CALORIC RESTRICTION IN YEAST

Chetana Thappeta, Yang Li , Nan Hao

The Department of Biological Sciences, University of California San Diego, 9500 Gilman drive, La Jolla, California

ABSTRACT

Caloric restriction is an intervention that is known to increase lifespan among different species ranging from yeast to mice. In Saccharomyces cerevisiae, previous studies suggest CR increases lifespan by regulating the abundance or localization of some longevity proteins. However, the specific target(s) for CR to regulate lifespan remains unclear. In yeast, there are several longevity genes that are known to affect lifespan drastically. But the molecular mechanisms that control those longevity genes to increase lifespan is still unclear. It is possible that CR might act through some of these genes by controlling their abundance or localization to affect lifespan. To test this hypothesis, we tagged *PNC1* and *SGF73* with green fluorescence protein (GFP) to monitor their abundance and localization with CR and without CR conditions. We found Pnc1 abundance is increased under CR conditions while the Sgf73 abundance has no significant change.

INTRODUCTION

Caloric restriction is restricting an organism's food supply so that it has less than the supply they would normally consume if they were given access to free nutrients. Caloric restriction is known to make species such as mice, dogs and yeast live longer but the mechanism behind this is not known. In yeast, caloric restriction usually refers to glucose starvation, which achieved by switching medium from 2% glucose to 0.02% glucose.

Caloric restriction is difficult to study in other organisms such as mammals because they take a long time to age but S. cerevisiae ages quickly. Previous studies suggest mechanisms that regulate aging are well conserved from yeast to mammals, such as Sir2, which makes yeast a great model organism to study aging. There are several longevity genes identified in yeast. Deletion or overexpression of those genes, such as PNC1 and SGF73, drastically changes the lifespan of yeast.

QUESTION AND HYPOTHESIS

Question: What is the mechanism for caloric restriction to regulate life span in yeast?

Hypothesis: Caloric restriction may regulate the abundance or localization of longevity protein(s).

METHODOLOGY

Time lapse microscopic technique was used to monitor the the change of abundance or localization of target proteins in single cells under caloric restriction conditions.

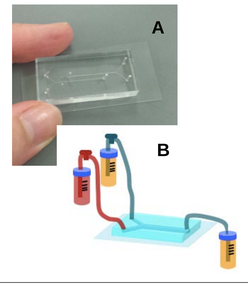
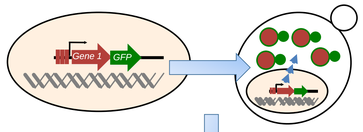
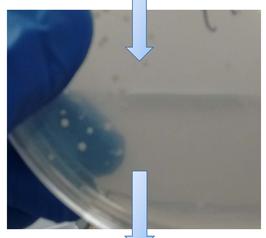


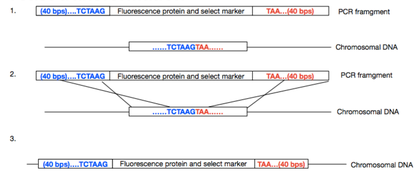
Figure 1. Diagram of microfluidics device and set up.
A. Each device contains two Y- shaped features. Each Y-shaped feature contains two medium loading ports and one waste port.
B. The two medium ports can connect to either a standard medium (without stimulation) or a medium with stimulation. The waste port is use to collect waste medium. The flow goes through one of the medium channels and exits from the waste port, so cells have constant access to fresh medium. Medium is connected to channels on the device through a tube. Medium tubes are located higher than waste tube.



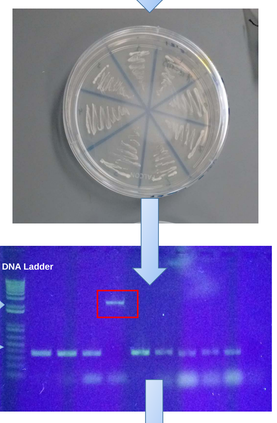
Genes are tagged with fluorescence protein to visually monitor their expression. PCR was done to get DNA fragments that contain GFP and HIS3 sequence. This fragment was inserted into the yeast genome by transformation:



Yeast transformation



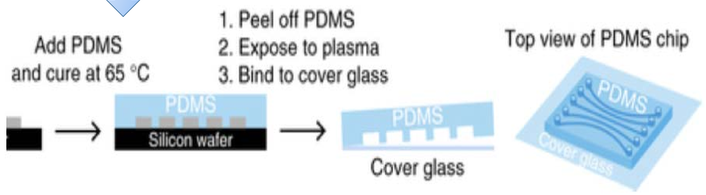
Yeast cells that have the insertion grow on the –HIS plate. Other cells that do not have homologous recombination die.



Colonies are selected and streaked onto another plate. PCR is done to test for the insertion

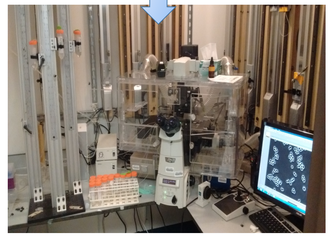
DNA gel electrophoresis
This is done to check the expression of the fluorescent marker

By comparing the distance travelled by each colony to the ladder, The tagged colony can be found. In the figure to the left only one colony has been tagged. This DNA is shown with a red rectangle around it.



Hanson, A.S., Hao, N., O'Shea, E.K., Nature Protocols, 2015

Creating a microfluidic device



The microfluidic device is placed inside a time lapse microscope. The microscope takes takes a picture every two minutes.

IMAGE ANALYSIS

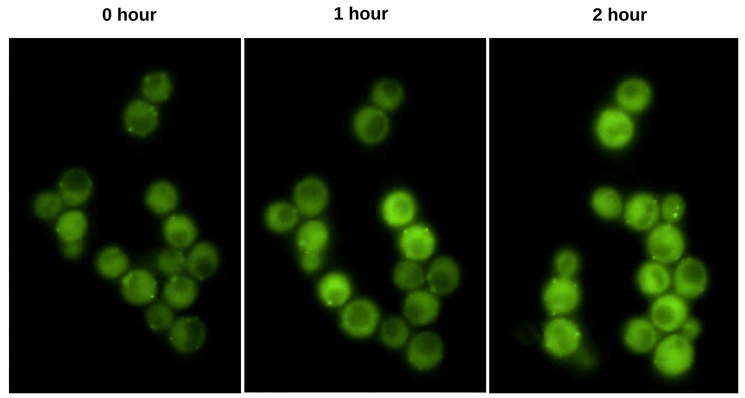
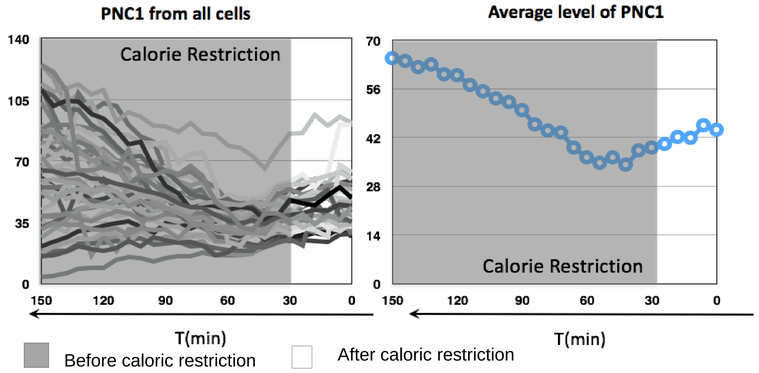


Figure 6. Changes of Pnc1-GFP abundance under CR conditions.
The pictures above shows S. cerevisiae cells. PNC1 is tagged with GFP, this makes the cells green. The whole cells is green because Pnc1 is localized in the cytoplasm . Cells start in medium with 2% glucose and then switch to medium with 0.02% glucose for caloric restriction. As time passes the images become brighter, this shows that the cells become more abundant with critical restriction.



CONLUSION

The abundance of the *PNC1* increases with caloric restriction but the abundance of *SGF73* does not. During caloric restriction the post translation modification could have been changed or critical restriction does not regulate *SGF73*.

FUTURE RESEARCH

- Observe S. cerevisiae when Pnc1 is double expressed and not expressed at all
- Observe other genes related to aging under CR conditions

ACKNOWLEDGMENTS

Meng Jin, Nick Csicsery, Ricky O'Laughlin, Yuan Zhao, Joydeb Sinha, Nan Hao