

Abstract

F-actin, a cytoskeletal component of microfilaments, plays an important role in fungal growth and development. It can be found in different forms, including cables and patches. Lifeact is a fluorescent reporter of actin dynamics in live cells. The Lifeact construct was transformed in to *Aspergillus nidulans* to monitor the dynamics of actin using time lapse imaging and fluorescence microscopy. Subapical collars, composed F-actin patches associated with endocytosis, have been found to be necessary components for polar growth in the hyphae of *Aspergillus nidulans*. Apical arrays of actin have been documented in the tips of other growing fungi. Similar patterns were noted in *Aspergillus nidulans*. A third structure, subapical actin webs (SAWs) are masses of F-actin cables that can be found distal to the apex. SAWs have been observed to have coordinated patterns in multiple hyphae, including movement of SAWs at approximately the same time and rate. Further analysis is required to understand this phenomena. Different growth and conidiation rates were recorded for the wild type and Lifeact strains. New transformants will be constructed for future studies to confirm these results.

Introduction and Objectives

The fungal cytoskeleton is responsible for cellular support, cytokinesis, as well as transport mechanisms, including exo- and endocytosis. The cytoskeleton is made up of three different types of protein polymers, microfilaments, microtubules and intermediate filaments, which each play different roles within the eukaryotic cell. Actin, in particular, can found in the cell in two different categories, monomeric globular actin or G-actin and filamentous F-actin. F-actin is formed by the polymerization of g-actin monomers, which will then aggregate to form patches and cables. Time-lapse microscopy is now used to visualize F-actin dynamics within the living fungal cells (Lichius, *et al.*, 2011).

In *A. nidulans* and *Neurospora crassa*, a subapical collar of cortical actin patches is located just behind the apex of the hyphae. The subapical collar has been shown to be required for polarized hyphal growth (Shaw, *et al.*, 2011)(Berepiki, *et al.*, 2010). In addition, F-actin cables are seen to form apical arrays in the apex and subapical actin webs (SAWs) distal to the tips in the growing hyphae of *A. nidulans*. Researchers are still unsure of the purpose for the SAWs and are currently studying the dynamics of the structures. In these experiments, the Lifeact construct, created by Laura Quintanilla, will be visualized in *A. nidulans* to reveal characteristics of the many actin structures. Time lapse microscopy will be used to monitor both subapical collars and SAWs inside the growing hyphae.

Results

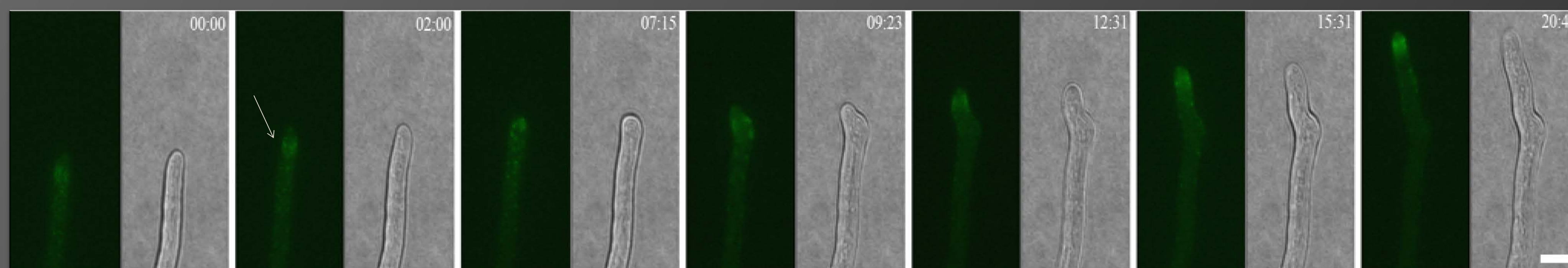


Fig. 1. Subapical collar present during hyphal growth. The sequence demonstrates the importance of the subapical collar for hyphal growth. In panels 00:00 to 02:00, the subapical collar is present in the growing hyphae (arrow). In panel 07:15, polarized hyphal growth stops simultaneously with the loss of the collar. Polarized growth initiates in panel 09:23 with the formation of the collar. Bar=5 μ m. Time=mm:ss.

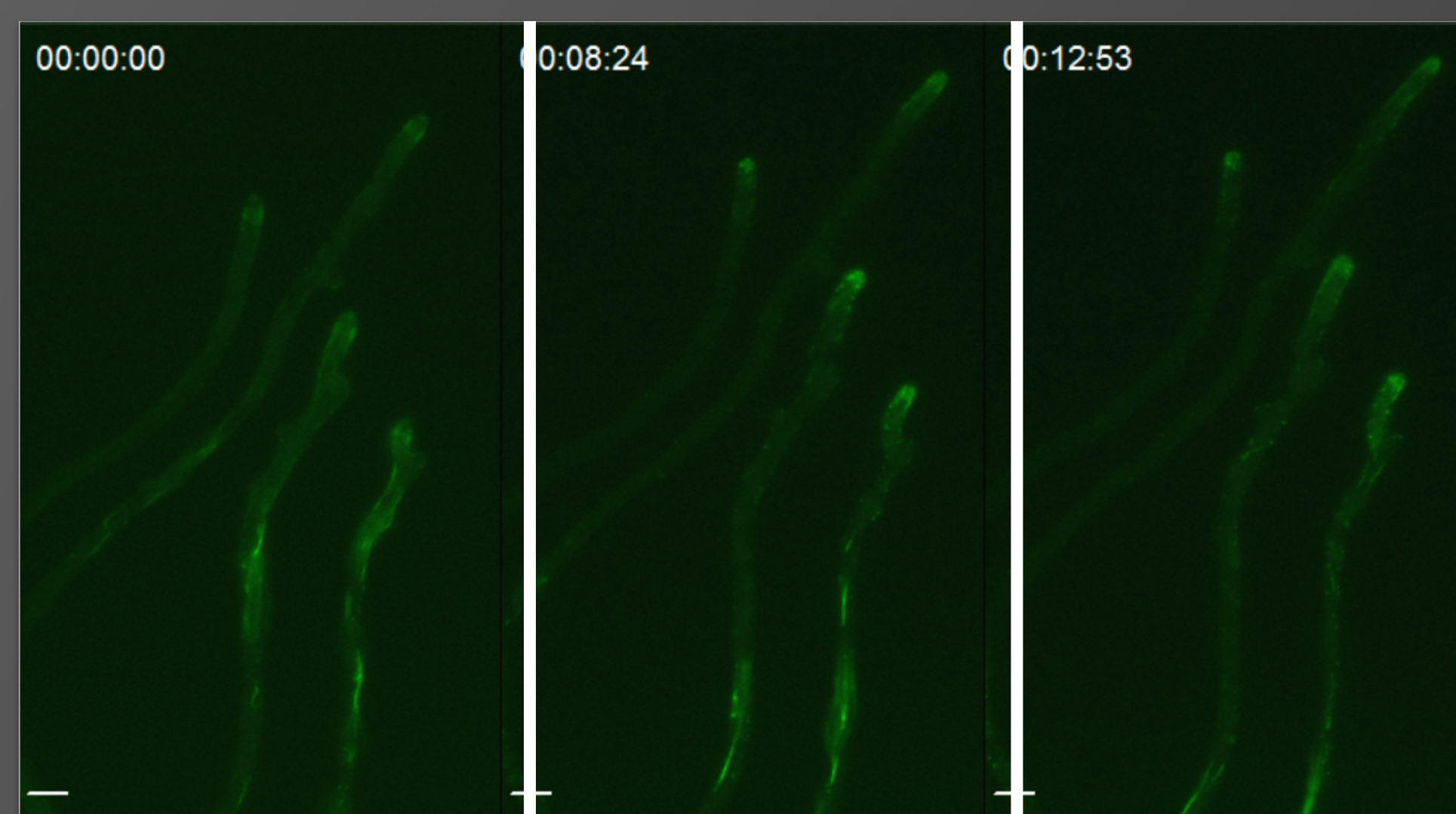


Fig. 4. SAW structures present in growing hyphae. In panels 00:00 through 12:53, SAWs are present in three of the four hyphae. Visualization is affected by the different focal planes of the hypha. SAW structures appear to have coordinated migration backwards through the independent hyphae at approximately the same time and rate. Scale bar=5 μ m. Time=hh:mm:ss.

Methods

Conidia Production

The conidia were collected from 7-day old cultures by cutting a 1 cm diameter block of agar from the center of a colony using a cork borer tube and washing with 1 ml of sterile H₂O. The solution was diluted to a 0.1x concentration before counting conidia using a hemacytometer and a basic compound microscope. The number of spores per cm² was then calculated using the formula, ((#spores*concentration*10)*1.27)/(10⁶). For each strain, n=6.

Mycelial Growth Rate

A 10 μ L solution, containing 10⁶ spores, was spot inoculated onto fully supplemented minimal medium. The cultures were incubated for two days before assaying the colony diameters. From day three to day seven, the diameter of the fungal strains was measured at approximately the same time of day using a ruler and cm units.

Time-lapse Microscopy

All time-lapse image acquisition was performed using a fluorescence microscope. A Prior shutter in each light path was used to create a dark period to avoid photo-toxicity to the cells. The reporter used was Lifeact, an actin binding protein domain fused to either green fluorescence protein (GFP) or red fluorescence protein (RFP). Each sample was incubated on fully supplemented minimal medium for approximately 21 hours under light at 28° C.

Fig. 3. Colony diameter comparison of wild type to Lifeact expressing strains. The graph shows the average colony diameter measurements over a 5 day period in two trials. These data indicate that Lifeact expression does affect fitness.

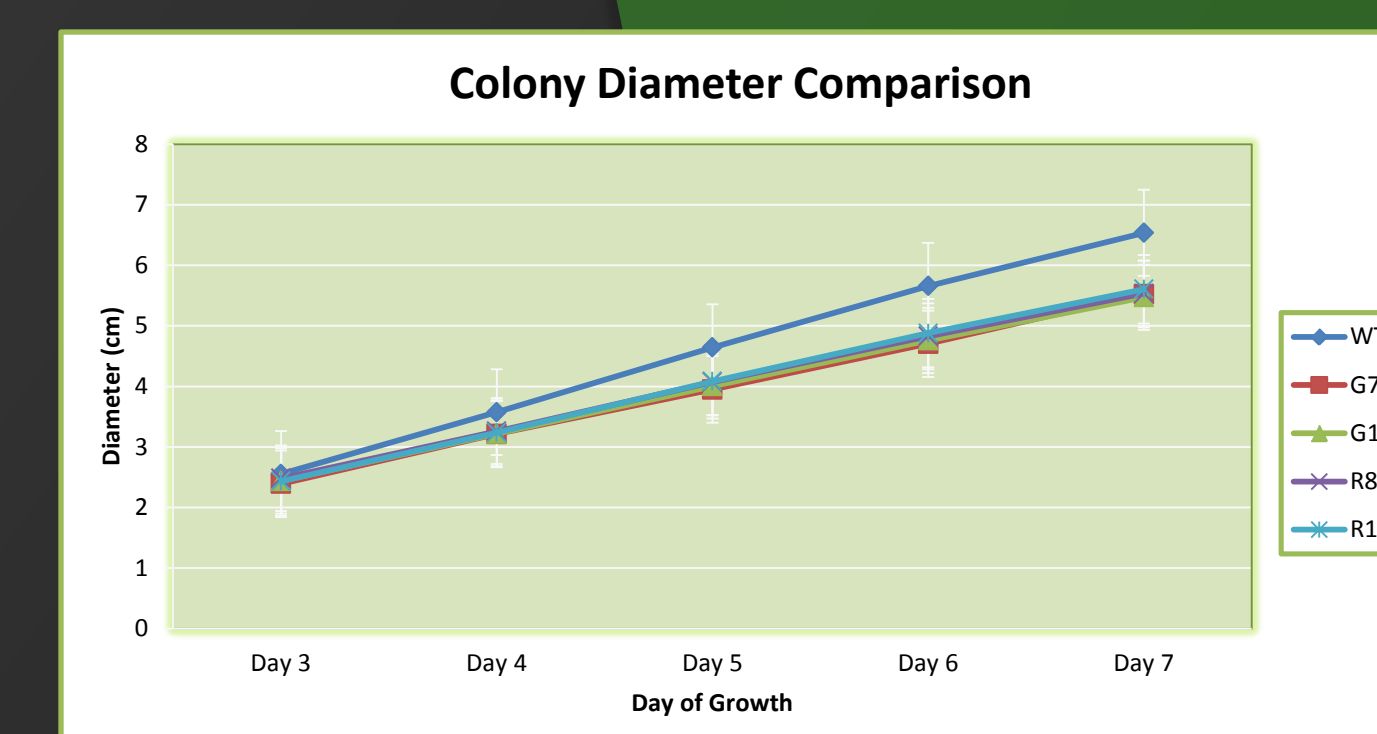
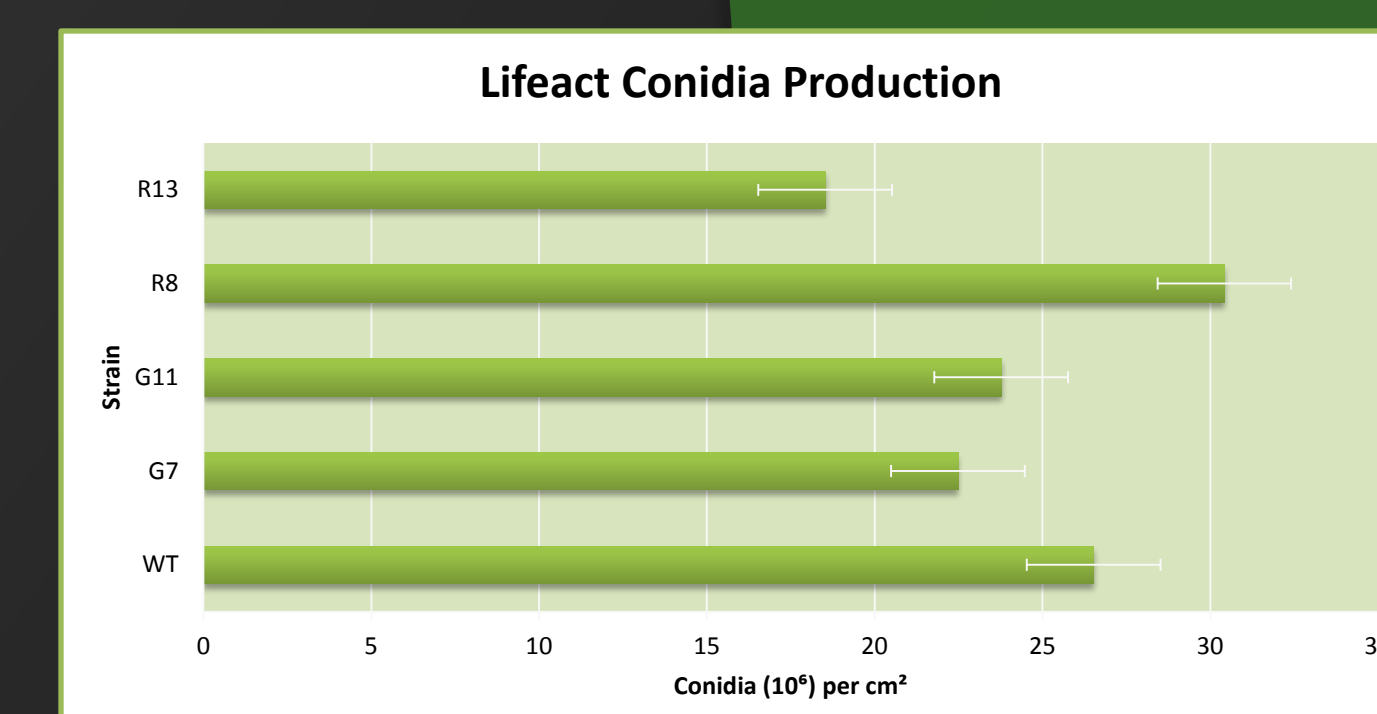


Fig. 2. Conidia production in the wild type and Lifeact expressing strains. The graph shows the average number of conidia per cm² produced by each strain in three trials. The wild type produces a similar number of conidia compared to three of the four Lifeact strains, suggesting that those three strains are as fit as the wild type.



Conclusion

- Subapical collars have been shown to be a necessary component for polarized growth. Hyphal tips which lack collars are likely to become abnormal or will cease further growth.
- Coordinated patterns have been observed in which SAWs move throughout multiple hyphae at approximately the same time and rate.
- Experiments to compare the fitness of the Lifeact expressing strains to the wild type were inconclusive. Conidia production appeared similar in the wild type and Lifeact expressing strains, whereas mycelial growth rates was reduced in Lifeact expressing strains in relation to wild type. New strains must be constructed to evaluate this further.

References

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