Investigating the role of NPFs in the Arp3ΔC phenotype

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Introduction

The arp2/3 complex is an assembly of seven protein subunits that nucleates branched actin networks involved in cellular functions such as endocytosis. Previous work has determined the complex is intrinsically inactive, and can be turned "on" by activators like ATP or proteins called nucleation promotion factors (NPFs). It has been hypothesized that the complex remains in an auto-inhibited state due to the c-terminus of the arp3 subunit. Deletion of the c terminus (arp3ΔC) results in a hyperactive arp2/3 complex in a purified in-vitro system. Strikingly, this complex is inhibited by the canonical NPF wiskott-aldrich syndrome protein (wsp1). These contrary phenomena are complicated further by the observation of endocytosis in-vivo. In S. Pombe fission yeast, arp3ΔC generates endocytic patches that have a reduced internalization percentage compared to wild type cells but assemble at nearly the same abundance. This suggests preferential binding of a single NPF to arp2/3 that polymerizes actin, but in an incorrect manner for endocytosis. Here, we will investigate involvement of dip1 amongst other NPFs in the Arp3ΔC phenotype. Dip1 has been shown to be involved in the temporal regulation of actin polymerization during endocytosis; deletion of this activator results in both decreased patch density and longer but more stochastic patch lifetimes before internalization. Utilizing S. pombe as a model organism, arp3ΔC will be combined with NPFΔ constructs to determine which activator is responsible for actin polymerization in the absence of the arp3 c-terminus.

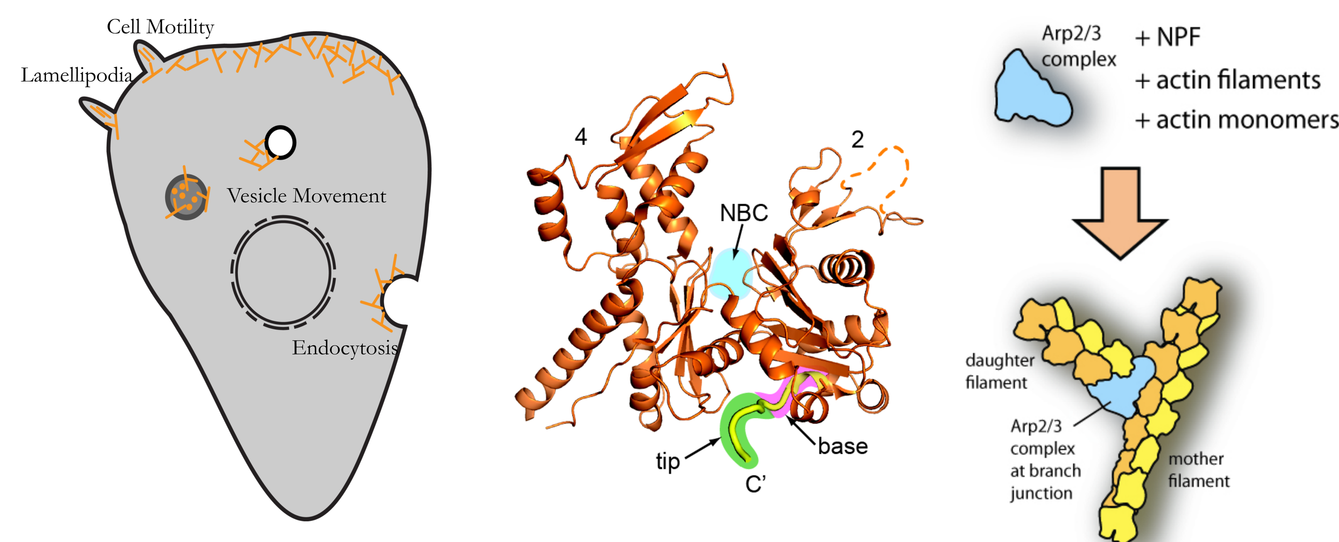
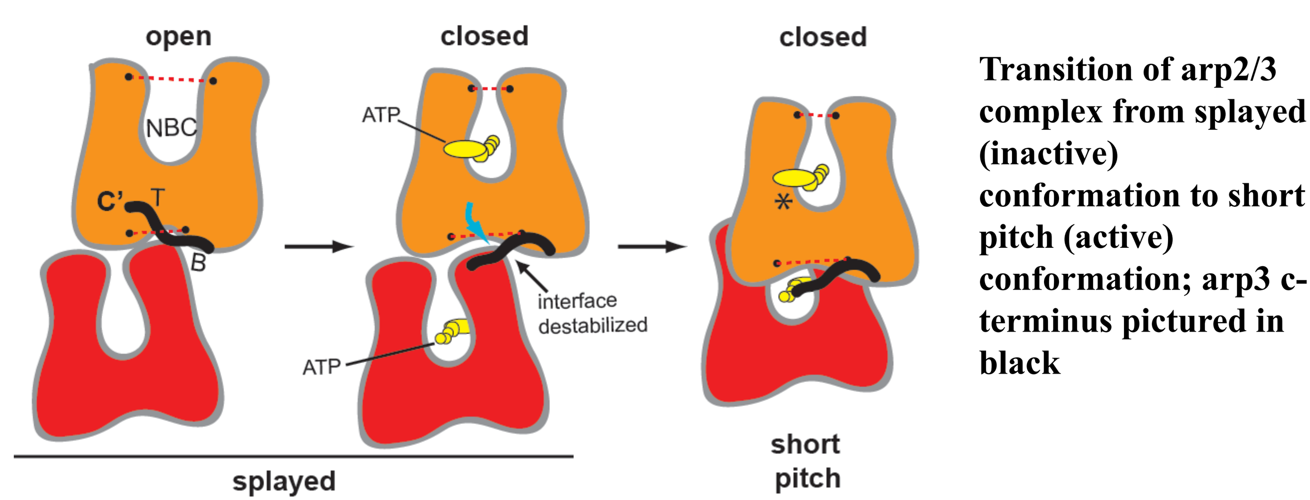
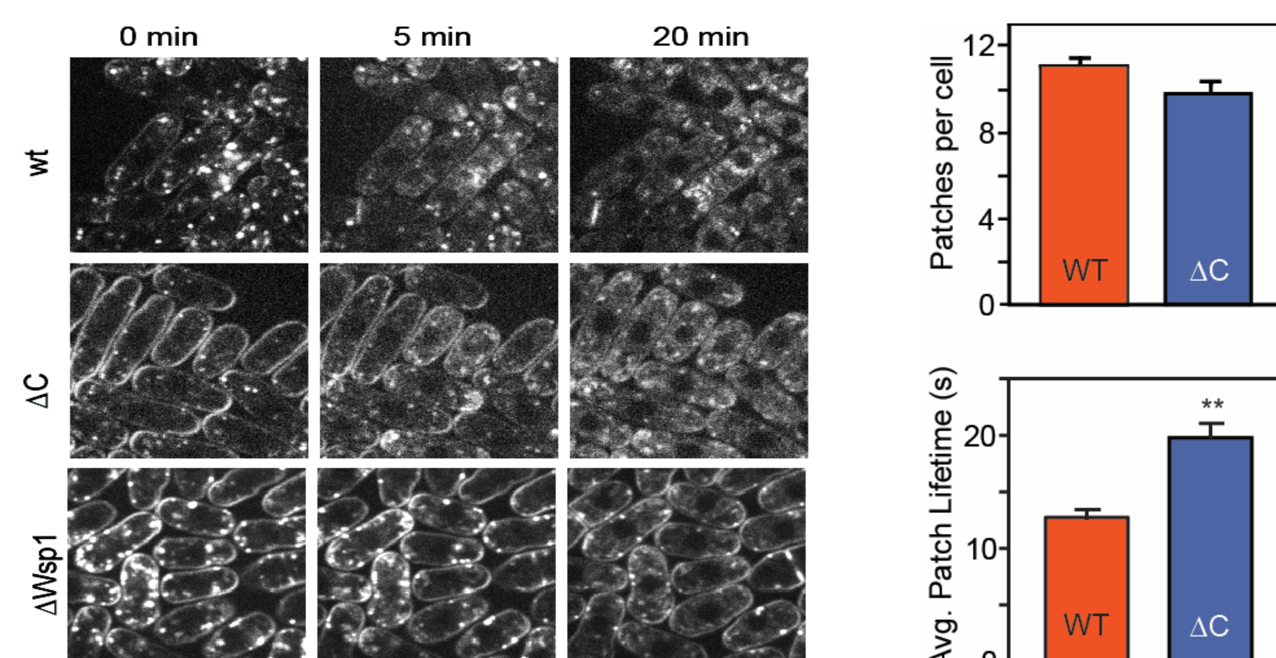


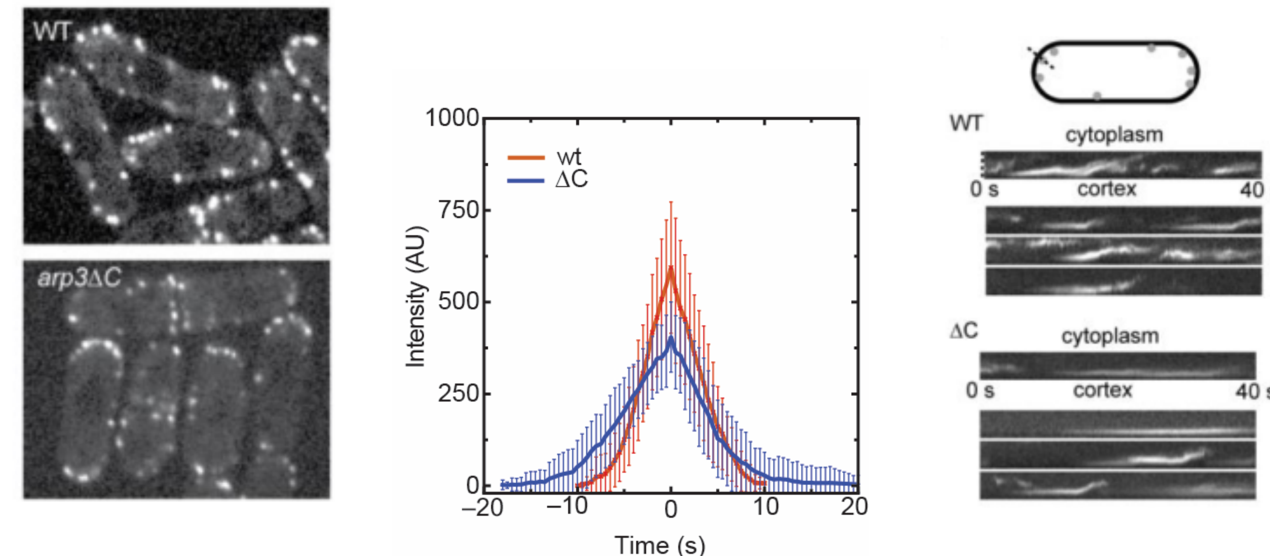
Illustration of S. Pombe cell with actin structures labeled (left), Close up of arp3 with c-terminus in yellow (center), illustration of branch formation (right)



Transition of arp2/3 complex from splayed (inactive) conformation to short pitch (active) conformation; arp3 c-terminus pictured in black



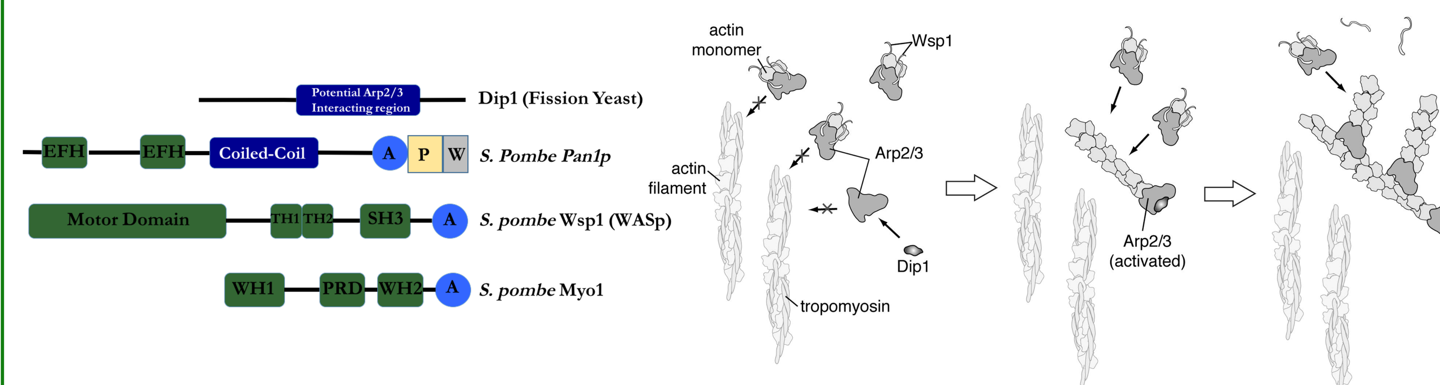
FM 4-64 dye endocytosis assay: Over 20 min time course the arp3dC mutant exhibited slower uptake of the dye compared to wildtype. Total endocytic patch number was relatively unchanged. A separate deletion of wsp1 yielded similar results.



Live cell images of wild type and arp3ΔC S. Pombe (left). In the arp3ΔC phenotype both patch assembly and disassembly is slowed; fluorescence intensity is also lower (center). Kymograph analysis reveals patches move off of the cortex much less frequently than wildtype. (right)

NPF Selection Rationale

In the arp3ΔC phenotype, endocytic patches fail to internalize but actin still accumulates to wildtype levels. Furthermore, in-vitro the NPF wsp1 becomes an inhibitor limiting branch nucleation. Therefore, if there is an NPF that is preferentially binding the arp2/3-arp3ΔC complex it is most likely binding with a mechanism dissimilar to wsp1. Previous study has determined the NPF dip1 binds with a non-wsp-like binding model in that it does not bind actin monomers or filaments when interacting with the complex. Unlike other NPFs, dip1 generates linear filaments with the arp2/3 complex that can serve to provide branch junctions. As described prior, the potential role of dip1 in the temporal regulation of endocytosis also adds to the likelihood of this NPF being involved in the observed defect of arp3ΔC. Nevertheless, the involvement other NPFs involved cannot be ruled out, the NPFs pan1p and myo1 are also noted to activate Arp2/3 but are not known to affect the timing of endocytosis.



NPFs and domains (left), illustration of dip1 and wsp1 activation (right)

Conclusions

From lifetime analysis, it was shown deletion of either dip1 or the active regions in pan1 in the presence of the arp3ΔC mutation rescues patch lifetime lengths and distributions from defective levels. It provides support for a hypothetical mechanism where the arp3ΔC-arp2/3 complex will abundantly nucleate filaments from activation by dip1 or pan1. Since there is preferential activation, the actin filament network in an endocytic patch will be structured differently and could hinder the regulating timing of endocytosis. This conclusion should be subjected to further scrutiny as it was thought wsp1-like binding mechanism as encountered in pan1 would be inhibiting to the arp3ΔC-arp2/3 complex. Additionally, wildtype levels of patch density could not be recovered in these two double deletions. A curious observation made during double mutant strain construction was arp3ΔC-wsp1ΔCA was unable to be produced, there were multiple false positives that appeared but never the correct construct. This could signify a potential lethality in the crossing or other method of transformation must be attempted. Further work will address the change of patch initiation rate in the arp3ΔC phenotype to further develop our mechanistic hypothesis.

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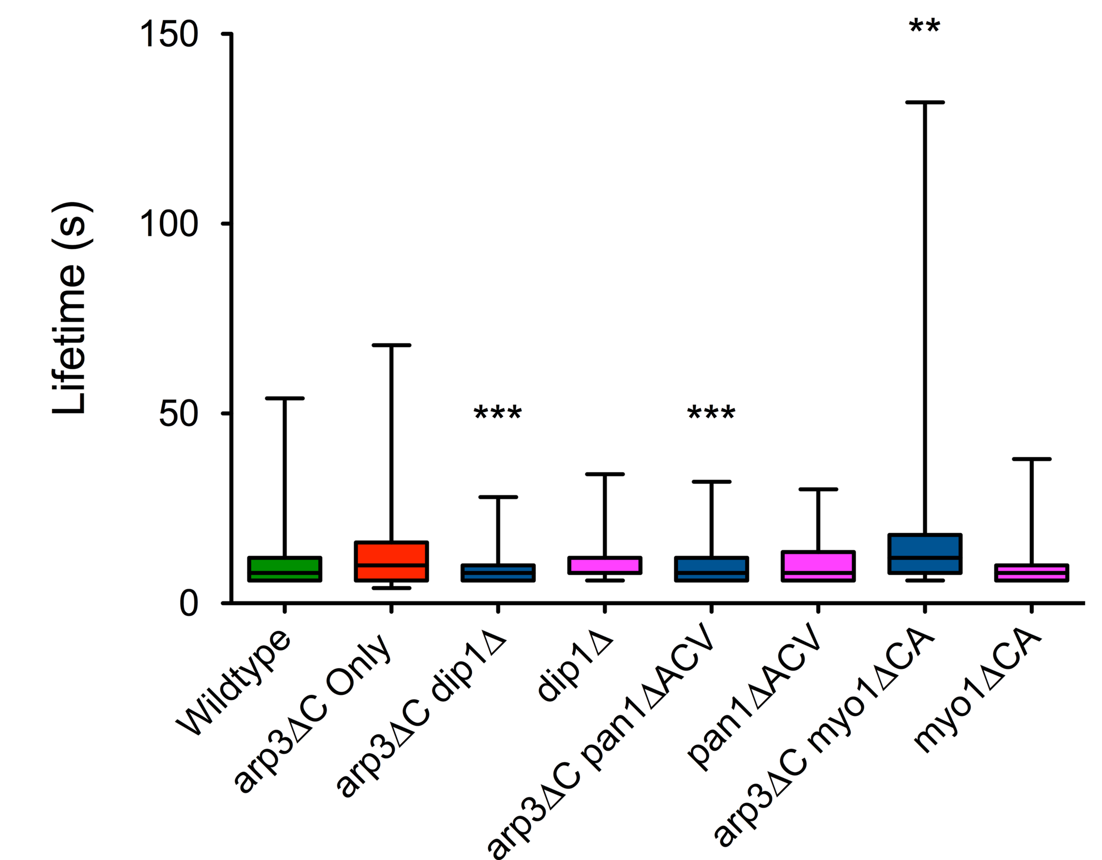
- Andrew Wagner for his continued mentorship

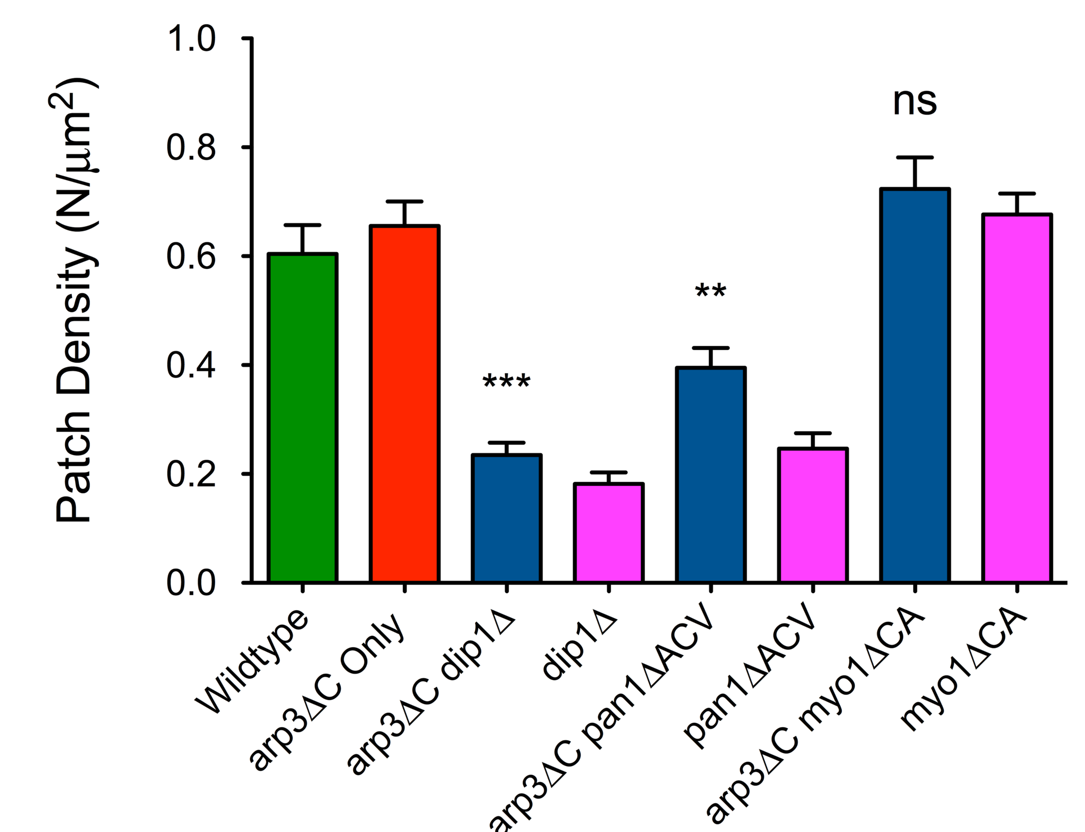
Current Hypothesis

The Arp3 c-terminus is a key regulatory feature that holds the Arp2/3 complex in the inactive splayed conformation in the absence of activators.

Results



Endocytic patch lifetimes of arp3ΔC-NPFΔ mutants (blue); included for comparison: wildtype (green), arp3ΔC (red), and NPFΔ (purple). Unpaired t-test p-values to arp3ΔC (shown): arp3ΔC-dip1Δ p<0.0001, arp3ΔCpan1ΔACV p<0.0001, arp3ΔC-myo1ΔCA p=0.0067. Unpaired t-test values to wildtype (not shown): arp3ΔC-dip1Δ p=0.0999, arp3ΔC-pan1ΔACV p=0.6785, arp3dC-myo1ΔCA p<0.0001



Endocytic patch densities of arp3ΔC-NPFΔ mutants (blue); included for comparison wildtype (green), arp3ΔC (red), and NPFΔ (purple). Unpaired t-test p-values to arp3ΔC (shown): arp3ΔC-dip1Δ p<0.0001, arp3ΔC-pan1ΔACV p=0.0035, arp3ΔC-pan1ΔACV p=0.0035, arp3ΔC-myo1ΔCA p=0.4169. Unpaired t-test values to wildtype (not shown): arp3ΔC-dip1Δ p<0.0001, arp3ΔC-pan1ΔACV p=0.0181, arp3ΔC-myo1ΔCA p=0.1972

References

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