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April 17, 2023

Abstract

Available from: Fritz-Laylin, L. K., Lord, S. J., & Mullins, R. D. (2016). Chytrid fungi construct actin-rich pseudopods, implicating actin regulators WASP and SCAR in an ancient mode of cell motility. doi:10.1101/051821



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CORRESPONDENCE:

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DATE RECEIVED:

October 17, 2016

DOI:

10.15200/winn.148087.70155

ARCHIVED:

December 04, 2016

KEYWORDS:

ASP2016Fall

CITATION:

Everett Hall, Luciane Silva, Chytrid fungi construct actin-rich pseudopods, implicating actin regulators WASP and SCAR in an ancient mode of cell motility, *The Winnower* 3:e148087.70155, 2016, DOI: 10.15200/winn.148087.70155

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INTRODUCTION

The manuscript titled “Chytrid fungi construct actin-rich pseudopods, implicating actin regulators WASP and SCAR in an ancient mode of cell motility” aims to investigate a specific mode of pseudopod-driven cell motility, coined α -motility, and to establish nucleation promoting factors WASP and SCAR/WAVE as its evolutionary signature. While SCAR’s role in pseudopod formation is firmly established, conflicting studies render WASP’s involvement more questionable. A survey of nucleation factors in eukaryotes suggests that pseudopod formation only occurs in organisms with both WASP and SCAR. Therefore, the authors hypothesize that the two factors are required for α -motility. To test this hypothesis, two approaches are used.

First, the authors characterize WASP and SCAR patterning during pseudopod formation in differentiated HL-60 cells, and find that both nucleation factors move together at the leading edge. They also show that WASP-KD results in a large reduction (~50%) in pseudopod formation and the appearance of an immotile cell population with abnormal protrusions. Next, they successfully predicted α -motility in *Batrachochytrium dendrobatidis* (*Bd*), a free-swimming, parasitic chytrid fungus that retains WASP and SCAR, but had not been known to exhibit pseudopod formation. In addition, they show that *Bd* pseudopods contain actin and that that inhibiting the Arp2/3 complex, an important component of actin-driven extensions, reduced pseudopod formation.

While the different approaches laid out in this manuscript provide a strong argument for the occurrence of α -motility only in organisms conserving WASP and SCAR, there is room for further refinement. Additional experiments and clarifications would allow further insight into the interplay between WASP and SCAR during α -motility, and allow easier interpretation of the results.

MAJOR COMMENTS

1. **Pseudopod formation in WASP-KD cells** – In figure 3, the authors show that pseudopod formation was reduced by half (from 80% to 40%) in WASP-KD cells compared to wildtype controls. While this change clearly implicates WASP in this process, the fact that the phenotype



was not fully penetrant weakens the predictive value of the WASP/SCAR evolutionary signature model. A more detailed structural and molecular analysis of pseudopod formation in WASP-KD cells could help reconcile this discrepancy. Could pseudopod formation in WASP-KD cells be driven by a separate, compensatory mechanism unrelated to WASP function? Or is the structure of pseudopods formed by WASP-KD cells different from those formed in wildtype cells?

2. **“Rhino” phenotype and migration in WASP-KD cells** –In figure 3, the authors show that 30% of KD cells exhibited a “rhino” phenotype with abnormal protrusions, and that these cells were completely immotile in chemoattractant experiments. The cell tracking figure (Fig. 3G) is broken down into three parts: Wildtype, All KD cells, and “Rhino” cells. However, it is not clear if the migration of “non-rhino” KD cells is reduced compared to wildtype. Adding a fourth section to this figure depicting the path of these cells would allow for a more comprehensive analysis of the different sub-populations within WASP-KD cultures. In addition, further experiments to determine how the “rhino” cells become initially malformed, such as observation of KD cultures over extended periods of time, would better characterize the WASP-KD phenotype.

MINOR COMMENTS

1. **Labels on phylogenetic tree** – Figure 1 depicts a phylogenetic distribution of eukaryotes and includes WASP and SCAR conservation, along with the presence of pseudopod formation in the various organisms. The figure is successfully shows the pattern of pseudopod formation only in organisms that retain both nucleation factors, but could be made more complete by adding the labels for the taxonomic groups on the bottom half of the figure, such as the ones present on the top half.
2. **WASP and SCAR localization** –In figure 2 and movie S1, the localization of fluorescently tagged WASP and SCAR complex are shown over time during pseudopod formation. It is clear that both nucleation factors concentrate at the leading edge, but the WASP punctate expression occasionally “anchors”, or moves in a retrograde fashion. Could this difference in expression perhaps provide insight into the function of WASP during pseudopod formation? In addition, the still images appear to show diffuse expression of both WASP and SCAR throughout the cell, not just at the leading edge. Could this simply be an artifact of the TIRF microscopy technique? Additional imaging experiments on fixed cells by fluorescent or confocal microscopy may allow enhanced detailing of WASP and SCAR’s differing expression patterns and help elucidate the role of WASP in pseudopod formation.
3. **Identity of the control vector(s)** –In figure 3A and 3F, a “KD+Control” shRNA vector is shown. In the figure legend, the control vector is described as an “empty vector rescue control”, while the text in the results section refers to “a control shRNA that targets a separate region of the WASP gene. Based off of the information provided, it is not clear whether both of these are referencing the same vector, or if there are multiple control vectors. Additional clarification on this point would allow easier interpretation of the results.
4. **Clarifications of polymerized actin figure** –In figure 3B, the authors show that WASP-KD cells exhibit reduced explosive actin polymerization as a result of chemoattractant treatment. The results are apparent, but initial interpretation of the figure may be improved by adding a figure key depicting the WT and KD groups. In addition, the small size of the error bars in the WT samples makes them difficult to see, giving the impression that the error bars are absent. Making the error bars a different color from the black squares would resolve this confusion.
5. **Ordering of the figures within sections** –In the results section of the manuscript, there are a few occasions where the references to the figures are disordered, requiring the reader to jump around the figure to follow the text. For example, the text pertaining to figure 3 lists the figure letters in the following order: A, E, C, D, F, G, B. Changing the figure arrangement is unrelated to the content, but it would improve the ease with which one reads the manuscript.