Aggregation of P-Bodies is Correlated With Remaining Cell Lifespan in *Saccharomyces cerevisiae* Cells That Experience Loss of rDNA Silencing

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ABSTRACT

Many age-related diseases in humans such as Alzheimer's involve intracellular protein aggregation, but much is still unknown about the molecular mechanisms involved. Characterizing these mechanisms is important for developing treatments for age-related illnesses [1,2,3]. My work investigates the relationship between cell life span and aggregation of processing bodies (P-bodies), which are cytoplasmic ribonucleoprotein granules that form inside cells experiencing stress and perform several functions that appear to benefit cells during stress [4]. Using GFP-tagged Dcp2 and RFP-tagged Xrn1 as P-body markers in *S. cerevisiae* with microfluidics and fluorescent microscopy to study single-cell lifespans, I demonstrated that P-bodies aggregated in aging cells that were not experiencing other forms of stress, and that P-body aggregation correlated to the remaining lifespan of cells. To investigate this link, I accounted for cell death mode phenotype. The correlation was strong for cells that underwent Mode 1 cell death but was not apparent in cells that underwent Mode 2 cell death. Additional research is needed to determine whether there are conditions under which there is a causal link between P-body aggregation and fatal single-cell pathogenesis and if so, whether these mechanisms are conserved in human cells and therefore possible targets for treatment of age-related illnesses.

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INTRODUCTION

Processing bodies (P-bodies) are membrane-less organelles involved in RNA regulation that are conserved in eukaryotic cells, comprised of RNA and proteins separated from the cytoplasm by liquid-liquid phase separation [4]. Composition varies, and components can occur elsewhere in cells [4]. P-bodies may form cytotoxic aggregates in aging cells, which may link them to single-cell senescence processes [4].

I used *S. cerevisiae* edited to express fluorescently tagged P-body markers to study P-body aggregation in single cells. Yeast typically undergoes 2 distinct death modes [5]. Each mode is characterized by specific protein and organelle aggregations before death, and Mode 1 is additionally characterized by loss of rDNA silencing not experienced by cells that undergo Mode 2 death (Table 1) [5]. Therefore, I hypothesized that P-body aggregation may be more significantly correlated in Mode 1 yeast cell death than in Mode 2.

Table 1: Late-Life Intracellular Correlations to Cell Death Modes

Cell Feature	Mode 1 Death [5]	Mode 2 Death [5]
Bud Shape	Elongated	Round
Mitochondria	No change	Aggregated
Nucleolus	Aggregated	No correlation
rDNA	Silencing lost	No change

METHODS

An *S. cerevisiae* strain with GFP-tagged Dcp2 and RFP-tagged Xrn1 (Table 2) was grown to mid-log growth to ensure young trapped cells.

1. Growth media washed buds from target cells in a microfluidics device (Fig. 1); images were taken with each filter at automated intervals.
2. Cells were analyzed against study inclusion criteria (alive and trapped at start of experiment, visible through death).
3. Bud time points were scored visually.
4. Time points for first appearance of bright P-bodies were scored visually (Fig. 3).

Table 2: Fluorescently Tagged P-Body Markers

Protein	Function [4]	Tag	Light Filter
Dcp2	mRNA decapping	GFP: mVenus	Blue
Xrn1	mRNA decapping	RFP: mRuby	Yellow-Green

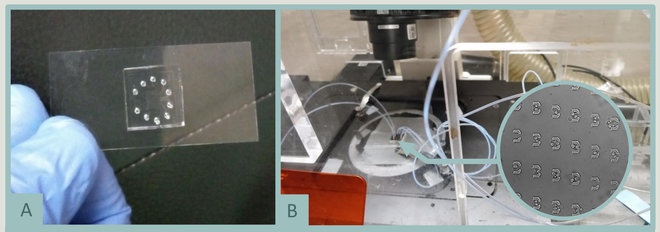

Figure 1: Microfluidics apparatus. A) A microfluidic device **B)** in incubated microscope box. Growth media flows from left to right, removing cells as they bud off.

RESULTS

Images were taken with each filter (bright field, blue, yellow-green) at 1,000 time points across four days. See Fig. 3 for selected images from an example cell. Manually scored data was recorded in an Excel sheet and used to produce Fig. 4.

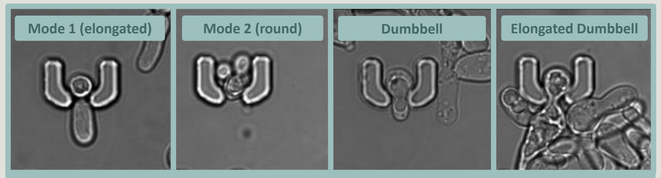

Figure 2: Yeast Cell Death Mode Phenotypes. Mode 1 and Mode 2 were the most common (n = 23 of 28).

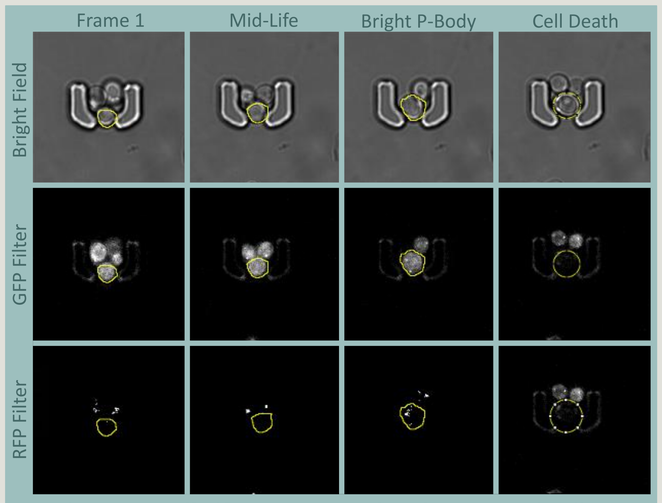

Figure 3: Cell Lifespan Example. The trapped cell is highlighted with yellow. These are 12 of 3,000 images captured for this cell, which suffered Mode 2 cell death.

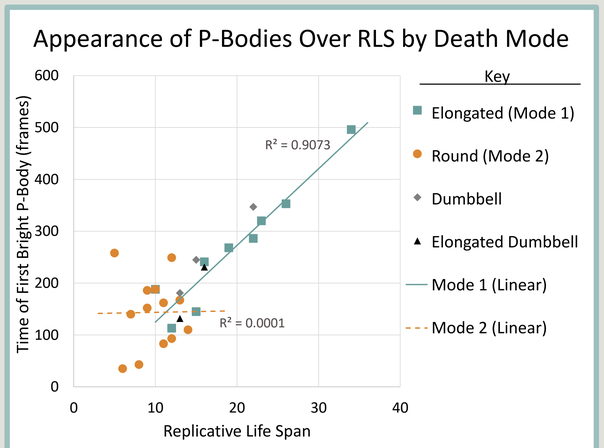

Figure 4: Initial Bright P-Body Appearance Over RLS. Yeast lifespan is measured in replicative lifespan (RLS), the number of cells that bud off during its lifespan. Each mark (n=27) represents 1 cell and the first appearance during its lifespan of bright/large fluorescent dots (P-bodies). 1 cell of 28 scored is not shown because it did not display bright spots.

DISCUSSION

P-body aggregation is strongly correlated to cell death in budding yeast cells that undergo Mode 1 cell death but not correlated in cells that undergo Mode 2 cell death (Fig. 4). The idea that P-body aggregation could be part of molecular mechanisms involved in single-cell aging might be true for some cells. and may or may not be conserved with human cells. This can be tested using senescent fibroblasts.

Use of microfluidics controlled more variables compared to traditional yeast dissection. To take full advantage of the benefits of the apparatus, computer analysis of fluorescence data is in progress to confirm the correlations found using visual scoring.

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