Molecular Cloning of *Dictyostelium* Filamin Lacking the Actin Binding Domain

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Abstract

Directed migration of cells is vital for a number of processes, including embryo development and uncoordinated migration of cancer cells. Cells can migrate directionally in response to mechanical stimuli, although how cells sense this type of stimulus is unclear. The social amoeba *Dictyostelium discoideum* is very similar to mammalian cells, making it an excellent model organism for studying migration. Previous studies in our lab showed that filamin, which is a protein that crosslinks actin cytoskeleton in the cells, is involved in the ability of cells to respond to mechanical cues. Filamin requires actin-binding domain (ABD) and the dimerization domain (DD) to work together to ensure binding to the actin cytoskeleton. To further understand the role of filamin in sensing physical stimuli, we plan to analyze filamin without ABD in *D. discoideum*. We are currently generating an expression construct for filamin without ABD. This procedure was successful up to the ligation process. Once ligation is successful and the construct is confirmed by sequencing, the plasmid will be transformed into *D. discoideum* and its response to mechanical stimuli will be tested.

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 the 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.**

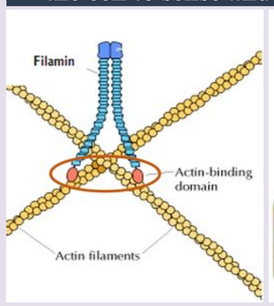

Figure A: Actin cytoskeleton with filamin crosslinks.
https://study.com/academy/lesson/actin-filaments-function-structure-quiz.html

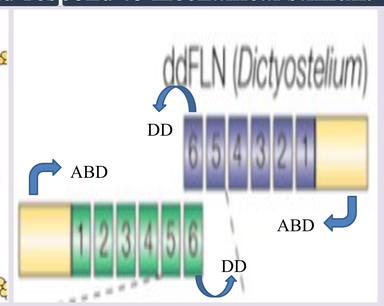

Figure B: *D. discoideum*'s filamin. ABD is located at the N-terminus (yellow) and DD is located at rod 6.

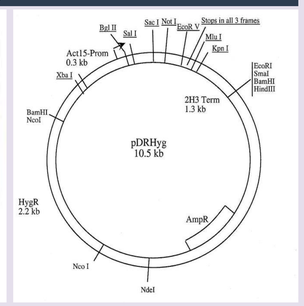

Figure C: pDRH plasmid. Filamin/no ABD gene will be inserted between Sal1 and Not1 restriction sites. mCherry fluorescent tag is not shown.

Approach

- 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).

Materials and Methods

Vector and Insert Preparation

PCR

PCR, or Polymerase Chain Reaction, is used to create multiple copies of a particular fragment of DNA *in vitro*. This is used to generate the filamin/no ABD insert.

Gel electrophoresis

In order to confirm if PCR worked, gel electrophoresis is used. It is a technique used to separate DNA fragments according to their size. The PCR product is verified based on its size (Figure 1).

Gel extraction

This technique is used to isolate a certain fragment of DNA from an agarose gel. The bands of interest are cut from the gel and the DNA is purified. Electrophoresis is performed to verify the size and purity, and determine the amount of the extracted product (Figure 2).

Digestion

The pDRH-mCherry-filamin vector is digested with Sal1 and Not1 enzymes and dephosphorylated. The filamin/no ABD insert is also digested with the same enzymes so it can be inserted into the same sites as the vector. Electrophoresis, followed by gel extraction of the fragments, is then done to purify the digested DNA (Figures 3 and 4).

Ligation

Ligation is the joining of two nucleic acid fragments. DNA ligase catalyzes the formation of phosphodiester bonds between the 3' hydroxyl of one terminus and the 5' phosphoryl which joins the ends of the two DNA fragments. The insert is ligated into the vector.

Transformation and Plasmid DNA Purification

E. coli bacteria transformed with ligated pDRH-mCherry-filamin/no ABD are grown and plasmid DNA is isolated. Isolated plasmid DNA is digested with SalI and NotI restriction enzymes to test for the presence of insert (Figure 5).

Results

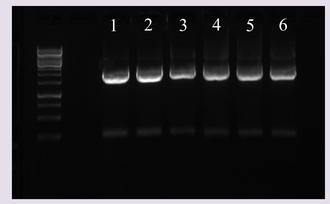

Figure 1: Large-scale PCR for filamin lacking ABD (1-6). The top bands (2238 bp) were cut out for DNA extraction. Note the DNA ladder on the left.

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

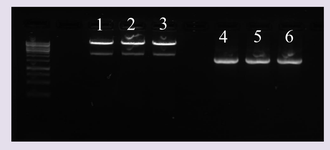

Figure 3: Digestion of the pDRH-mCherry-filamin vector (1-3) and filamin/no ABD insert (4-6) with SalI and NotI. The pDRH-mCherry fragment of the vector at 10 kb and insert at 2.2 kb were cut out for gel extraction. The bottom band of the vector is the full-length filamin gene.

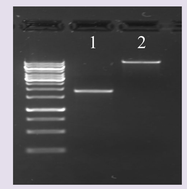

Figure 4: DNA extraction test after digestion. Vector (2) and insert (1) show correct sizes.

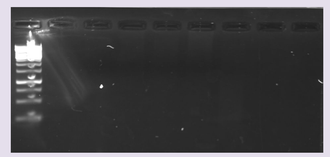

Figure 5: Digestion of miniprep with SalI and NotI. Plasmid DNA isolated from bacteria transformed with the ligated pDRH-mCherry-filamin/no ABD. DNA failed to be isolated and showed no visible bands.

pDRH/mCherry vector and filamin/no ABD insert were successfully prepared (Figures 3 and 4). Ligation of the insert and vector yielded unexpected results (Figure 5).

Moving Forward

Ligation and transformation of *E. coli* with the ligation product will be repeated. Once expression construct is successfully generated, we will test the response of *D.discoideum* lacking filamin or expressing filamin with or without ABD to mechanical stimulation.

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

I would like to thank Dr. Yulia Artemenko and SUNY Oswego's Department of Biological Sciences in assisting and supporting this research. This work is supported by NSF-RUI grant no. 1817378 (to Y.A.).