The Role of α-actinin in *Dictyostelium discoideum* Response to Mechanical Stimuli

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INTRODUCTION

Dictyostelium discoideum

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

Cell Motility

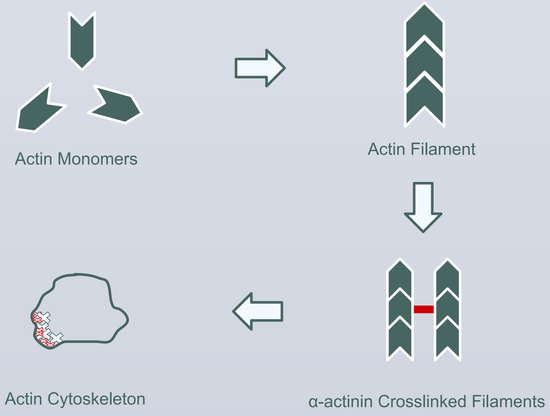
- Cells respond to various chemical and mechanical stimuli.[2]
- Stimuli direct cell movement.[2]
- Although all stimuli appears to activate similar signal transduction networks, how cells sense mechanical stimuli remains unclear.[3]

Actin Cytoskeleton

- Made up of globular actin monomers[2]
- Polymerizes to form long filaments[2]
- Filaments concentrated at cell cortex[3]
- Filaments extend the plasma membrane leading to cell movement.[2]
- Activation of the signal transduction network can bias actin polymerization allowing for directed cell migration.[3]
- An intact actin cytoskeleton is necessary for cell response to mechanical stimuli.[3]

α-actinin

- Actin-binding protein[2]
- Crosslinks actin filaments[2]
- Implicated in sensing mechanical pressure[4]



HYPOTHESIS

α-actinin is involved with the response to mechanical stimuli in *Dictyostelium discoideum*.

METHODS & RESULTS

CLONING & TRANSFORMATION

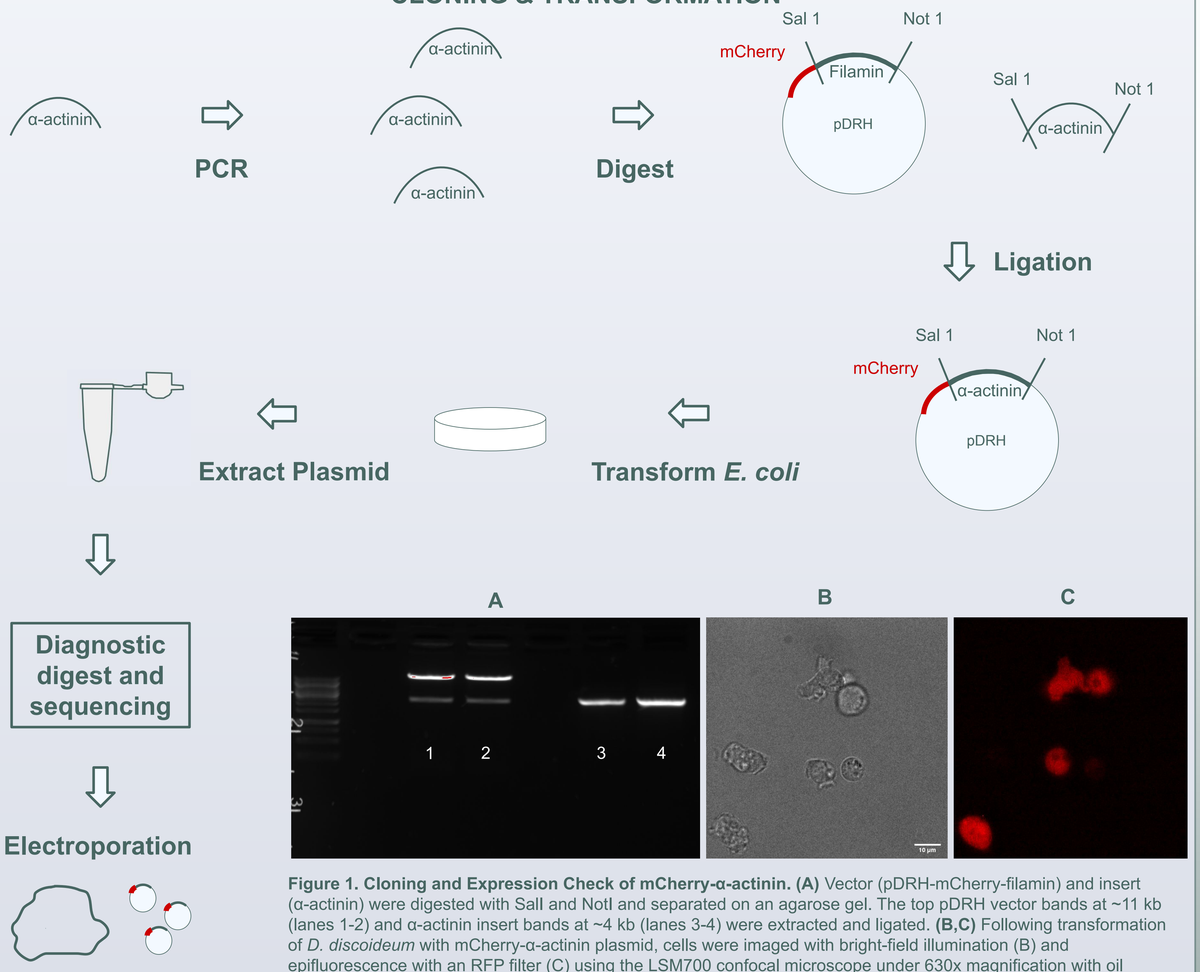


Figure 1. Cloning and Expression Check of mCherry-α-actinin. (A) Vector (pDRH-mCherry-filamin) and insert (α-actinin) were digested with SalI and NotI and separated on an agarose gel. The top pDRH vector bands at ~11 kb (lanes 1-2) and α-actinin insert bands at ~4 kb (lanes 3-4) were extracted and ligated. **(B,C)** Following transformation of *D. discoideum* with mCherry-α-actinin plasmid, cells were imaged with bright-field illumination (B) and epifluorescence with an RFP filter (C) using the LSM700 confocal microscope under 630x magnification with oil immersion.

SHEAR FLOW ASSAY

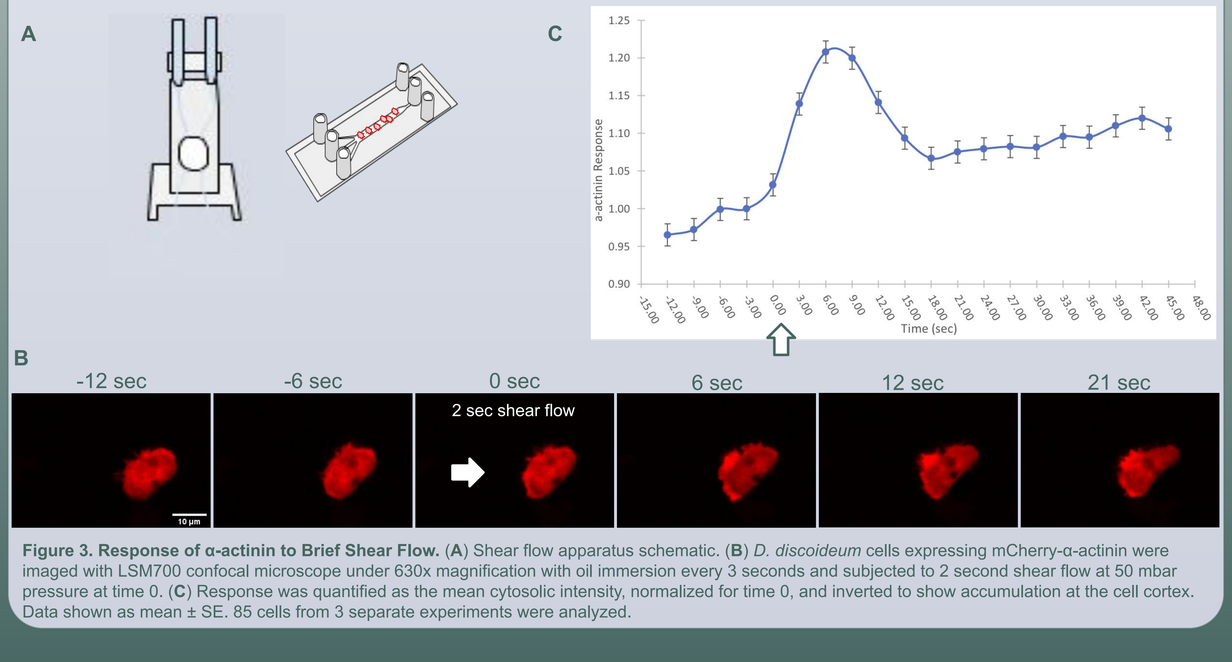


Figure 3. Response of α-actinin to Brief Shear Flow. (**A**) Shear flow apparatus schematic. (**B**) *D. discoideum* cells expressing mCherry-α-actinin were imaged with LSM700 confocal microscope under 630x magnification with oil immersion every 3 seconds and subjected to 2 second shear flow at 50 mbar pressure at time 0. (**C**) Response was quantified as the mean cytosolic intensity, normalized for time 0, and inverted to show accumulation at the cell cortex. Data shown as mean ± SE. 85 cells from 3 separate experiments were analyzed.

CONCLUSION

pDRH-mCherry-α-actinin plasmid was successfully cloned.

***Dictyostelium discoideum* cells were successfully transformed with pDRH-mCherry-α-actinin plasmid.**

Shear flow assay data indicates α-actinin increases in concentration at the cell cortex in response to brief mechanical stimuli.

- Peak α-actinin accumulation at the cell cortex occurred at 6 sec.
- a-actinin levels at the cell cortex did not return to pre-shear flow levels after 45 seconds.

FUTURE DIRECTION

Test activation of the signal transduction network with a biosensor in α-actinin-null cells expressing mCherry-α-actinin (rescue) or vector (null) to determine if α-actinin is necessary for sensing and/or responding to mechanical stimuli.

Determine any properties of α-actinin that make the protein mechanosensitive.

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

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