Analysis of random and directed migration of *Dictyostelium discoideum* mutants

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Abstract

Dictyostelium discoideum is a model organism due to its similarities with human cells. *Dictyostelium* cells lacking an adhesion and migration regulator KrsB were mutagenized to look for candidates that can rescue these cells. Three mutants will be characterized by comparing their random and directed migration to wild-type and KrsB-null controls.

Introduction

Cell Migration

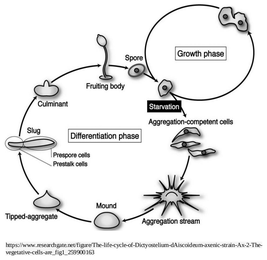
- Cells migrate as a response to environmental stimuli including chemical, electrical, and nutritional gradients.
- Researching cellular migration can improve our understanding of processes like embryogenesis, wound healing, inflammation, and metastasis.

Chemotaxis

- A type of directed cell migration in which the cells move up a chemical gradient.[1]
- Chemotaxis can be studied through methods such as small population assays.[1]

Dictyostelium discoideum

- Acts as an ideal model for cell migration and medical research.[2]
- Soil-dwelling social amoeba that shares similarities with human cells, like leukocytes.[3]



https://www.researchgate.net/figure/The-life-cycle-of-Dictyostelium-dAlscoideum-axenic-strain-Ax-2-The-vegetative-cells-are_fig1_259900163

Fig 1: **Life cycle of *Dictyostelium discoideum.***

Kinase Response to Stress B (KrsB)

- A homologs of HIPPO and MST1/2 that acts as a negative regulates of cell spreading and substrate attachment.[4]
- Excess adhesion of KrsB-null reduces directional movement.[4]
- Prolongs the stream phase of multicellular aggregation.[4]

Restriction Enzyme-Mediated Integration (REMI) Screen

- Provides a method to generate large collection of *D. discoideum* mutants.[5]
- Mutagenesis is achieved through the transformation of cells with DNA carrying antibiotic resistance in the presence of a restriction enzyme, which creates breaks in the genome.[5]
- Previous students generated a collection of mutants in the cells lacking KrsB with the goal to find new regulators of this protein.[5]
- Comparing the differences between the mutant, wildtype cells and KrsB-null cells allows determination of whether the mutant is a rescue or not.[5]

We hypothesize that the mutated cells that resemble wild-type cell morphology will demonstrate a higher success rate in random and directed migration when compared to KrsB-null cells.

Materials and Methods

D. discoideum Cell Culture

Wild type (WT), Krsb-null, REMI Mutant #s 2.5-1-7. 2.5-1-19, 5-1-10

Plaques on a Bacterial Lawn

- **Prepare SM/5 agar plates**
- **Collect cells and count**
- **Plate 25-50 cells on the agar plate along with *K. aerogenes* bacteria**
- **Incubate plates at 20°C**
- **Image plaques on day 13 with the Leica M205 FA Stereomicroscope**

Small Population Assay

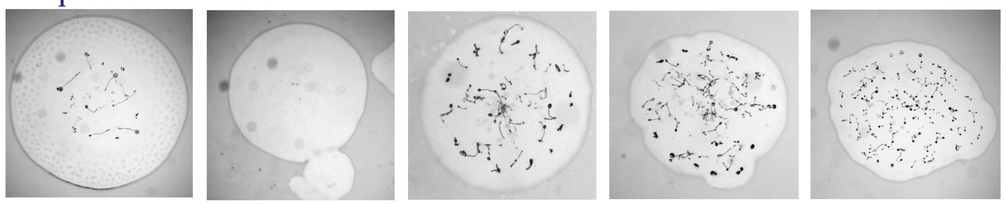
- **Collect, count and wash cells with 1 x Phosphate Buffer (PB)**
- **Prepare a 1-well chambered cover glass with 0.8% noble agar**
- **Dilute cells to 6.7 x 10^5 cells/mL**
- **Plate 1.5 µl drops of cells and 1.5 µl 1 x PB OR 1 mM folic acid ~ 3 mm apart**
- **Image cells every 30 sec for 15 min with the Zeiss LSM 700 Confocal Microscope**

Cell Morphology

- **Collect, count and plate 2x10^5 cells/mL in a 24-well plate in growth media**
- **Image cells with the Leica DMi1 microscope**

Results

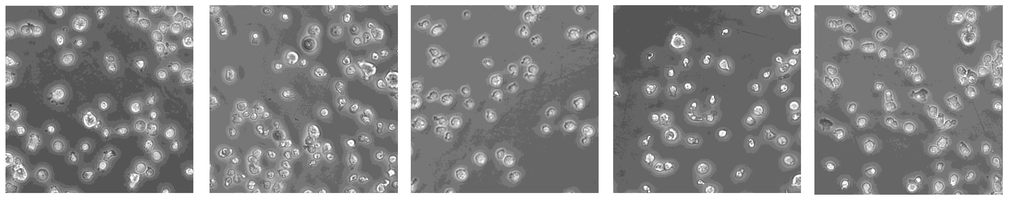
Plaques on a Bacterial Lawn



Ax2RK (WT) | **KrsB – null** | **REMI 2.5-1-7** | **REMI 2.5-1-19** | **REMI 5-1-10**

Fig 2: **The plaques compare the morphology produced by REMI Mutants 2.5-1-7, 2.5-1-19, and 5-1-10 vs. WT and KrsB –null cells. Plaques were imaged a week and half after plating using the Leica M205 FA stereomicroscope at 19.5x magnification.**

Cell Morphology



Ax2RK (WT) | **KrsB – null** | **REMI 2.5-1-7** | **REMI 2.5-1-19** | **REMI 5-1-10**

Fig 3: **Comparison of cell morphology of WT, KrsB-null, and REMI Mutants 2.5-1-7, 2.5-1-19, and 5-1-10 in a 24-well plate. Images were acquired with the Leica DMi1 microscope at 200X magnification with phase contrast.**

Results

Small Population Assay

0 Minutes

15 Minutes

Fig 4: **Small population assay was performed for WT, KrsB-null cells and REMI mutants with folic acid. Images were acquired every 30 sec for 15 min with the Zeiss LSM 700 Confocal Microscope at 100X magnification under brightfield illumination. A representative image of one of REMI 2.5-1-19 is shown at time 0 and 15 min of imaging. Cells were imaged approximately 15 minutes after plating.**

Conclusions and Future Directions

- The SM/5 plate plaques yielded unexpected results:
  - all mutant plates demonstrated rescue of the phenotype compared to KrsB-null plaques
  - KrsB-null plaques did not show proper development
- We plan on quantifying random and directed migration for the timelapse images acquired in the small population assay.
- If time permints, we will quantify cell area in the cell morphology images.
- If time permits, we will replate cells on a bacterial lawn to see if the observed plaques are consistent or were the result of an error.

References

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