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

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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).

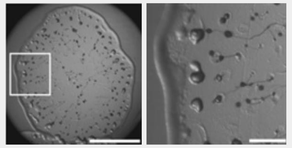


Fig 1: A plaque formed by wild-type *D. discoideum* cells grown on a bacterial lawn. The edge of the colony appears to be smooth.

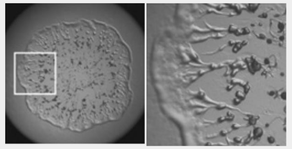
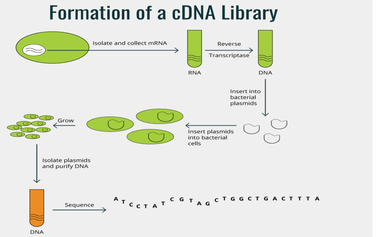


Fig 2: A plaque formed by *D. discoideum* cells lacking *krsB* grown on a bacterial lawn. The edge of the colony appears to be rough (left) and there is an expansion of the region with streams towards the center.

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

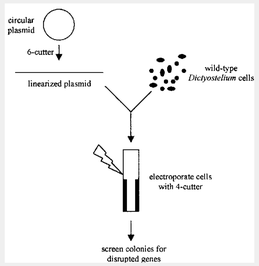
Our main goal is to use two complementary approaches to elicit mutations in cells lacking *krsB* to find new regulators or effectors of KrsB.

- **cDNA library** is a collection of cloned complementary DNA, which contains only exons and is primarily used for cloning and expressing specific genes.
- The characteristic of a cDNA library to contain partial as well as full length clones of a given cDNA allows for gene complementation and suppression.



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

- **Restriction Enzyme-Mediated Integration (REMI) mutagenesis** works by introducing restriction enzymes into cells which increases the rate of insertion of the plasmid into cognate restriction sites in the genome so that random mutants can be easily identified via plasmid recovery along with flanking sequences that define the mutated gene (4).



https://www.semanticscholar.org/paper/A-user%27s-guide-to-restriction-enzyme-mediated-in-Guerin-Larochelle/3f533b74bd4153494f0d3b78140637cf53638a9/figure/0

Acknowledgments

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Approach

Transform *krsB*-null cells with cDNA library*

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Grow transformed cells on a bacterial lawn

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Pick cells from plaques with rescued phenotype (after 4 days)

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Grow cells and isolate plasmid DNA via miniprep

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

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Sequence cDNA insert

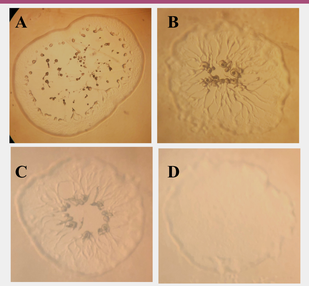

Fig 3: Test to determine whether plaque assay can be used to identify mutants that show rescued morphology compared to *krsB*-null *D. discoideum* cells. (A, B) *krsB*-null cells transformed with empty vector pDM317 (control) and pDM317/ *krsB* (rescue) were plated on a bacterial lawn at a ratio of 50:1. **(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)**.

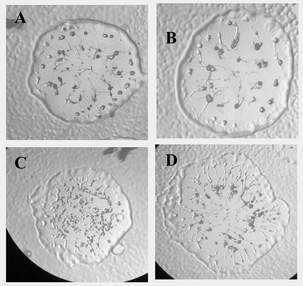

Fig 4: Plaques formed by colonies of *krsB*-null cells transformed with cDNA library. (A, B) Sample phenotypes of mutants with rescued plaque morphology showing smooth edges. **(C)** One plaque with morphology that does not resemble *krsB*-null or rescue. **(D)** Sample plaque showing morphology that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

*** Denotes a step that differs from REMI mutagenesis methodology**

Conclusions and Future Directions

- REMI mutagenesis is underway and the cell lines have been transformed with the restriction enzymes and plasmid DNA. Cells will be grown and plated on bacterial lawns to identify the unique phenotypic variations seen in *krsB*- cells when the knockout has been rescued or made more severe. Further genetic analysis must be done thereafter to identify the mutated genes.
- Identification of the sequences of cDNA inserts in the isolated mutants is in progress. This includes amplification of plasmids isolated from the *D. discoideum* mutants in Stbl2 bacteria, PCR and/or restriction digest analysis to identify plasmids with the cDNA insert, and sequencing to identify the genes.
- Further research must be done to begin characterizing the behavior of the mutants once the presence of a single cDNA insert is confirmed for those cell lines.

Methods and Results – cDNA Mutagenesis

Transformation and selection of *krsB*-null cells with 3 μg of cDNA library and 3 μg of pREP

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To enrich for poorly adherent cells, the plate was rotated at 200 RPM for one hour.

↓

Both floating and adherent cells were plated separately on a bacterial lawn

↓ →

29 plaques were identified with either a rescue-like or severe phenotype.

↓ →

A secondary screen was performed resulting in 5 plaques with a rescue-like phenotype and 5 with a more severe one consistent with the identified morphology in the initial screen.

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A tertiary screen was performed on the 10 plaques of interest. All morphologies were consistent with ones identified in the initial and secondary screens.

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Mutants were frozen and plasmid DNA was extracted for future growth and analysis.

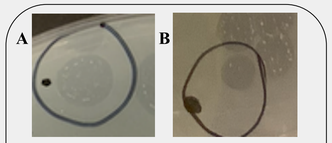

Fig 5: Enrichment was done to select for rescue-like cells that may have reduced adhesion compared to the *krsB*-null cells **(A)** Morphology of rescue-like plaque; **(B)** Morphology of severe plaque.

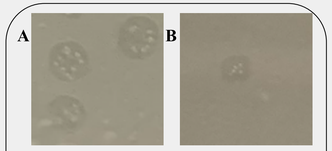

Fig 6: Secondary screen done to confirm the morphology of the mutants. (A) Morphology of rescue-like plaque; **(B)** Morphology of severe plaque.

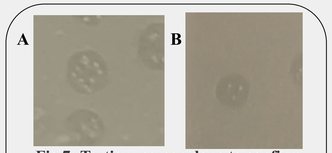

Fig 7: Tertiary screen done to confirm the morphology of the mutants. (A) Morphology of rescue-like plaque; **(B)** Morphology of severe plaque.

References

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