

Elucidating the Role of Paxillin B in Septation in *Aspergillus nidulans*

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Introduction

Paxillin B (also referred to as PaxB) is a scaffolding protein found in *Aspergillus nidulans*, though it is not very well characterized in this species. This protein does, however, share structural and functional similarities to paxillin proteins found in mammalian and yeast cells. These proteins have been found to play a role in regulating actin networks, which are required for performing functions such as regulating cell motility and cytokinesis (Zhang 2016). In fungal cells, it has been seen that actin networks are a crucial component of cell growth, by means of both hyphal growth and septation (Schultzhaus 2016). In this experiment, the gene for *PaxB* was modified in various manners to determine its location, function, and interactions with other proteins predicted to play roles in septation.

Methods

Modified *PaxB* genetic sequences (e.g. GFP/RFP tagging, gene deletion) were obtained using Fusion PCR. In the case of deleting the gene, *PaxB* was replaced with an *AfRiboB* selective marker, while in the case of tagging the gene, cassettes containing GFP and RFP—along with a selective marker—were added directly downstream of the gene, but before its stop codon. The strain A1145 was transformed with the fusion pieces, using the Hill lab KCl-based transformation protocol. After the transformants displayed sufficient growth, they were viewed using light and fluorescence microscopy. Transformants lacking a copy of *PaxB* underwent sexual reproduction with a strain containing Lifeact::RFP—a small molecule that binds to actin and fluoresces red. A strain lacking a copy of *PaxB* (R1940) was transformed through the Hill lab KCl-based transformation protocol with GFP-tagged chitin synthase (*ChsA::GFP*) generated through fusion PCR. Transformants of this experiment were then viewed using light and fluorescence microscopy.

References

Zhang W, Huang Y, and Gunst, S.J. (2016). P21-Activated kinase (Pak) regulates airway smooth muscle contraction by regulating Paxillin complexes that mediate actin polymerization. *J Physiol*.

Schultzhaus Z, Quintanilla A, Hilton A, et al. (2016). Live Cell Imaging of Actin Dynamics in the Filamentous Fungus *Aspergillus nidulans*. *Microsc Microanal*, 2, 264-274.

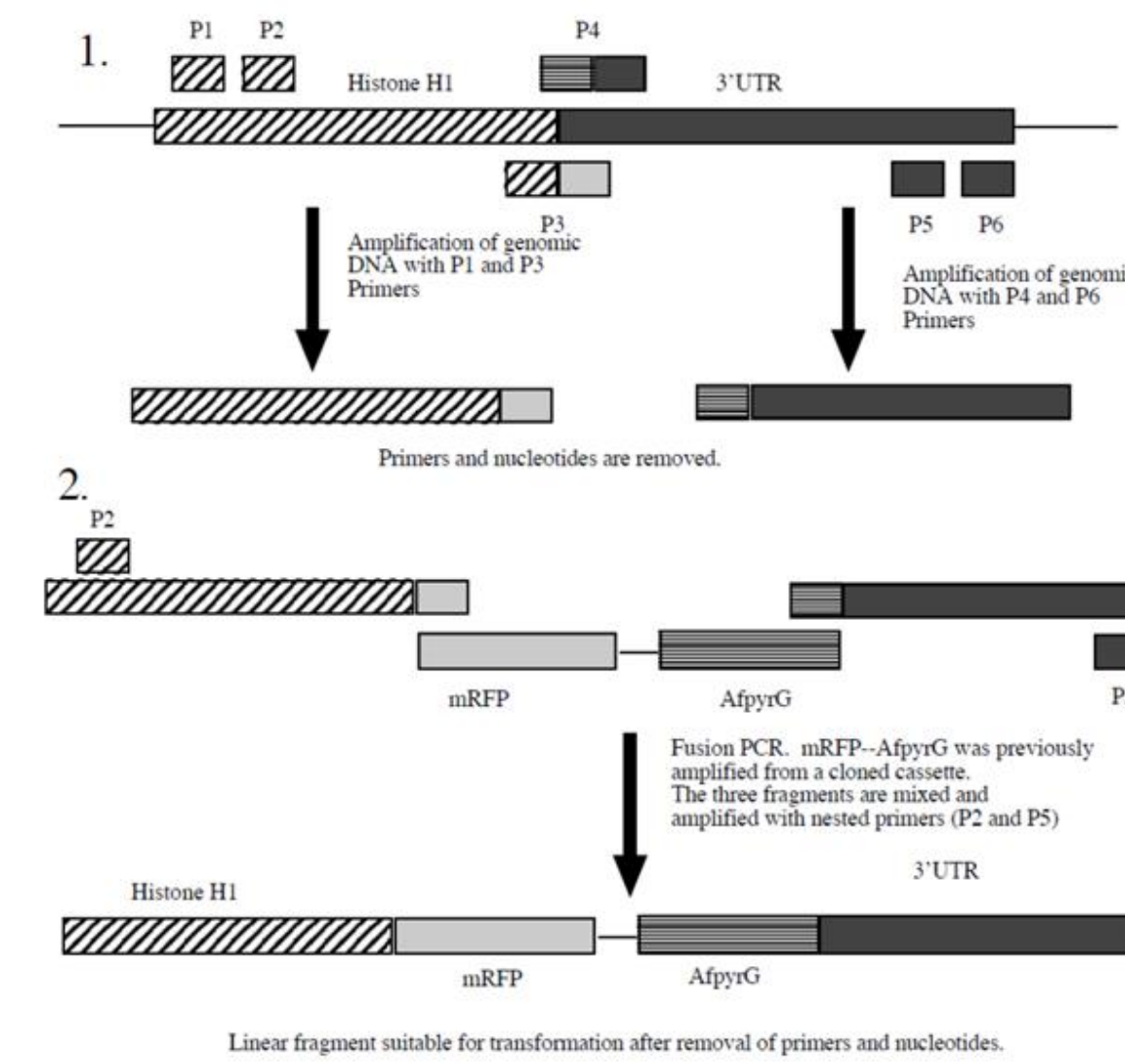


Figure 1: The process of Fusion PCR. P1, P2, P3, etc. are primers that amplify regions of interest in the DNA.



Figure 2: GFP-tagged PaxB protein in a wild-type background

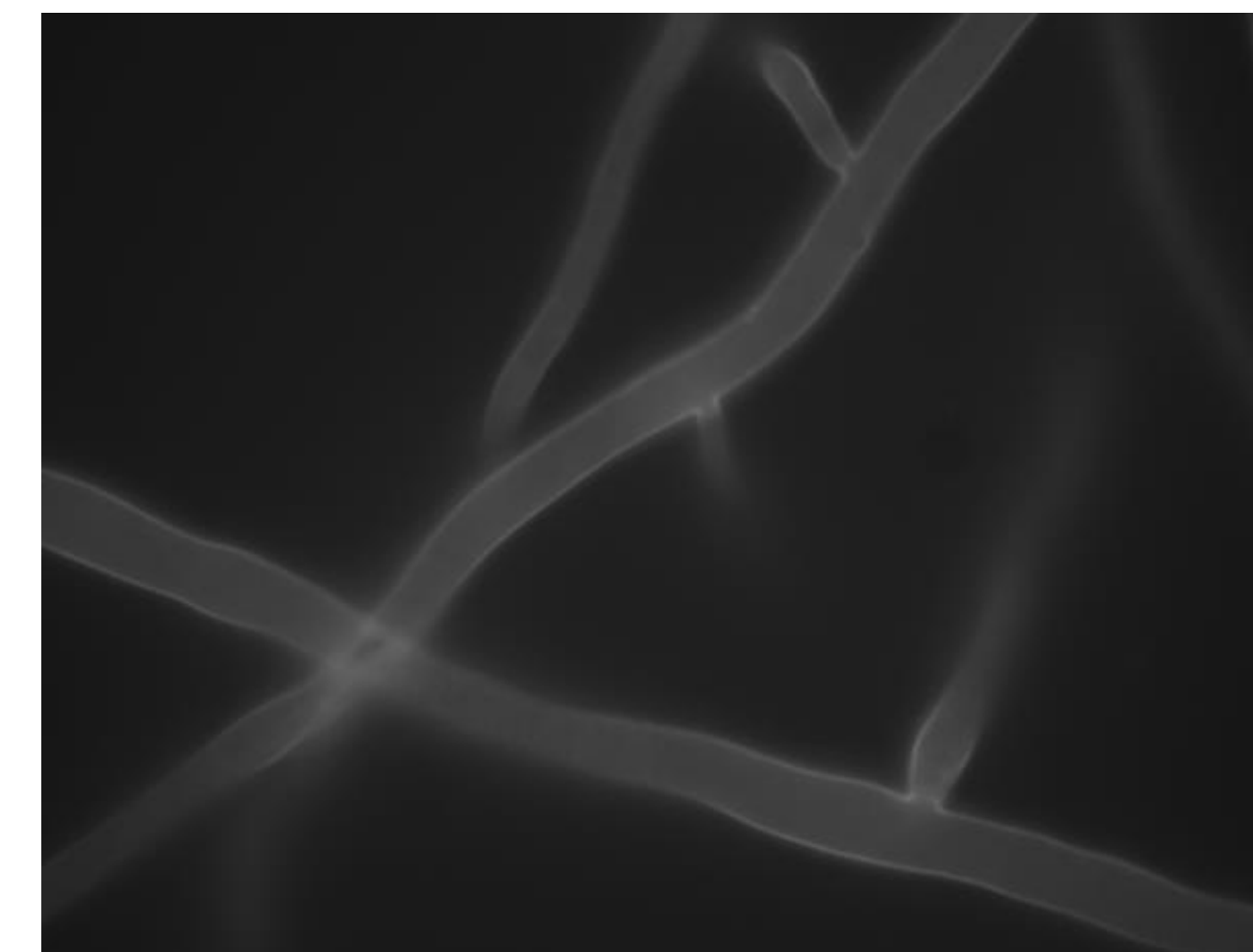


Figure 3: *A. nidulans* hyphae after deleting *PaxB* from the genome

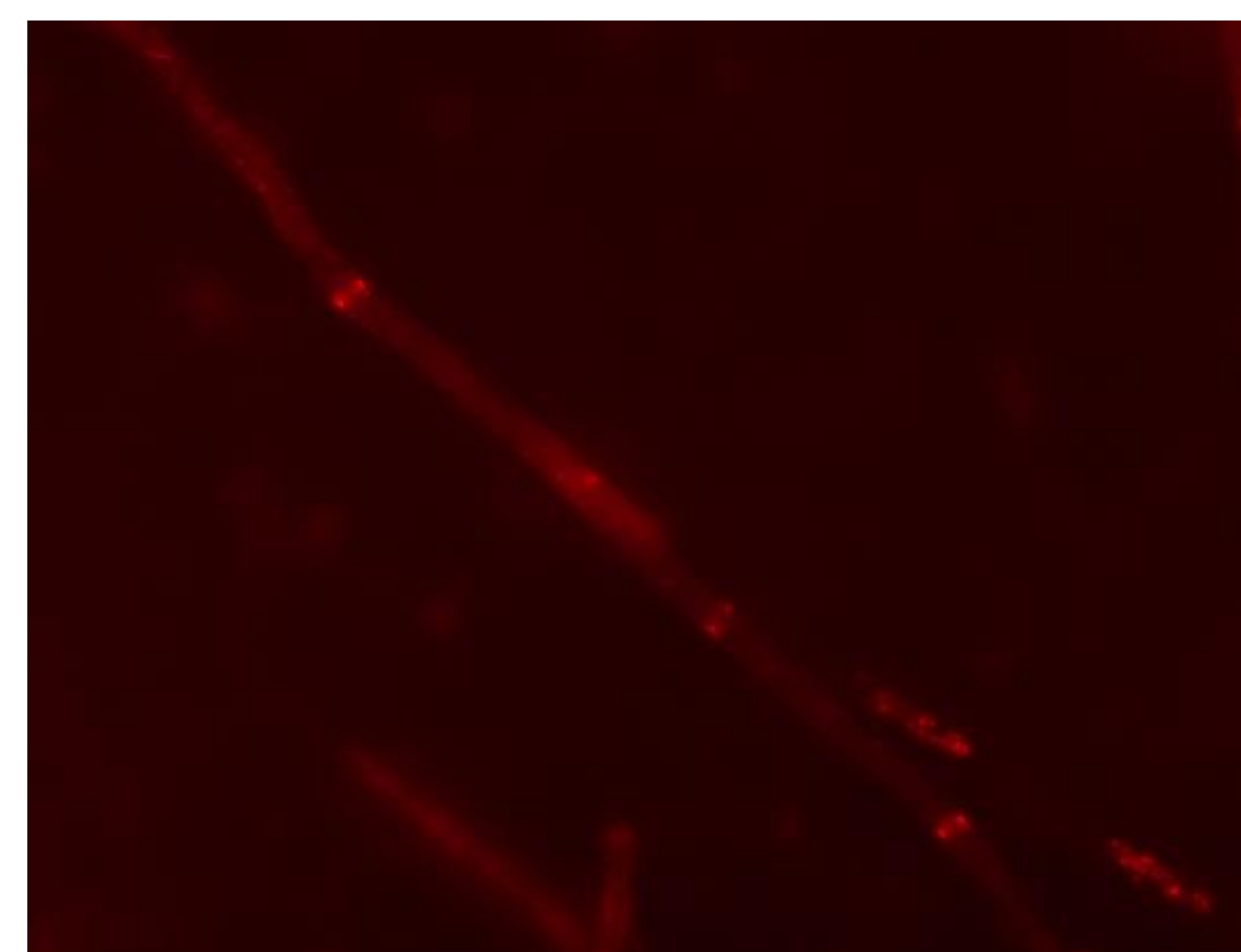


Figure 4: RFP-tagged Lifeact in a *PaxB*-deleted strain

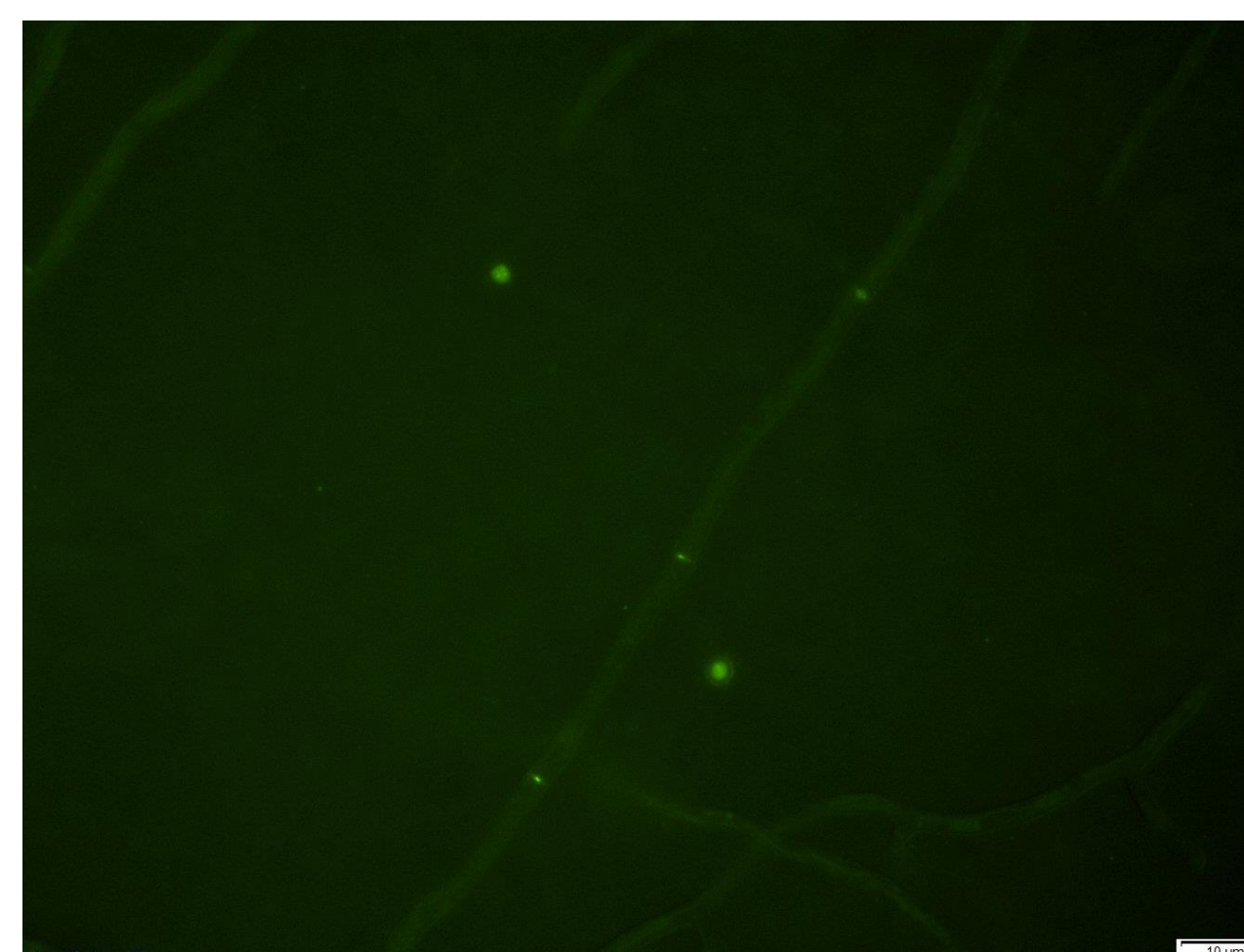


Figure 5: GFP-tagged ChsA in a wild-type background. In the *PaxB*-deleted strain, these signals are weaker and less frequently visible

Conclusions

PaxB localizes to forming septa, though it is not found in mature septa, implying that the role of PaxB is limited to serving as a scaffold to proteins that regulate septum formation rather than maintenance. Removal of *PaxB* from the genome leads to an aseptate phenotype, indicating that PaxB is necessary in the role of septum formation. Formation of the contractile acto-myosin ring can be seen when PaxB is absent, yet no septa form; these results show that PaxB is not needed for actin localization but is needed for ring constriction. Localization of the chitin synthase ChsA to maturing septa can also be noted when PaxB is absent, though its signal appears to be significantly weaker, indicating some level of inhibition of ChsA localization when *PaxB* is deleted from the genome.

Future Work

Initial testing using the Hill lab GFP-Trap protocol has shown that PaxB is in too little abundance or otherwise difficult to isolate from a protein homogenate. As such, a project is underway to upregulate the production of PaxB using an *AlcA(p)::PaxB::GFP* construct generated via Fusion PCR. Once this strain is generated, we aim to perform the GFP-Trap protocol on PaxB to determine potential binding partners via mass spectrometry. Additionally, quantitative analysis of changes in ChsA::GFP signal intensity will be performed to determine to what extent the deletion of *PaxB* affects the localization of ChsA.

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