Arp2/3 complex-nucleated, light-dependent filopodia in amoeboid algae perform bidirectional cyclosis

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P1080 / B82

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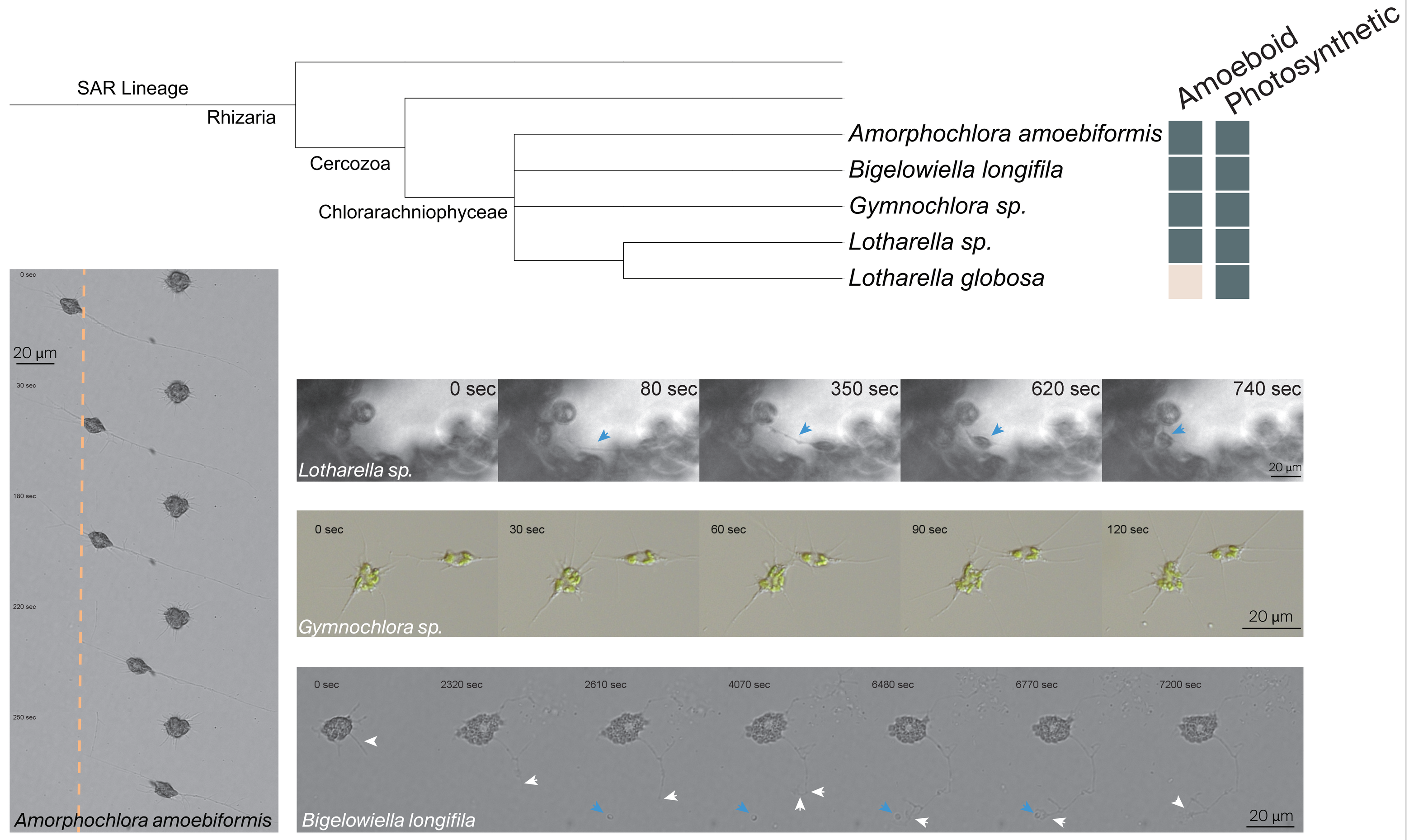
Background

Chlorarachniophytes are endosymbiotic amoeboid algae



Full project narative available at : **bit.ly/diverse-cyto**

Strains available at: **National Center for Marine Algae and Microbiota (NCMA)**
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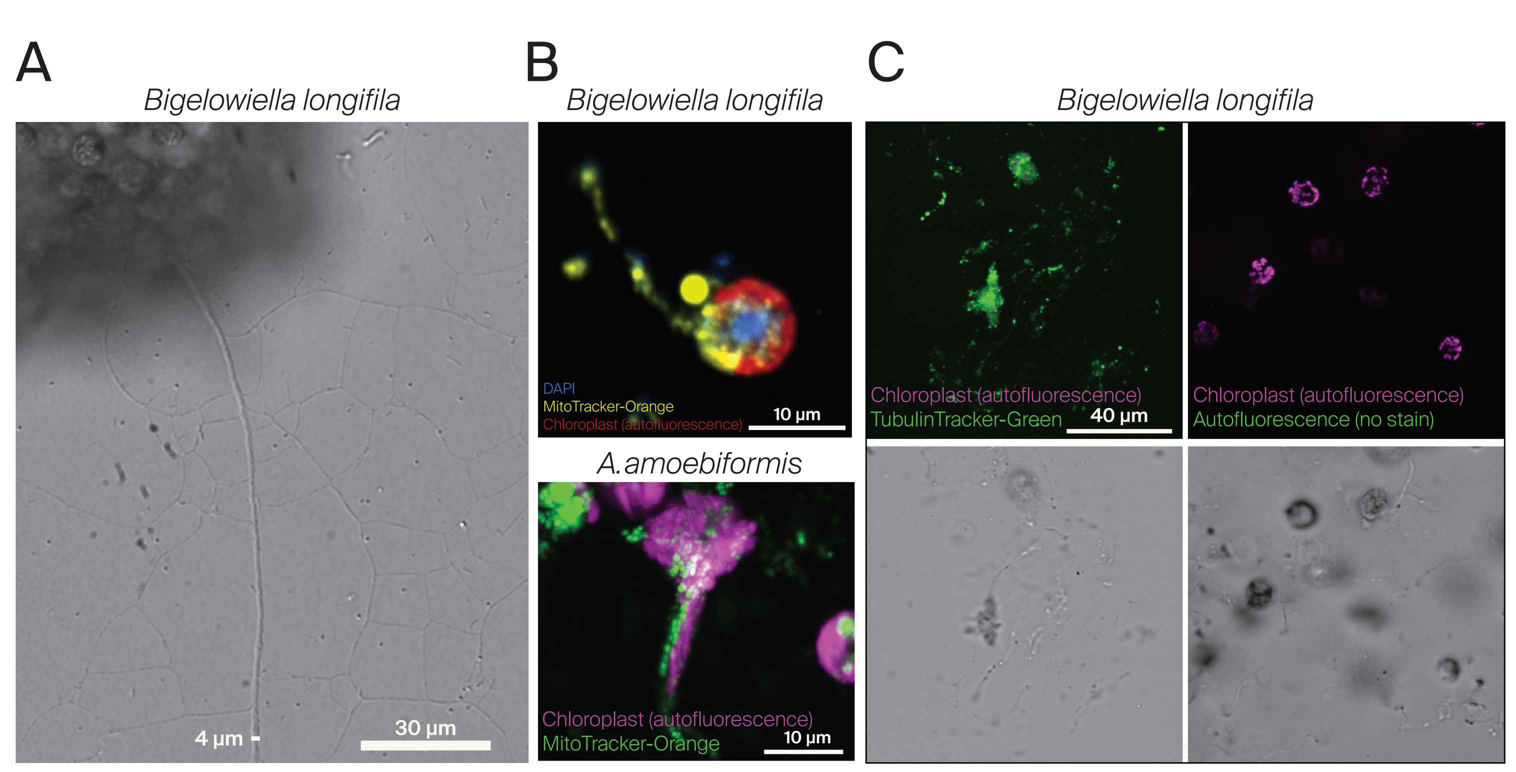
Chlorarachniophytes (Chlor-green / Arachnio- spider) are a group of amoeboid cells that underwent secondary endosymbiosis by engulfing a unicellular green algae (1). These cells carry out photosynthesis while also forming intricate extensions which contribute to cell motility and predation.

Key questions:

A. Which cytoskeletal components give rise to these extensions?
B. How are these extensions initiated and regulated?
C. What specific functions do these extensions have?

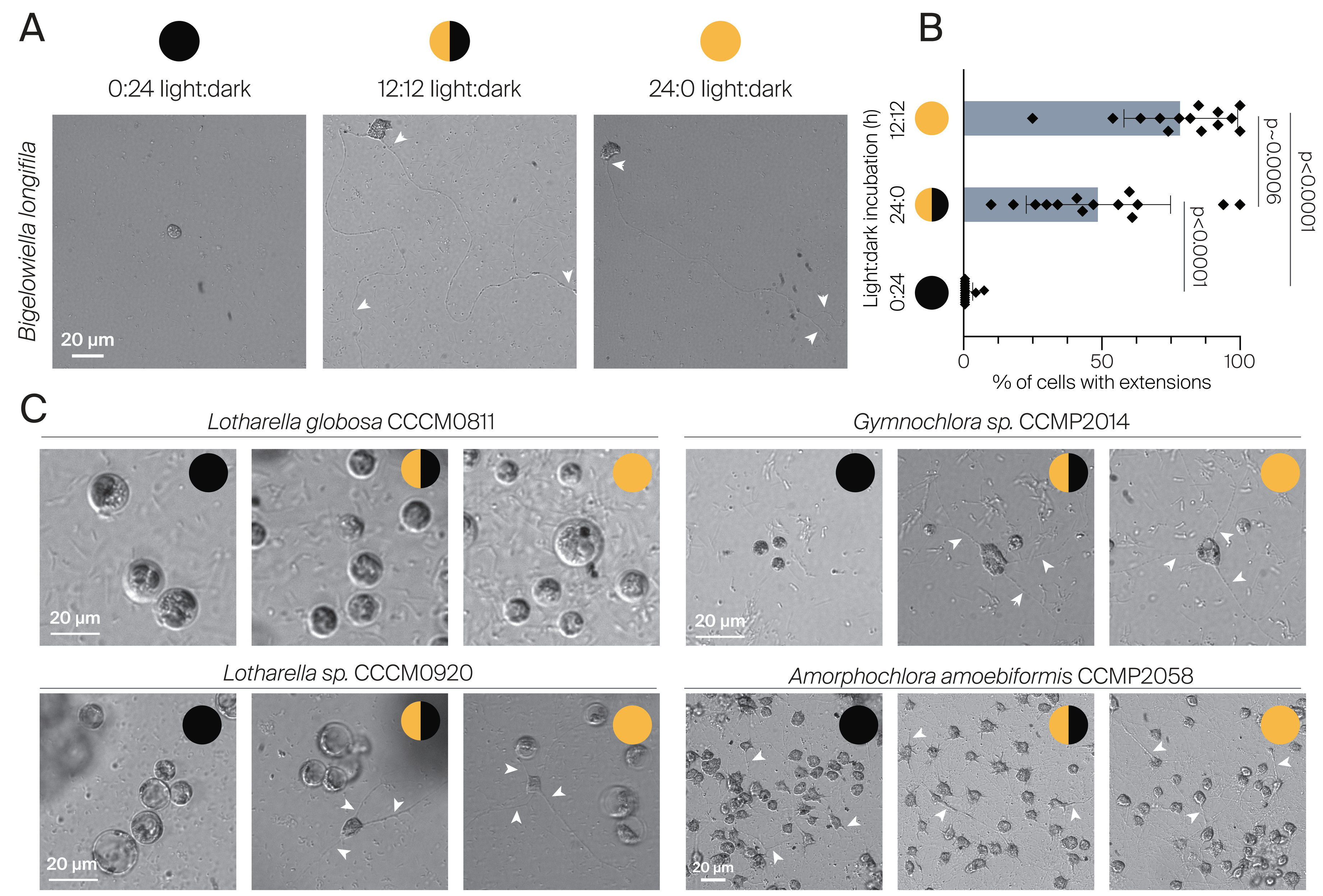
Findings & Results

1) Mitochondria and Chloroplasts are trafficked through extensions



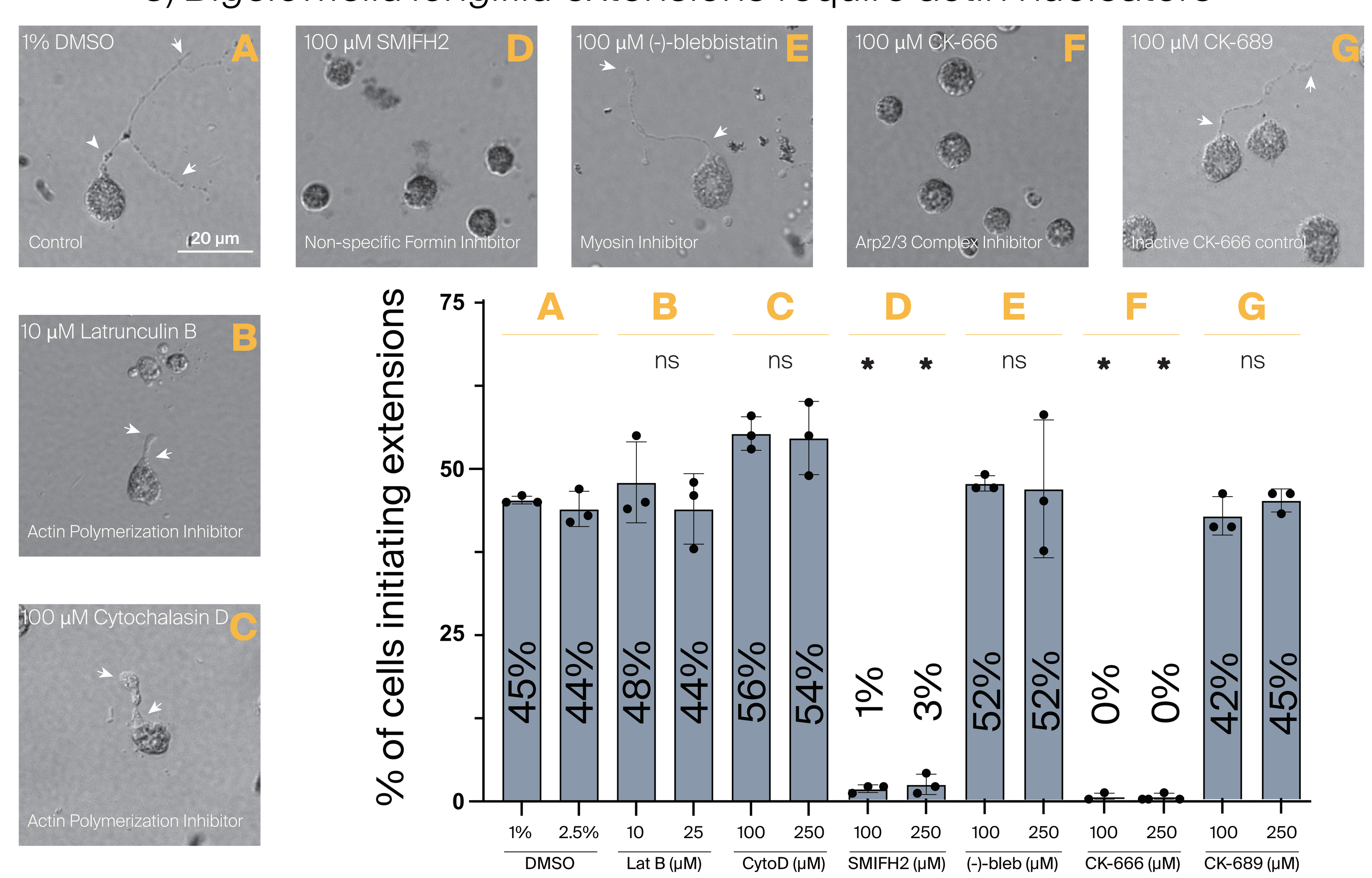
Visualization of extension components. (A) DIC image of *Bigelowiella longifila* cells forming a massive network with tubes up to 4 µm wide. Rapid cytoplasmic transfer was observed throughout all extensions in the network. (B) Live cell microscopy uncovered mitochondria (MitoTracker™-Orange CMTMRos), Chloroplasts (autofluorescence), and DNA (DAPI) being trafficked through these extensions in *Bigelowiella longifila* and *Amorphochlora amoebiformis*. The chloroplasts observed in the *Amorphochlora amoebiformis* extension quickly retracted into the cell body following exposure to intense light. (C) (Top Left) Tubulin Tracker™ Green was taken up by *Bigelowiella longifila* extensions, suggesting tubulin is also trafficked through these structures. (Bottom panels) DIC images of the islands of *Bigelowiella longifila* extensions. (Top Right) Confirmation that unstained *Bigelowiella longifila* islands do not autofluoresce in the same channel as Tubulin Tracker™ Green.

2) Extension formation is light-dependent in some species



Effects of light on extension formation. Chlorarachniophytes were grown on coverslips over 2 .5 days under constant darkness (0:24 Light:Dark; black circle), cyclic light (12:12 Light:Dark; yellow:black circle), or constant light (24:0 Light:Dark; yellow circle). All light sources were full visible spectrum white light. (A,C) Representative images of chlorarachniophyte species with or without filopodia. Arrowheads indicate filopodial extensions. (B) Quantification of panel A. Dataset includes 14 individual samples per variable.
Total number of cells per variable: 0:24 (349 cells); 12:12 (505 cells); 24:0 (609 cells).

3) *Bigelowiella longifila* extensions require actin nucleators



Effect of cytoskeletal inhibitors on extension initiation. *Bigelowiella longifila* cells were treated with cytoskeletal inhibitors for 3 hours while adhering to coverslips. After 3 hours, PFA was added to a final concentration of 4% to fix the cells without disturbing extensions. Cells were then imaged by DIC microscopy. Yellow letters (A-G) indicate corresponding representative images and bars on the bar graph.
10 µM & 100 µM (25 µM & 250 µM) conditions were compared with the 1% (2.5%) DMSO control * $p<0.0001$; ns: not significant.
Bar graphs represent 3 independent experiments.

Conclusions & Summary

- Extensions undergo bidirectional transport trafficking organelles including mitochondria and chloroplasts
- Extension formation is prevented by actin nucleation inhibitors, CK-666 and SMIFH2; however, SMIFH2 inhibition was recently shown to be non-specific (2).
- Strangely, inhibiting actin polymerization did not reduce the frequency of extensions forming, but altered their morphology
- *Bigelowiella longifila* actin might be resistant to actin polymerization inhibitors, similar to the divergent *Chlamydomonas reinhardtii* actin NAP1 which is resistant to Latrunculin B treatment (3).

Next Steps

These structures are dependent on the actin cytoskeleton, yet we've been unable to visualize actin in these cells. Recent work developed transformation methods in chlorarachniophyte species (4). We're creating strains with fluorescently tagged Actin Binding Proteins to visualize dynamics in live cells. Further, the amoeboid chlorarachniophyte transcriptomes were recently sequenced (5) and assembled (6). We are actively combing these datasets to determine which actin binding proteins are present in these species.

I'd appreciate feedback on any of this work, but I'm especially curious about the following:

- Can you hypothesize additional functions for these extensions?
- What cargo would you expect to see being trafficked through these extensions?

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