Mechanism of Filamin Action in Response to Mechanical Stimuli

Sarah Buckler, Yulia Artemenko

Department of Biological Sciences, SUNY Oswego, Oswego NY

ABSTRACT

Molecular mechanisms by which cells sense and directionally migrate in response to mechanical perturbation, which is critical in homeostasis and many diseases, are not well understood. *Dictyostelium discoideum* cells exposed to a brief burst of shear flow show rapid and transient activation of multiple components of the signal transduction network, a response that requires an intact actin cytoskeleton of the cell. However, exactly what aspect of the actin cytoskeleton network is responsible for sensing and/or transmitting the signal is unclear. Previous data from our laboratory suggested that actin crosslinking protein filamin is involved in the ability of cells to respond to shear flow. In this study we further characterized the mechanism of filamin action in this response. Filamin itself showed rapid and transient relocalization from the cytosol to the cortex following 2 sec stimulation with shear flow. To detect activation of the signal transduction network in the presence or absence of this actin-binding protein, we used fluorescently-tagged Ras binding domain biosensor that detects active Ras and was previously shown to relocalize to the cortex following mechanical stimulation. Reduced responsiveness of the network to stimulation with shear flow in the absence of filamin was specific to mechanical stimuli since response to global stimulation with a chemoattractant was comparable between cells with or without filamin. To understand how filamin might be regulating shear flow-induced responses we generated truncation constructs of filamin lacking either the actin binding domain or the dimerization domain. Studies are underway to determine whether these truncation constructs are able to rescue the reduced response of filamin-null cells to brief stimulation with shear flow, which will offer insight into the molecular mechanism of filamin action in this context.

INTRODUCTION

Dictyostelium discoideum

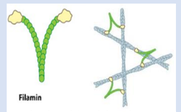
- Social amoeba[1]
- Contains many genes homologous to higher eukaryotes[1]
- Useful for studying cell motility, chemotaxis, signal transduction, etc.[1]

Directed Cell Migration

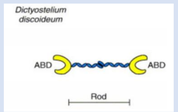
- Cells respond to various stimuli, including chemical and mechanical cues.[2]
- Activation of the signal transduction network can bias actin polymerization allowing for directed cell migration.[3]
- Although chemical and mechanical stimuli appear to activate similar signal transduction networks, **how cells sense mechanical stimuli remains unclear**.[3]
- An intact actin cytoskeleton is necessary for cell response to mechanical stimuli.[3]

Actin-binding Protein - Filamin

- Crosslink actin filaments[2]
- Stabilize 3D actin webs[5]
- Link actin to plasma membrane[5]
- Implicated in sensing mechanical pressure[4]
- Previously shown in our lab that response to acute mechanical stimulation is reduced in the absence of filamin in *D.discoideum*



https://www.mechanobio.info/cytoskeleton-dynamics/actin-crosslinking/



Popowicz et al., 2004.

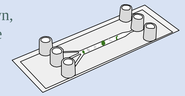
HYPOTHESIS

Filamin's actin-binding domain and/or dimerization domain is needed for filamin-mediated response to mechanical stimulation in *Dictyostelium discoideum*.

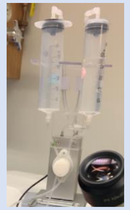
APPROACH

***Dictyostelium* cells** (WT, filamin-null) expressing **mCherry-tagged filamin** and **GFP-tagged Ras-binding domain (RBD)**

Collect vegetative cells grown on a bacterial lawn, wash in buffer, and plate



Ibidi µ-Slide III ^{3in1}



- Image with epifluorescence every 3 sec for 20 frames
- After 5 frames, stimulate cells with shear flow for 2 sec at 50 mbar pressure

RESULTS

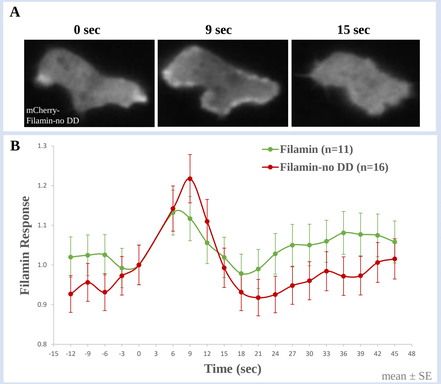


Figure 1. Filamin-no DD transiently relocalizes to the cortex in response to acute stimulation with shear flow. Wild-type cells expressing mCherry-tagged filamin or filamin lacking DD were imaged with epifluorescence microscopy every 3 sec. Cells were stimulated with shear flow for 2 sec at time 0. (A) Representative images showing increased accumulation of filamin lacking DD at the cortex 9 sec after stimulation. (B) Response was quantified as the inverse of drop in cytosolic intensity of mCherry-filamin signal.

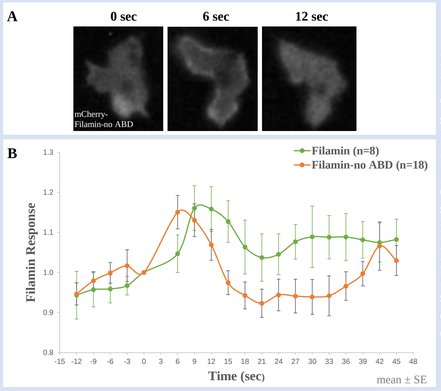


Figure 2. Filamin-no ABD transiently relocalizes to the cortex in response to acute stimulation with shear flow. Wild-type cells expressing mCherry-tagged filamin or filamin lacking ABD were imaged with epifluorescence microscopy every 3 sec. Cells were stimulated with shear flow for 2 sec at time 0. (C) Representative images showing increased accumulation of filamin lacking ABD at the cortex 6 sec after stimulation. (D) Response was quantified as the inverse of drop in cytosolic intensity of RFP filamin.

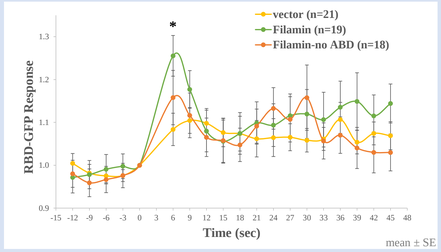


Figure 3. Response to acute stimulation with shear flow appears to be reduced in filamin-null cells expressing filamin-no ABD compared to cells with wild-type filamin. Vegetative filamin-null cells expressing RBD-GFP, as well as empty vector (pDRH), mCherry-tagged filamin or filamin lacking ABD were stimulated with shear flow for 2 sec at time 0. RBD-GFP response (relocalization from the cytosol to the cortex) was quantified as the inverse of drop in cytosolic intensity. *P<0.05 for vector vs. filamin.

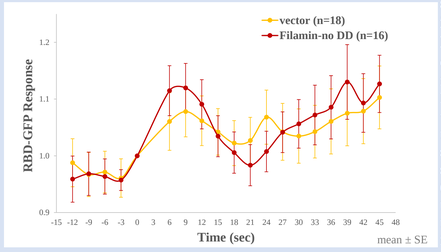


Figure 4. Response to brief stimulation with shear flow is similar in filamin with no DD compared to cells with empty vector. Vegetative filamin-null cells expressing RBD-GFP, as well as empty vector (pDRH), or mCherry-tagged filamin lacking DD were stimulated with shear flow for 2 sec at time 0. RBD-GFP response (relocalization from the cytosol to the cortex) was quantified as the inverse of drop in cytosolic intensity. No significant differences were found between the cell lines.

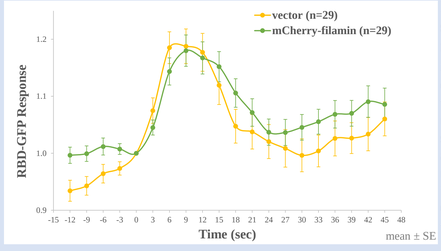


Figure 5. Response to stimulation with folic acid is not affected by lack of filamin. Vegetative filamin-null cells expressing RBD-GFP, as well as empty vector (pDRH) or mCherry-tagged filamin were stimulated with 100 µM folic acid at time 0. Response was measured as an inverse of drop in cytosolic intensity of RBD-GFP. No significant differences were found between the cell lines.

CONCLUSIONS AND FUTURE DIRECTIONS

- **Filamin's role appears to be specific to mechanical stimuli.**
  - Filamin is not involved in the response to chemical stimuli.
  - Effects of filamin are not due to altered adhesion of filamin-null cells to the surface *(data not shown)*.
- **Filamin's actin-binding domain and dimerization domain may not be needed for the re-localization to the cortex in response to shear flow stimulation**
  - In wild-type cells filamin without ABD or DD translocated to the cell cortex similarly to full-length filamin in response to shear flow.
  - More data is needed to confirm the role of these domains in filamin's translocation.
- **Filamin's actin-binding domain and dimerization domain may contribute to filamin's response to brief stimulation with shear flow.**
  - Filamin-null cells expressing filamin without ABD or DD responded similarly to cells expressing empty vector, suggesting ABD and DD are required for filamin-mediated response of cells to shear flow, although the response was also not significantly different between cells with full-length filamin vs. filamin with no ABD.
  - More data is needed to confirm ABD and DD's role in this response.

REFERENCES

1. dictyBase. Dictyostelium discoideum: Model System in Motion. http://dictybase.org/tutorial/ (accessed Aug 18, 2020).
2. Pollard, T. D. (2016). Actin and Actin-Binding Proteins. *Cold Spring Harbor Perspectives in Biology*, *8*(8). doi:10.1101/cshperspect.a018226
3. Artemenko, Y., Devreotes, P. N. Assessment of *Dictyostelium discoideum* Response to Acute Mechanical Stimulation. *J. Vis. Exp.* (129), e56411, doi:10.3791/56411 (2017).
4. Luo, T., Mohan, K., Iglesias, P. *et al.* Molecular mechanisms of cellular mechanosensing. *Nature Mater* 12, 1064–1071 (2013). https://doi.org/10.1038/nmat3772
5. Sunderland, M. E., "Dictyostelium discoideum". *Embryo Project Encyclopedia* (2009-06-10). ISSN: 1940-5030

ACKNOWLEDGMENTS

This work was supported by NSF-RUI grant no. 1817378 (to Y.A.) and RISE travel funds (to S.B.)