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Video article

The appressorium of the rice blast fungus *Magnaporthe oryzae* remains mitotically active during post-penetration hyphal growth

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ABSTRACT

To investigate the mitotic dynamics of an appressorium, we used live-cell confocal imaging of a fluorescence-based mitotic reporter strain of *Magnaporthe oryzae*. We present evidence that the *M. oryzae* appressorium remains viable and mitotically active well after host penetration. These results suggest the potential roles of the appressorium during post-penetration proliferation of invasive hyphae. Our studies also revealed that a mitotic appressorial nucleus undergoes extreme constriction and elongation as it migrates through the penetration peg in a manner analogous to mitosis during cell-to-cell movement of invasive hyphae. Understanding the mechanisms underlying these pathogen-specific nuclear dynamics may provide new targets for disease control.

1. Introduction

Many fungi form a specialized infection structure, called an appressorium, to directly penetrate plant hosts (Howard and Valent, 1996; Ryder and Talbot, 2015). Recent studies have shown that the appressorium is considerably more complex than previously thought. It serves as the site of initial secretion of fungal effectors, and its development requires highly orchestrated events such as cytoskeleton reorganization and cell cycle regulation (Dagdas et al., 2012; Kleemann et al., 2012; Martin-Urdiroz et al., 2015; Ryder and Talbot, 2015; Saunders et al., 2010). There are questions yet to be answered, including how long the appressorium remains viable after host penetration, what mechanisms control division and migration of an appressorial nucleus, and whether the appressorium plays additional roles during post-penetration stages of infection. Here, we provide insights into some of these questions by studying the economically important rice blast disease caused by *Magnaporthe oryzae* (Khang and Valent, 2010). On the host surface, *M. oryzae* produces a single-celled appressorium, from which develops a penetration peg for breaching the plant surface. After penetration, the peg expands to form a filamentous invasive hypha, which subsequently differentiates into bulbous invasive hyphae (IH). The appressorium provides a nucleus to the first IH cell, which continues to grow and divide for about 12 h in the first-invaded cell, and then IH move into adjacent cells via IH pegs (Kankanala et al., 2007; Veneault-Fourrey et al., 2006). Recently, Jones et al. (2016a) developed a fluorescence-based mitotic reporter strain of *M. oryzae* and provided evidence that IH undergo semi-closed mitosis and that a mitotic nucleus shows extreme constriction and elongation when migrating through the IH peg to nucleate IH growing in an adjacent host cell. In this study, using the mitotic reporter strain coupled with high tempo-spatial resolution imaging, we show that the appressorium remains mitotically active during IH proliferation and that the appressorial nucleus undergoes extreme constriction and elongation through the penetration peg.

2. Results and discussion

2.1. The M. oryzae appressorium remains viable and mitotically active after host penetration

During live-cell imaging of rice cells infected with an *M. oryzae* strain expressing histone H1-mRFP and cytoplasmic EYFP, we unexpectedly observed that the appressorial cytoplasm and nucleus remained fluorescent even after IH had already spread into adjacent cells (Fig. 1A). Interestingly, we also observed two nuclei in an appressorium at the similar infection stage (Fig. 1B). These observations led us to hypothesize that the *M. oryzae* appressorium remains viable and even mitotically active while IH proliferate inside host cells. Upon further investigation, we found, consistent with this hypothesis, that 89.2% of appressoria contained one or two fluorescent nuclei at the 10–27-nuclear IH stage during first host cell colonization: of the 379 appressoria, 226 had one nucleus

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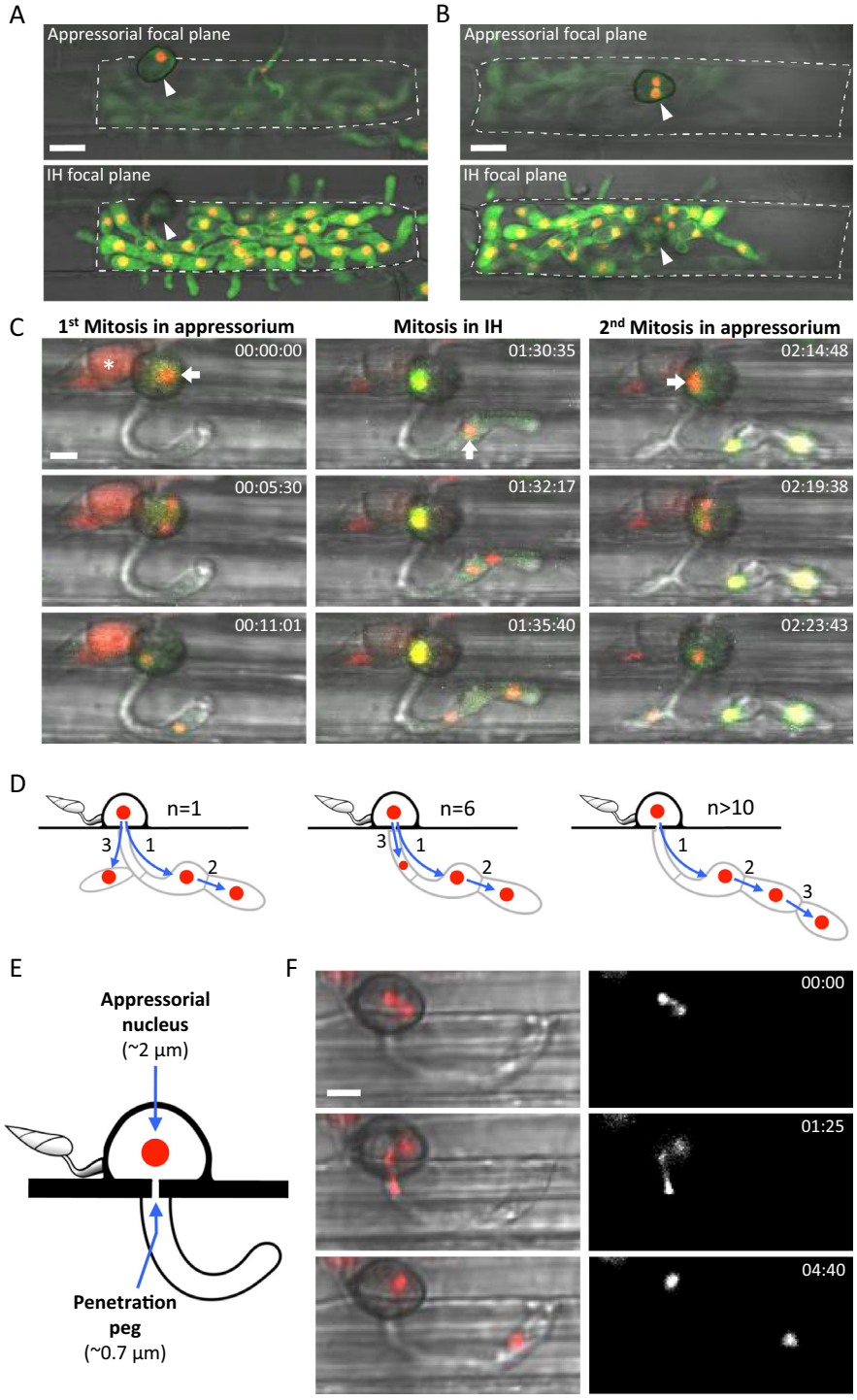

Fig. 1. Appressorial mitotic dynamics of *M. oryzae* during invasion of rice cells. (A) Confocal images showing two focal planes of the same *M. oryzae* strain CKF110 infection at 36 h post inoculation (hpi). This *M. oryzae* strain expresses histone H1-mRFP (red) and cytoplasmic EYFP (green). The IH focal plane (bottom) is 7 µm below the appressorial focal plane (top). A single nucleus in the appressorium (white arrowhead) is clearly visible in the appressorial focal plane. The IH focal plane shows highly branched IH that have spread into adjacent rice cells. Bar = 10 µm. (B) Another infection at the same growth stage as (A). The IH focal plane (bottom) is 8 µm below the appressorial focal plane (top). Note that there are two nuclei in the appressorium (white arrowhead). Bar = 10 µm. (C) Time-lapse confocal images selected from Video 1, showing three rounds of mitosis during initial host colonization by *M. oryzae* CKF1962, starting at 25 hpi. This strain expresses histone H1-tdTomato (red; nuclear localization throughout the cell cycle) and GFP-NLS (green; nuclear localization during interphase but cytoplasmic localization during mitosis). The first mitosis is appressorial (left column), followed by mitosis in IH (middle column), and finally another appressorial mitosis (right column). Nuclei entering mitosis are denoted with white arrows. The conidium (white asterisk) is undergoing autophagic cell death. Times are shown in hours:minutes:seconds. Bar = 5 µm. (D) Schematic representations of the observed sequences of the first three rounds of mitosis after host penetration. Red circles indicate nuclei. Numbers associated with arrows indicate the order of nuclear divisions. Arrows indicate the direction of nuclear migration. The diagram on the left represents the mitotic sequence of the infection site in Fig. 1C and Video 1. The other sequences (middle and right) are of independently observed infection sites. n = number of observed infection sites. (E) Schematic diagram illustrating the question of how an appressorial nucleus (~2 µm in diameter) can migrate through the narrow penetration peg (~0.7 µm in diameter) to enter the filamentous IH. (F) Time-lapse single plane confocal images of *M. oryzae* CKF1962 invading a rice cell. Left: merged tdTomato and bright-field; right: tdTomato alone (shown in white). Sequence shows the first appressorial nuclear division and migration supplying a nucleus to the filamentous IH. The middle panel shows an extreme elongation and constriction of a mitotic nucleus as it migrates through the penetration pore. Times are shown in minutes:seconds. Bar = 5 µm.