Investigating Aggregation of P-Bodies, Single Cell Aging, and Death Modes in *Saccharomyces cerevisiae* With Microfluidics and Automated Fluorescence Microscopy

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ABSTRACT

This ongoing project investigates the relationship between single cell life span, cell death mode, and aggregation of processing bodies (P-bodies), a molecular process which may be indicated in age-related illnesses. Specialized strains of *S. cerevisiae* created with fluorescently tagged P-body markers Dcp2, Xrn1, and Lsm4 are grown in microfluidics devices that trap single cells and capture fluorescence data over their full lifespans. Cells are visually inspected against study inclusion criteria, then the distribution of fluorescence in included cells is analyzed over individual cell lifespans for P-body aggregation using various software. P-body aggregation data is analyzed against remaining cell lifespan to investigate whether there is a correlation between P-body aggregation and the remaining lifespans of cells. Data is further analyzed to investigate possible correlations between yeast cell death modes and P-body aggregation.

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INTRODUCTION

Older humans endure many age-related diseases [1] such as Alzheimer's that involve aggregation of proteins [2,3]. However, much is still unknown about the composition and mechanisms involved [1]. Developing treatments for age-related illnesses requires characterizing these aggregates and the mechanisms by which they develop [1,3].

Processing bodies (P-bodies) are membrane-less organelles conserved in eukaryotic cells, comprised of RNA and proteins separated from the cytoplasm by liquid-liquid phase separation [4]. Composition can vary, and components can be found elsewhere in cells [4]. P-bodies may form toxic aggregates [4]. While they have functions related to mRNA degradation, many functions are still unknown [4].

To study P-bodies, this project uses 3 fluorescently tagged components (Dcp2, Lsm4, and Xrn1) in specialized strains of budding yeast *S. cerevisiae*, combined with computer-automated microscopy and microfluidics. Yeast undergoes two distinct death modes [5]. Each death mode is characterized by specific pathways that result in specific protein and organelle aggregations [5].

This project investigates correlation between aggregation of P-bodies and remaining cell lifespan and yeast cell death modes to establish whether aggregation of P-bodies merits further study in single-cell aging.

METHODS

Two *S. cerevisiae* strains each have tagged Dcp2, and either tagged Lsm4 or Xrn1 (Table 1). Cultures are grown to mid-log growth for each run to ensure observed cells are young at the start.

1. Growth media washes buds from target cells in a microfluidics device (Fig. 1); images are taken with each filter at automated intervals.
2. Cells are analyzed manually against study inclusion criteria (alive and trapped at start of experiment, visible through death). (Fig. 2)
3. Bud time points and diameters of target cells at budding are recorded across target cell lifespan.
4. ImageJ and R are used to analyze fluorescence data and death modes of target cells.

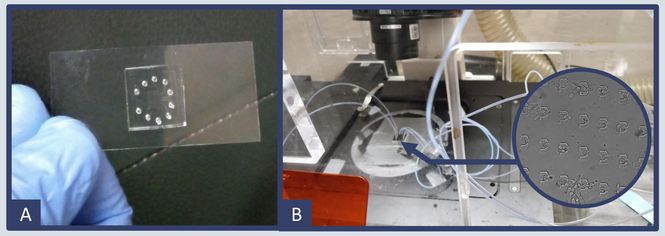

Figure 1: Microfluidics apparatus. A microfluidic device with **A:** 8 channels and **B:** in incubated microscope box. During an experiment, growth media flows left to right to remove budded cells from target cells. The apparatus is protected from background light with blackout curtains.

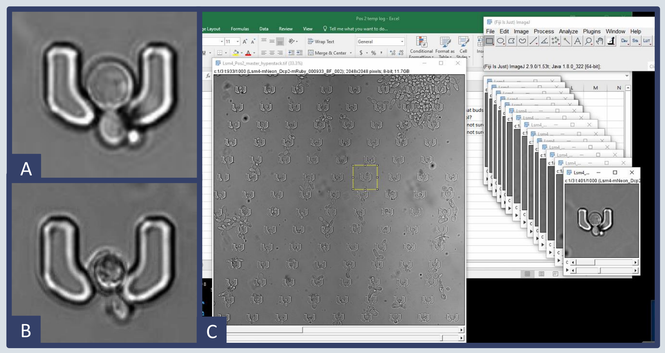

Figure 2: Selecting cells that meet inclusion criteria: in the trap at experiment start, visible until death. **A:** Living cell in trap. **B:** Dead cell in trap. **C:** Selected cells are placed in their own files for future use in computer analysis of distribution of fluorescence brightness.

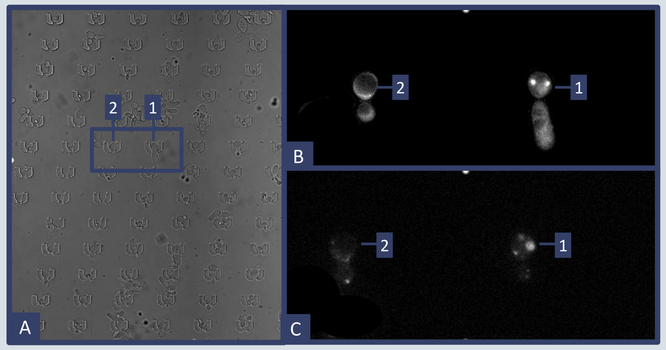

Figure 3: Results from one position of a microfluidic channel. Numbers correspond to cell death mode. Images taken near cell death. Trapped cells marked in **A** (bright field) correspond to the cells in **B** Dcp2 (GFP) and **C** Lsm4 (mCherry).

Table 1: Fluorescently Tagged P-Body Markers

Protein	Function	Tag	Light Filter
Dcp2	mRNA decapping [4]	GFP	Blue
Lsm4	P-body maintenance [4]	mCherry	Yellow-Green
Xrn1	mRNA decapping [4]	mCherry	Yellow-Green

RESULTS

For each run of each microfluidic channel, images were taken with each filter (bright field, blue, yellow-green) at 1,000 time points across four days. Individual hyperstacks of these images were created for analysis for each cell that met inclusion criteria (Fig. 2).

By visual inspection, Mode 1 cell death showed greater aggregation than Mode 2 death across P-body markers (Fig. 3).

DISCUSSION

This two-quarter project is still in progress. Use of microfluidics controlled more variables and reduced hands-on time spent on the experiments compared to traditional yeast dissection.

Many cells met inclusion criteria, but software did not successfully isolate target cell data and exclude data from nearby cells. Further adjustment to this analysis protocol will be necessary.

Visual inspection indicated correlation between P-body aggregation and cell death mode (Figure 3), but computer analysis of the data will determine whether this correlation is real. This computer analysis will occur after the necessary analysis protocol updates allow isolation of target cell data.

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