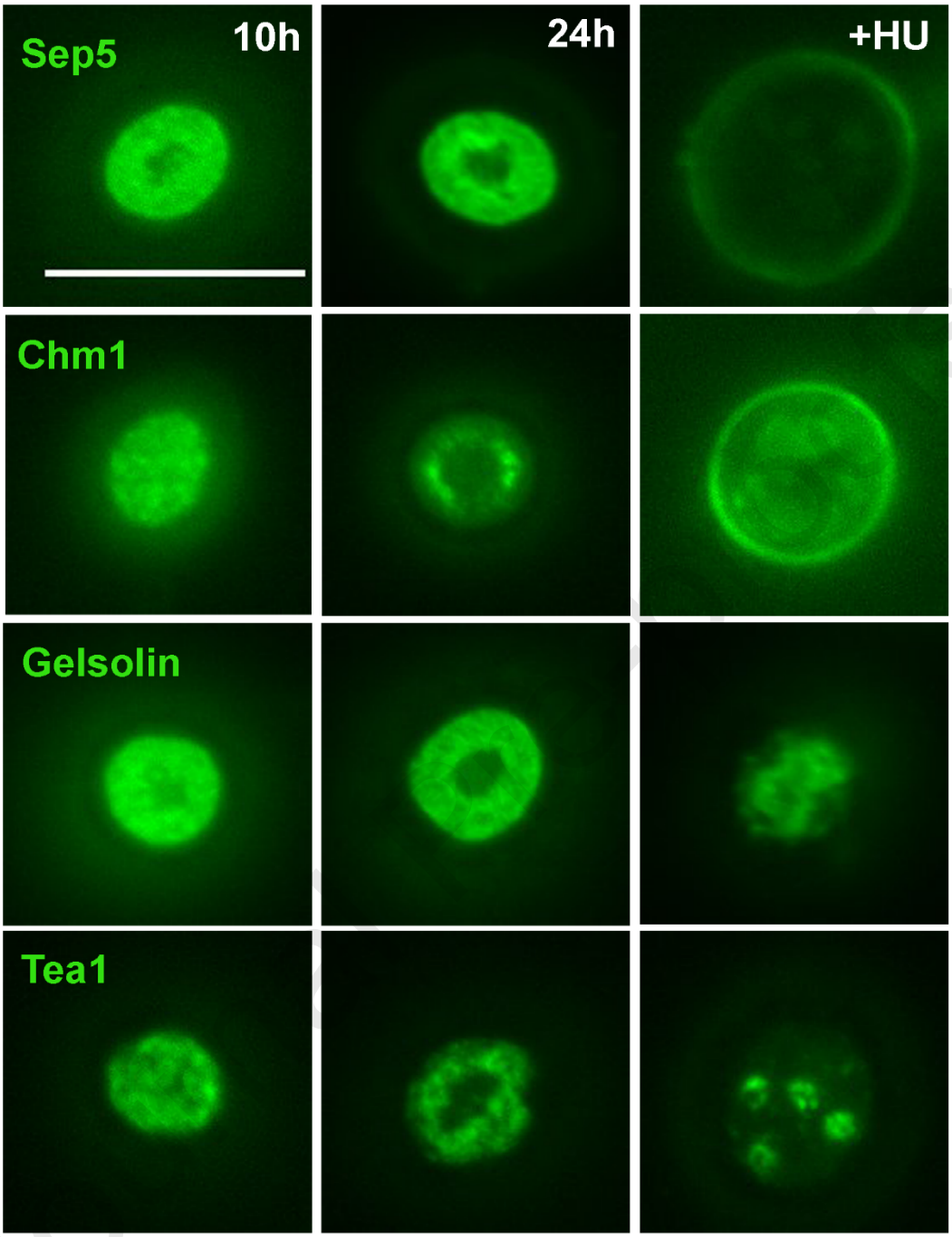


Figure 5

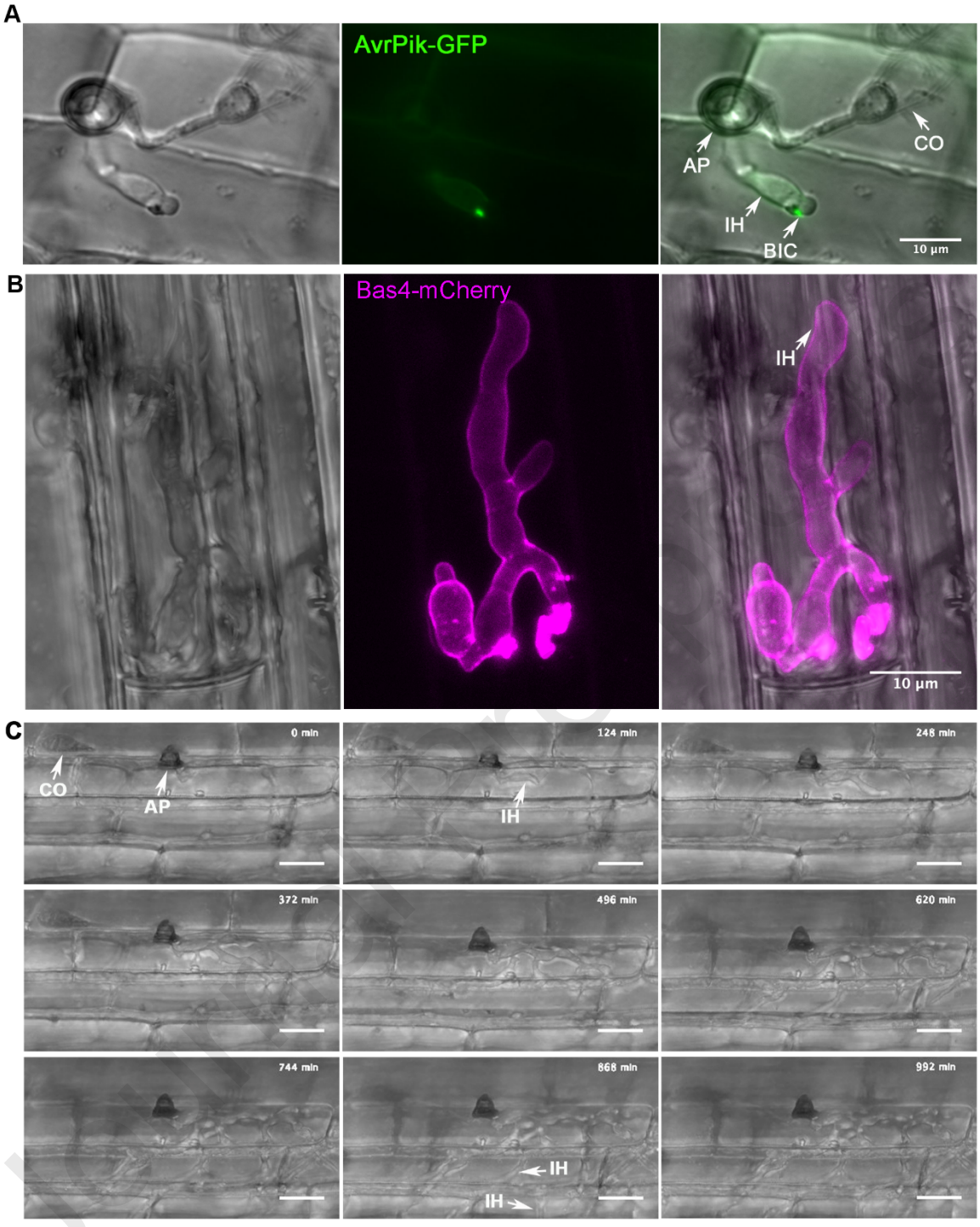

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Figure 6