Understanding the Role of Amphiphysin in Polarized Growth of Filamentous Fungi

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Abstract
A complete understanding of the underlying growth and development of filamentous fungi remains largely unknown. Current research interests concerning filamentous fungi such as Aspergillus nidulans have to do with their characteristic polarized growth. It is thought that endocytosis is required for the continuation of such growth. The proteins AmpA and AmpB are believed to play a role in the final step(s) of endocytosis. In this study, strains were examined in which ampA or ampB were down regulated. Phenotypic variables that were considered included: radial growth rate, conidiation, and germination. The down regulation of ampB resulted in reduced germination and conidiation compared to wild type. It is worth noting that AmpA and AmpB might have overlapping function. This hypothesis could be tested by crossing the ampA and ampB strains to each other to produce the double mutant. When ampB was repressed, this resulted in abnormal development of the fungus.

Introduction and Objectives
Polarized growth has been a perplexing issue in understanding the overall structure and functions within filamentous fungi. Over the past decade, it has become generally accepted that the maintenance of this polar growth is a direct result of a precise balance between exo- and endocytosis (see Shaw et al. 2011). The investigated genes, ampA and ampB are thought to play an integral role as part of the scission machinery of filamentous fungi during endocytosis (see Youn et al. 2010; see Peñalva 2010). The niaA promoter was inserted in front of the ampA and ampB. This promoter represses expression in the presence of ammonia. Objectives included:
- Determine the role of the Amphiphysins (AmpA/AmpB) as they relate to morphology and physiology
- Compare growth and phenotypic expression between wild type, ampA, and ampB
- Hypothesis: When the expression of the proteins AmpA and AmpB are repressed abnormal growth and development will occur in the fungus

Methods
Radial Growth
- Wild type, ampA, and ampB were plated three times (each) on Minimal Media (MM) with
  - Nitrate (KNO3), Ammonia (NH4Cl), Nitrate w/ 0.4M Salt, Ammonia w/ 0.4M Salt, Nitrate w/ 0.8M Salt, or Ammonia w/ 0.8M Salt (see Higuchi et al. 2009)
- Measured hyphal extension each day for 4 days

Conidiation
- Plated wild type, ampA, and ampB on nitrate- and ammonia-based media
- Used standard concentration of 1x10^8 spores/mL
- 0.79 cm^2 sections of culture were collected after 5 days incubation at 28 degrees C
- These sections were then homogenized in 1 mL ddH2O and spore concentrations were measured using a hemocytometer

Germination
- Incubated conidia in liquid ammonia- or nitrate-based media
- 4000 conidia were used for each of three replicates
- Germination was assessed after 7 hours (n=450)
- Percentage germinated was then calculated

Results
The colony growth rates of the mutants were compared to wild type on inductive and repressive media. No significant difference was noted for the mutants when compared to wild type.

Conclusion
- Abnormal radial growth was not observed when either ampA or ampB were repressed
- Conidiation and germination were both significantly affected by the down regulation of ampB; the down regulation of ampA reduced conidiation
- Further evidence is required to fully understand the role of the proteins Amphiphysin A&B
- AmpA and AmpB might be functionally redundant, producing normal hyphal growth and development even when one is repressed

References

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Figure(s) 1&2. Data for colony size after 4 days and germination after 7 hours. Wild type produced more conidia than ampA and ampB on ammonia-based media. Wild type germinated better than ampB on ammonia, but not ampA. Germination was defined as the emergence of a germ tube.

Figure(s) 3&4. Data for Colony Diameter after 4 days and Germination after 7 hours. Wild type produced more conidia than ampA and ampB on ammonia-based media. Wild type germinated better than ampB on ammonia, but not ampA. Germination was defined as the emergence of a germ tube.

Figure 7. Germination after 7 hours in nitrate (top) and ammonia (bottom) liquid media. No difference was observed in ampA [(1)(4)]. Wild type germinated better than ampB in ammonia [(6)- w.t. ammonia; (5)- ampB ammonia]. Arrow indicates a germinated conidia. In order from left to right: ampA, ampB, wild type.