Characterization of Filamin's Involvement in Response to Mechanical Stimuli

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Abstract

Directed migration of cells is vital for a number of processes, including uncoordinated migration of cancer cells. The social amoeba *Dictyostelium discoideum* is very similar to mammalian cells, making it an excellent model organism for studying migration. Cells can migrate directionally in response to mechanical stimuli, such as shear flow, although how cells sense this type of stimulus is unclear. Previous studies in our lab showed that filamin, which is a protein that cross-links actin cytoskeleton in cells, is involved in the ability of cells to respond to mechanical cues. We have now shown that filamin does not affect response to chemical stimuli, suggesting that filamin's role is specific to responsiveness to mechanical stimuli. Filamin requires the actin-binding domain (ABD) and the dimerization domain (DD) to work together to ensure proper binding and crosslinking of the actin cytoskeleton. To further understand filamin's role in sensing physical stimuli, we generated an expression construct for filamin with no ABD. Future studies will test the ability of filamin without ABD to rescue the reduced response of filamin-null cells in a mechanical assay.

Social Amoeba *Dictyostelium discoideum*

- *D. discoideum* has an unusual life cycle, including periods of both unicellular and multicellular stages.
- *D. discoideum* is a valuable model in human-disease analysis, as well as studying cell-cell communication and roles in cell shape changes. [1]

Filamin

- Filamins are large actin binding proteins that stabilize three-dimensional actin webs and link them to cellular membranes (Figure A).[2]
- *D. discoideum's* filamin is composed of actin-binding domain (ABD) located at the N-terminus, followed by 6 rod domains. Dimerization is mediated through rod 6 (Figure B). [3]
- Filamin can regulate multiple cellular functions including its involvement as the mechanotransduction element of the cytoskeleton. [3]
- **The focus of this study is to test whether filamin requires its ABD to allow the cell to sense and respond to mechanical stimuli.**

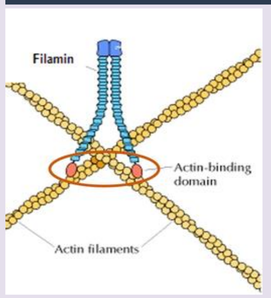


Figure A: Actin cytoskeleton with filamin crosslinks.
https://study.com/academy/lesson/actin-filaments-function-structure-quiz.html

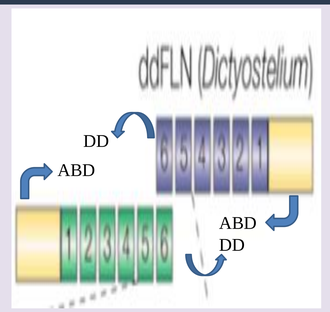


Figure B: *D. discoideum*'s filamin. ABD is located at the N-terminus (yellow) and DD is located at rod 6.

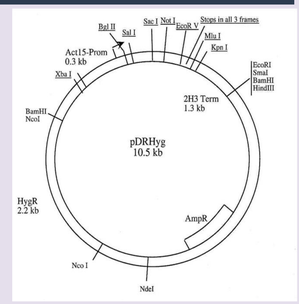
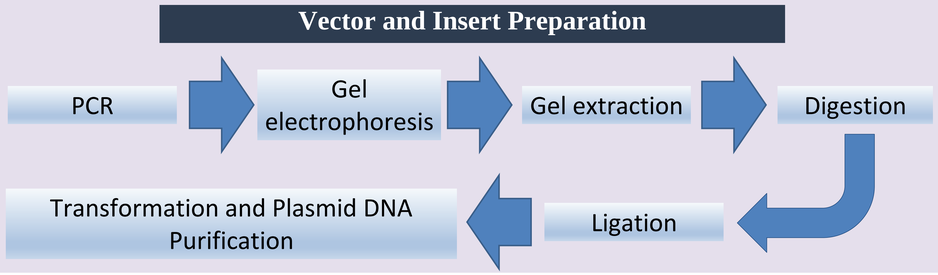


Figure C: pDRH plasmid. Filamin/no ABD gene will be inserted between Sal1 and Not1 restriction sites. mCherry fluorescent tag is not shown.

Approach and Methods

- Measure response of filamin-null cells expressing mCherry-filamin or vector to a chemoattractant (folic acid) to determine if filamin's role is specific to mechanical stimuli.
- Test whether ABD regulatory domain is required for filamin's function in response to mechanical stimulation
  - Generate an expression construct for filamin without ABD by inserting the appropriate filamin fragment amplified by PCR into the Sal1 and Not1 restriction sites of the pDRH-mCherry vector (Figure C).



Results

Response of filamin-null cells to stimulation with a chemoattractant

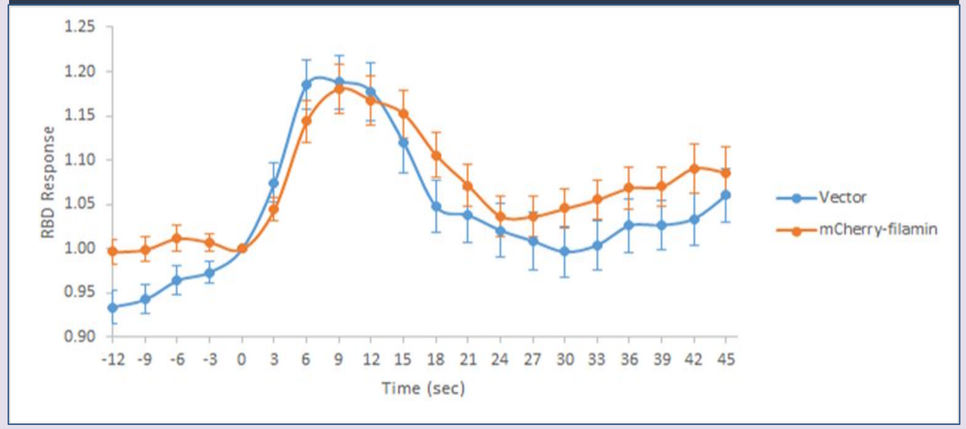


Figure 1. Response of filamin-null cells expressing pDRH vector or mCherry-filamin, as well as GFP-tagged Ras Binding Domain (RBD) to stimulation with folic acid. Cells grown on a bacterial lawn were collected, washed, plated in 8-well chambers, and imaged with epifluorescence microscopy every 3 seconds for 1 min. 100 µM folic acid was added after frame 5 (time 0). Response was measured as an inverse of a drop in cytosolic intensity of RBD-GFP. Data shown are mean+/-SE; n=29, collected from several wells in one experiment.

Results

Cloning of filamin lacking ABD

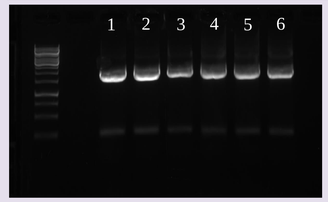


Figure 2: **Large-scale PCR for filamin lacking ABD (1-6).** The top bands (2238 bp) were cut out for DNA extraction. Note the DNA ladder on the left.

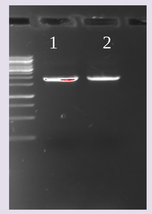


Figure 3: **DNA extraction test for filamin lacking ABD.** Lanes 1 and 2 show that the product of the expected size (2238 bp) was successfully purified.

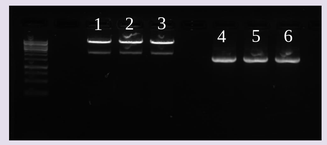


Figure 4: **Digestion of the pDRH-mCherry-filamin vector (1-3) and filamin/no ABD insert (4-6) with SalI and NotI.** The pDRH-mCherry fragment of the vector at 10 kb and insert at 2.2 kb were cut out for gel extraction. The bottom band of the vector is the full-length filamin gene.

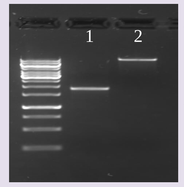


Figure 5: **DNA extraction test after digestion.** Vector (2) and insert (1) show correct sizes.

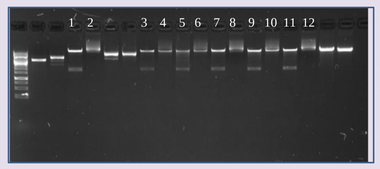


Figure 6: **Diagnostic digestion of plasmid DNA isolated from bacteria transformed with the ligated pDRH-mCherry-filamin/no ABD.** Plasmids were digested with SalI and NotI. Clones in lanes 1,3,5,7,9,11 gave the expected sizes. Corresponding uncut plasmids are shown in lanes 2,4,6,8,10,12.

Several clones were verified by sequencing and large amounts of plasmid were prepared.

Moving Forward

- Since filamin-null cells responded to stimulation with a chemoattractant similarly in the presence or absence of filamin, it appears that filamin is not involved in response to chemical stimuli. Thus, its role is likely specific to mechanical stimuli.
- To further understand the role of filamin in sensing chemical and mechanical stimuli, an expression product with no ABD was successfully prepared and will be tested for its ability to rescue the phenotype of filamin-null cells in a mechanical stimulation assay.
- Cloning of filamin with no DD is in progress.

References

[1]Mondal S, Burgute B, Rieger D, Müller R, Rivero F, et al. (2010) Regulation of the Actin Cytoskeleton by an Interaction of IQGAP Related Protein GAPA with Filamin and Cortexillin I. PLoS ONE 5(11)
[2]Sunderland, Mary E., "Dictyostelium discoideum". *Embryo Project Encyclopedia* (2009-06-10). ISSN: 1940-5030
[3]Su, W., Mruk, D. D., & Cheng, C. Y. (2012). Filamin A: A regulator of blood-testis barrier assembly during post-natal development. *Spermatogenesis*, *2*(2), 73–78.

Acknowledgements

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