Actin Patterns of *Aspergillus nidulans* during growth

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Abstract

The following research was conducted while participating in the Bioenvironmental Sciences Undergraduate Research Scholars (BURS) program. Apical growth patterns in a model of filamentous fungus *Aspergillus nidulans* were analyzed. Among the many organisms that exhibit tip growth, *A. nidulans* is easily amenable to molecular genetic experimentation (N. Taheri-Talesh et al., 2007). The strains used contain Lifeact (an actin binding protein domain) fused with green or red fluorescent protein (GFP or RFP). This construct allows for the visualization of actin patches, cables, and rings. The cytoskeletal protein actin is involved in a variety of functions including: cell polarity, exocytosis, endocytosis, cytokinesis, and organelle movement (Berepiki, et al., 2010). These sub-units coalesce into three different structures including: a sub-apical collar, Sub-apical Actin Web (SAW), and an apical actin array. The sub-apical collar is usually located within the first 2 µm from the growing tip. The Apical Recycling Model states that the sub-apical collar of endocytic patches contributes to the balance of endocytosis and exocytosis at hyphal tips influencing growth and shape of the hyphae. Since, the sub-apical collar has been found in *A.nidulans* and *Neurospora crassa*, there is a high probability that it could be found in all filamentous fungi (Shaw, et al, 2011). The SAW is usually found within an average distance of 25 µm from the tip in 23% of cells (n=196). The apical actin array is found in 27% of growing hyphae and emerging branches. The array is characterized by a retrograde movement of actin cables referred to as treadmilling. Thus, visualizing actin behavior, especially in the SAW and apical array, is key to further understand the role of actin in relation to fungal tip growth.

Introduction and Objectives

Fungi or their products are present in a wide range of foods, drinks, drugs, chemicals that are necessary for our survival. Fungi are also significant pathogens in agriculture and human health. For example, cereal grain rusts, from the *Puccinia spp*. that caused famine in the Middle East thousands of years ago. (Hudler, 1998). As well as emerging threats such as rice and wheat blast. Filamentous fungi exhibit polarized growth. Invasive polarized growth is necessary in most disease systems. Since the actin cytoskeleton is necessary for apical growth (Berepiki, et al., 2010), our goal is to document actin dynamics in the model fungus *A. nidulans* . Therefore, the following will be investigated:

•Document actin dynamics in *A. nidulans* during apical growth.

•Establish whether formation of an apical actin array is present in lateral branch formation.

•Determine whether expression of the Lifeact construct has a fitness consequence by assessing growth patterns such as conidia production and colony diameters.

Methods

•Strains used in this study include: Wild Type (A773); Lifeact GFP expressing strains G10 and G15; Lifeact RFP expressing strains R3 and R11. Cultures were grown on synthetic *Aspergillus* minimal media overnight at 28.0˚C.

•The Lifeact construct was obtained from Nick Reed's lab (Berepiki, et al., 2010), transformed into wild type of *A. nidulans*.

•Transmitted and fluorescent images for the time-lapse sequences were acquired at intervals of 45-50 s to limit phototoxicity caused by continuous exposure of live cells to light.

•To establish whether an apical actin array is present in lateral branch formation we examined 313 branches.

•For fitness test all strains were replicated at least six times. Colony diameter was measured daily from Day 3 to Day 7. On Day 7, a 1 cm diameter block was cut from the center of all cultures. Conidia were washed from the block and counted on a hemacytometer.

Results

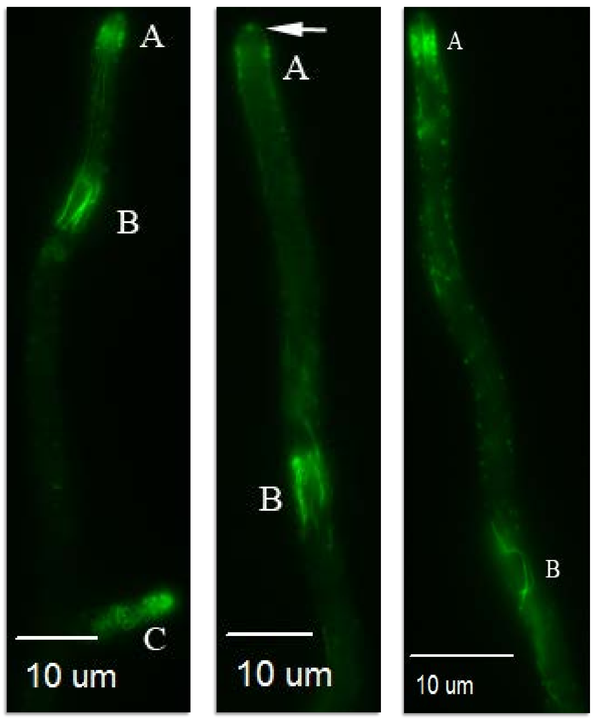

Figure 1. Actin labeled with Lifeact GFP in growing cells of A. nidulans. All three actin structures are shown. A) sub-apical collar, found in 50% of growing cells (n=196). B) Sub-apical Actin Web (SAW), found in 23% of growing cells. C) apical actin array in a lateral branch, found in 27% of growing cells. The arrow points to the Spitzenkörper.

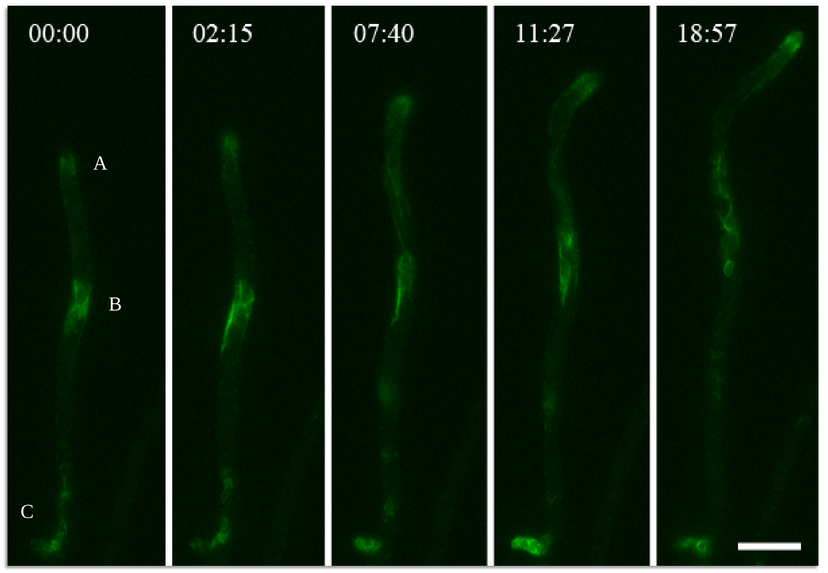

Figure 2. Timelapse of a single growing cell showing all three actin structures. A) the cortical sub-apical collar of endocytic actin patches is persistent throughout the sequence. The Apical Recycling Model predicts that the sub-apical collar contributes to a balance of endocytosis and exocytosis promoting tip growth. B) The SAW persists throughout the sequence. Actin cables form and retract from the front face of the SAW (07:40), whereas the back face is stable. C) apical actin array. The apical actin array contributes to lateral branch formation as seen continuously throughout the sequence. Bar 10µm. Time mm:ss.

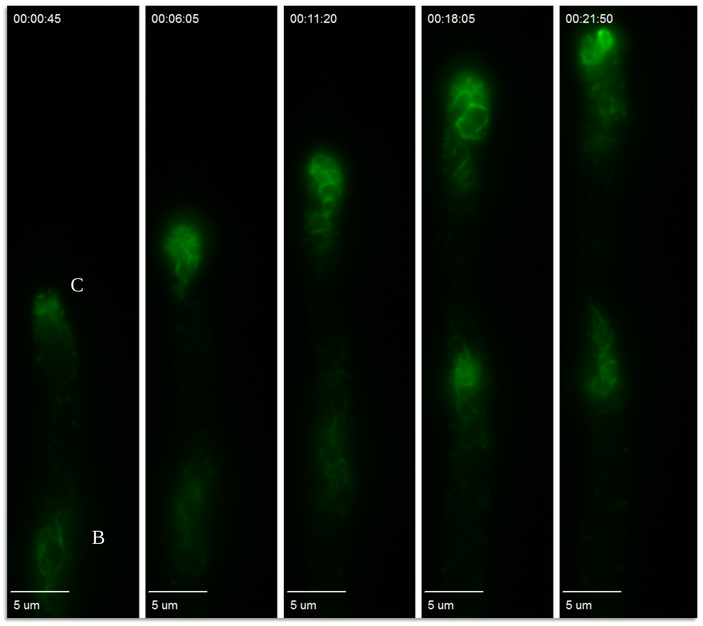

Figure 3. Timelapse of a growing live cell displaying an apical actin array and a SAW. B) The SAW maintains a consistent distance from the apical actin array in this growing cell. C) The apical actin array is associated with apical growth at the hyphal apex or at newly formed lateral branches.We can see the characteristic treadmilling or backwards flow of actin cables. Not visible here a condensation of actin cables, seen in 00:18:05, corresponds to a new branch forming. Time hh:mm:ss.

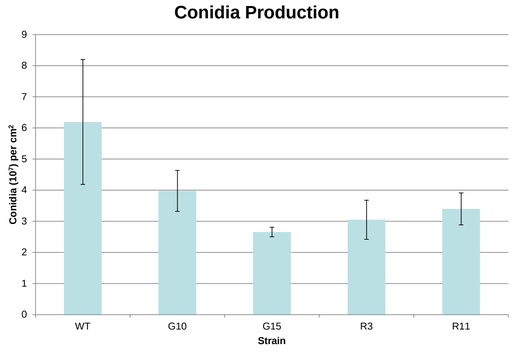

Figure 4. Average conidia produced per strain (n=6). Each Lifeact expressing strain produced approximately half as many conidia as wild type.

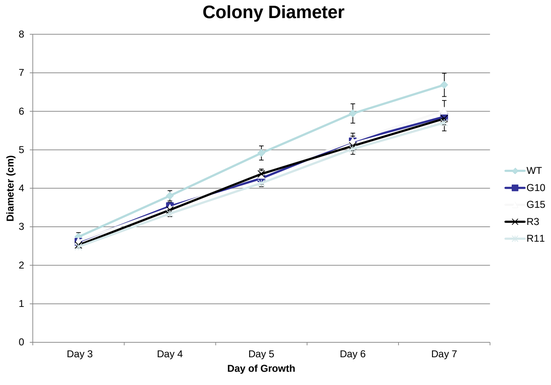

Figure 5. Average colony diameter measured over 7 days for wild type and Lifeact expressing strains (n=6). Wild type grew at a faster rate than strains containing Lifeact.

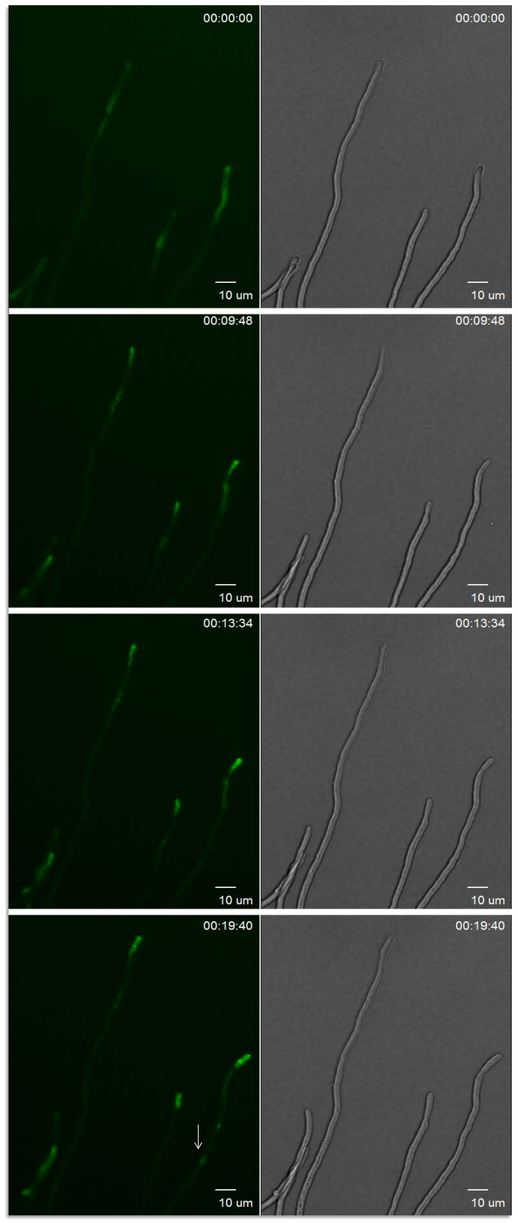

Figure 6. Timelapse of multiple growing hyphae displaying synchronized cable formation and retrograde movement. The two middle hyphae at 00:00 display a sub-apical collar. By 09:48 an apical actin array formed masking the sub-apical collar. In the hyphae to the right, at 19:40 a branch is beginning to form (arrow). SAW behavior is duplicated in all hyphae as it maintains a continuous distance from the growing apex. Time hh:mm:ss.

A sample size of 313 branches was quantified for the presence of an apical actin array.

- 60% had an apical actin array
- We noted no correlation between branch length and the presence of an apical actin array.

Conclusion

•We identified three actin structures in growing hyphae of *A. nidulans*: sub-apical collar, Sub-apical Actin Web (SAW), and the apical actin array.

•A condensation of actin cables formed prior to branch formation.

•Wild type strains produced more conidia and grew at a faster rate compared to those strains with the Lifeact. This indicates that this Lifeact construct affected the fitness of the strains. Therefore, future work will utilize other available Lifeact constructs.

References

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