Actin and Myosin contribute to cell wall integrity in the diatom *Phaeodactylum tricornutum*

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P1663 / B687

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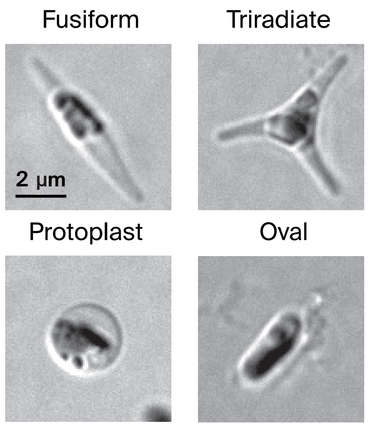
Background

Phaeodactylum tricornutum cells take on 3 morphotypes with altered expression of actin binding proteins



	Severs	Caps	Bundles	Nucleates	O-F	O-T
Cofilin	■				+4	+5
Tropomodulin		■			+4	+3
Gelsolin	■	■			+2	+2
Villin	■	■	■		-	+2
Capping Protein, α		■			+2	+2
Capping Protein, β		■			+1	+1
Formin				■	-	-2
Ysc84			■		-2	-2
WASH				■	-	-2
Dynamin			■		-1	-2
Formin				■	-	-2

Transcriptomic data (ref. 1)
Protein Functions (ref. 3)

O-F : fold change from oval to fusiform
O-T : fold change from oval to triradiate

For more, read the full project narrative: **bit.ly/diverse-cyto**

Strains available at: **National Center for Marine Algae and Microbiota (NCMA)**
UTEX Culture Collection of Algae

The diatom *Phaeodactylum tricornutum* takes on 3 main morphotypes: Fusiform, Triradiate, and Oval. Transcriptomics studies revealed actin severing and capping proteins are upregulated while nucleators and bundlers are downregulated in certain morphotypes (1), suggesting the actin cytoskeleton plays an important role in cell morphology decisions. Interestingly, *Phaeodactylum* and all other sequenced diatoms lack an obvious Arp2/3 complex ortholog. This complex is responsible for nucleating branched actin networks and is essential in many species. Further, diatoms integrate silica into their cell walls; however, recent work suggests silica nanoparticles alter actin filament localization and dynamics (6,7). Here we aim to understand how the actin cytoskeleton functions in this seemingly unfavorable environment.

Key questions:

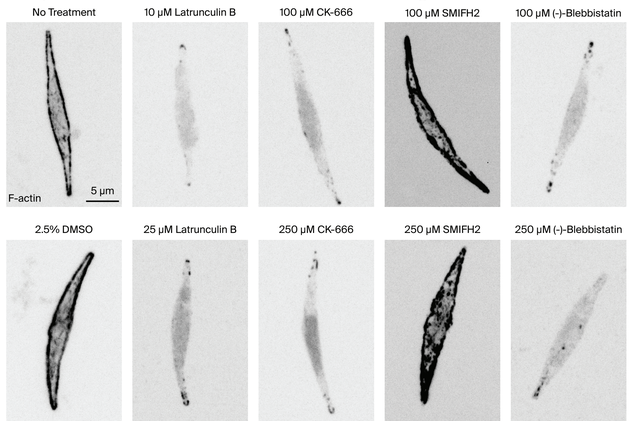
A. What, if any, regulators of actin dynamics are required for morphotype switching?

B. What dictates whether the Arp2/3 complex is essential in a given species?

C. How does cell wall integrity intersect with actin regulation in organisms with silica-based cell walls?

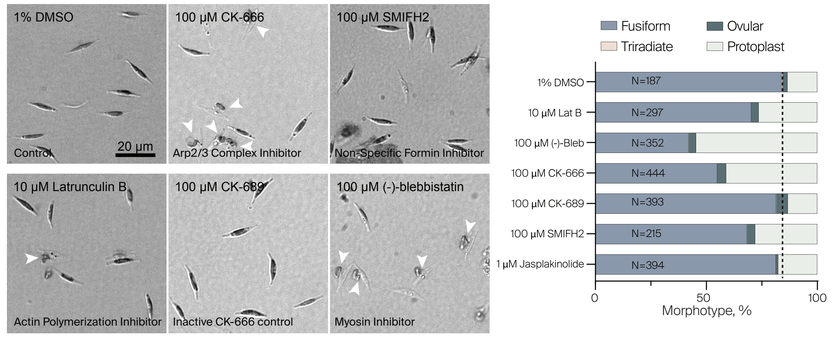
Findings & Results

1) Actin filaments localize along the cell cortex



Actin filament localization in fusiform cells. Representative maximum intensity projections of z-stack images of fusiform UTEX646 *Phaeodactylum tricornutum* cells stained with phalloidin-atto 488 after the 2 hour indicated treatment. Notably, treatment with the Arp2/3 complex inhibitor CK-666 strongly impacted actin localization even though these cells lack an Arp2/3 complex.

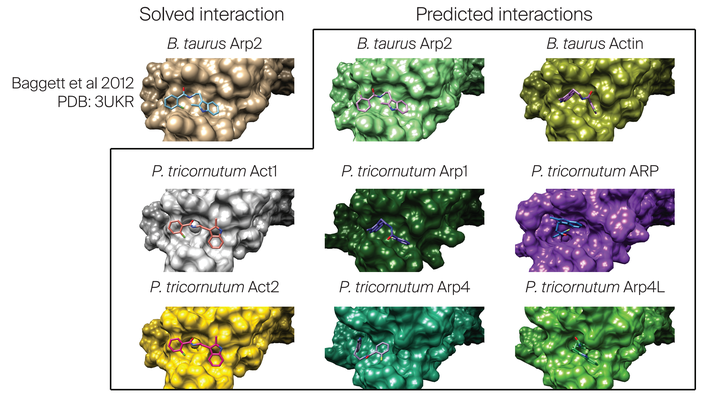
2) Myosin and Arp2/3 complex inhibitors induce protoplast formation



**Recent experiments have yielded 0% protoplasts across conditions*

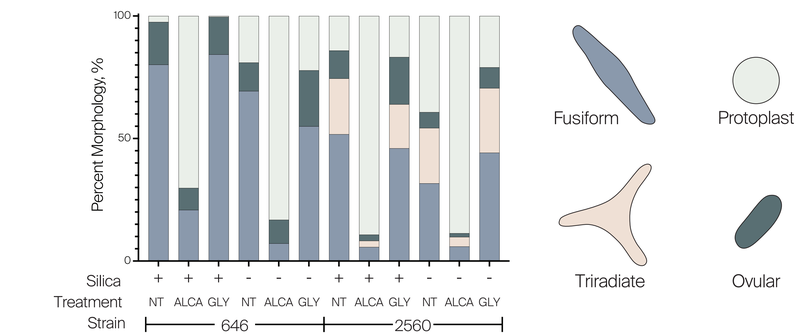
Effects of cytoskeletal inhibitors on *Phaeodactylum* morphology. Representative DIC images of *Phaeodactylum* cells after 2 hours of the indicated treatment. Arrowheads indicate emerging protoplasts. Quantification is representative of 2 independent experiements on seperate days; however, **these data are highly inconsistent and likely dependent on the life cycle, cell cycle, or other not yet identified variables.**

3) Simulations indicate CK-666 might bind *Phaeodactylum* actin



Modeling CK-666 interactions with actin and actin-related proteins. The solved structure of CK-666 bound *Bos taurus* Arp2 (PDB:3UKR) (4) was used as a template to simulate CK-666 docking to *P. tricornutum* actin-like protein structures which were predicted using Phyre One-to-One threading software. Docking was performed using Chimera and AutoDock Vina.

4) Silica reduces the frequency of protoplast formation



Effects of Alcalase® and silica on cell morphology. Cells were treated with Alcalase® enzyme (ALCA) (0.3 Anson Units / mL), glycerol as a negative control (GLY), or fresh media (NT; No Treatment) for 1 hour while rotating. Cells lacking silica were grown in Guillard's F/2 media without silica in polymethylpentene erlenmeyer flasks. Cells with silica were grown in standard Guillard's F/2 media in borosilicate erlenmeyer flasks. Data is representative of 3 individual experiments.

Conclusions/Summary

Our current findings suggest actin and myosin regulate cell wall integrity, possibly by trafficking cell wall components to the cortex. Further, our results indicate that the Arp2/3 complex inhibitor CK-666 could be inhibiting actin in diverse species. Interestingly, growing cells in the absence of silica resulted in a increased protoplast formation, similar to cells treated with cytoskeletal inhibitors.

Next Steps

We are actively working to map actin-dependence across the life cycle in this species. Our inability to reproduce the findings in Figure 2 suggest variability across the >50 hour life cycle that we hope to parse out and provide rigorous data to support our findings. Additionally, we're excited to learn if these cells can indeed form branched actin networks while lacking an Arp2/3 complex. Finally, we are interested in using these cells to investigate recently reported silica-induced changes in the actin cytoskeleton.

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

- Do you have any tips on protoplast isolation? Our inability to reproduce cytoskeleton-induced protoplast formation could be because the protoplasts could be easily ruptured when the cells are handled.
- Why would SMIFH2 increase the intensisty of F-actin staining?
- How can we test CK-666 specificity in a more rigorous way?

Leave Feedback!

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> *Understanding the evolution of actin-binding proteins across diverse species*

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If you decide to share anyway, yay! That's what science is all about. If your input is useful, we will include you as a contributor to the publication and explain that your role was in providing "Critical Feedback," likely with an additional description of what you shared.

tl,dr — If you're not ready for everyone to know about something, please refrain from sharing it with us.

Contributors (A–Z)

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Cameron MacQuarrie • Conceptualization, Formal Analysis, Investigation, Methodology, Writing, Visualization

Megan Hochstrasser • Editing, Visualization

Atanas Radkov • Formal Analysis, Investigation, Methodology, Visualization

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