Genetic Screening to Find Novel Regulators of Tumor Suppressor Homolog Kinase Responsive to Stress B (KrsB)

Emily Fingar, Ali Khan, Swin Ratnayake, Yulia Artemenko

Department of Biological Sciences, SUNY Oswego

Background

- *Dictyostelium discoideum* is a species of soil-living amoeba belonging to the phylum Amoebozoa (1). It is commonly known as social amoeba.
- KrsB is a homolog of tumor suppressors Hippo and MST1/2 and is a negative regulator of cell spreading and substrate attachment (2).
- Excessive adhesion of KrsB−null cells reduced directional movement and prolonged the streaming phase of multicellular aggregation (2).

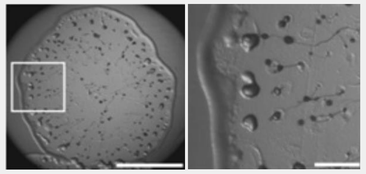


Fig 1: A plaque formed by wild-type *D. discoideum* cells grown on a bacterial lawn. The edge of the colony appears to be smooth.

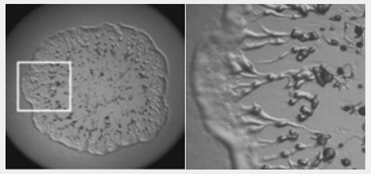
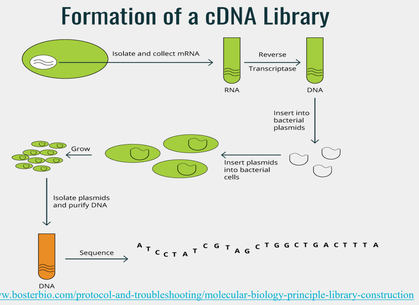


Fig 2: A plaque formed by *D. discoideum* cells lacking KrsB grown on a bacterial lawn. The edge of the colony appears to be rough (left) and there is an expansion of the region with streams towards the center.

- The active phosphorylated form of KrsB acts to decrease adhesion to the substrate (2).

Our main goal was to express a cDNA library in cells lacking KrsB to find new regulators or effectors of KrsB.

- cDNA library is a collection of cloned complementary DNA which contains only exons and is primarily used for cloning and expressing specific genes.



https://www.bosterbio.com/protocol-and-troubleshooting/molecular-biology-principle-library-construction

Conclusions and Future Directions

- Plating of cells transformed with the cDNA library yielded 210 plaques on 15 SM/5 plates. Cells from 12 plaques showing a morphology different from *krsB*-null were collected and grown (Fig. 4). Plasmid DNA was isolated from all 12 mutants.
- Although PCR using primers flanking the cDNA insert was performed on plasmids from all 12 mutants, only two mutants showed a single band that could be purified and sequenced. This verifies our method which can work for the identification of the insert, although our mutant collection needs to be expanded.
- Two additional transformations of cells with the cDNA library were completed. The first attempt produced only 7 mutants. The second attempt was performed on a larger scale but was determined unsuccessful because of issues with the stock cell line used for the transformation.
- Following isolation of additional mutants, we will confirm their phenotypes and identify the cDNA inserts. Each gene will then be further characterized to determine its role in the KrsB pathway.

Approach and Results

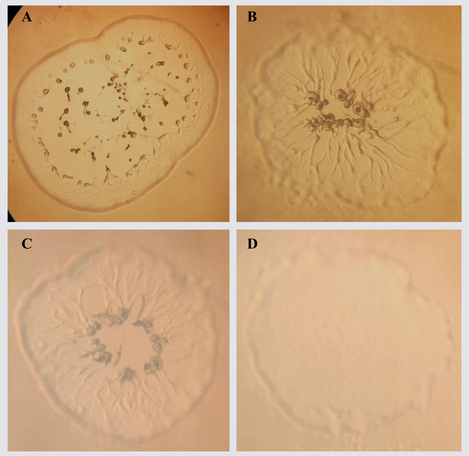


Fig 3: Test to determine whether plaque assay can be used to identify mutants that show rescued morphology compared to *krsB*-null *D. discoideum* cells. (A, B) *krsB*-null cells transformed with empty vector pDM317 (control) and pDM317/KrsB (rescue) were plated on a bacterial lawn at a ratio of 50:1. **(A)** An example of a rescue plaque with a smooth edge. **(B)** An example of the *krsB*-null phenotype with a rough edge and streamer morphology. **(C, D)** *krsB*-null cells transformed with an empty vector pDM317 (control). Most plaques showed a rough edge and streamer morphology **(C)**, although a few had aggregationless phenotype **(D)**.

Transform KrsB-null cells with cDNA library

Grow transformed cells on a bacterial lawn

Pick cells off of plaques with rescued phenotype (after 4 days)

Grow cells and isolate plasmid DNA via miniprep

Amplify the cDNA insert via PCR to check for presence of insert

Sequence cDNA insert

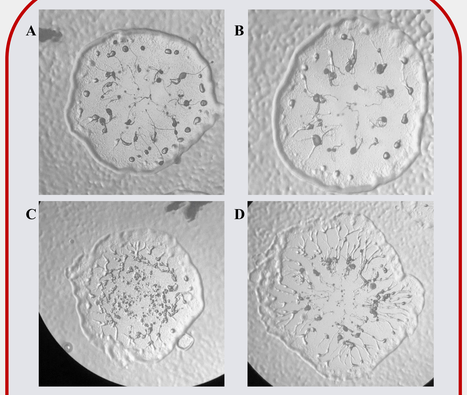


Fig 4: Plaques formed by colonies of *krsB*-null cells transformed with 3.5ug cDNA library. (A, B) Sample phenotypes of mutants with rescued plaque morphology showing smooth edges. **(C)** One plaque with morphology that does not resemble *krsB*-null or rescue. **(D)** Sample plaque showing morphology that is typical of *krsB*-null phenotype.

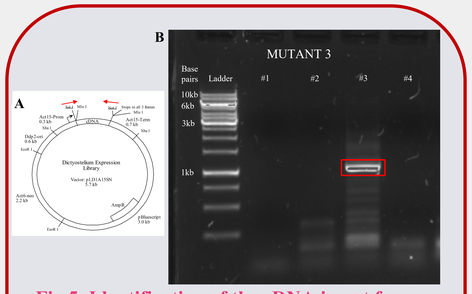


Fig 5: Identification of the cDNA insert for mutant #3. (A) A schematic showing the position of PCR primers in the cDNA library plasmid. (B) Gel shows that the cDNA insert from plasmid DNA isolated from mutant #3 was amplified and produced an ~ 1 kb band.

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References

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