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Measuring environmental & genetic effects on cell differentiation kinetics in yeast

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Measuring Environmental & Genetic Effects on Cell Differentiation Kinetics in Yeast

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Abstract

The MAPKinase pathway is commonly involved in cell differentiation and growth processes. To study the kinetics of this pathway in regards to the effects of several genetic and environmental factors, we have chosen the popular model of pheromone response in yeast, *Saccharomyces cerevisiae.* Our data showed significant differences based on strain that seem to match previous data. Surprisingly, there were no measurable trends based on concentration, but results indicate that temperature may be positively correlated with response rate and may vary between strains. Future experiments will be aimed towards statistically defining the effects of each variable and creating a mathematical model that can be used to predict the outcomes of untested temperature and concentration conditions.

Goals

- Discover the effects of changing temperature and concentration of pheromone on differentiation kinetics by measuring the rate of cell morphology changes.
- Numerically characterize each factor's effects on differentiation kinetics in two strains of yeast.

Hypotheses

- We expected S288c to have a higher rate of response because of its known increase in mating efficiency.
- Temperature's effect on the response rate was expected to be similar to it's effect on growth. Highest rate of response at 30° C with a strain dependent interaction causing a higher relative rate in YJM145 at 35° C.
- Based on gene expression results, we expect higher concentration will increase response in both strains.

Background

Yeast

- Two mating types (a, α) only differentiate into mating-competent shmoo in response to the other's mating pheromone.
- Process controlled my a MAPK pathway

(Erdman & Snyder 2001)

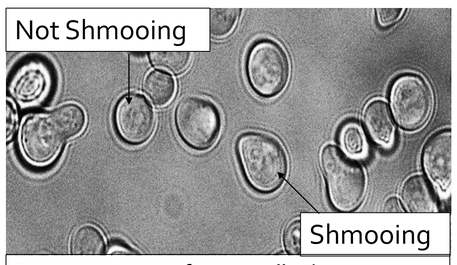


Figure 1: Image of yeast cells showing shmooing and non shmooing cells.

MAPK Pathway

- Mitogen Activated Protein Kinase
- Pheromone response in yeast is a very popular model for study.
- Errors in this type of pathway are commonly linked to cancer.

(Burotto et al. 2014)

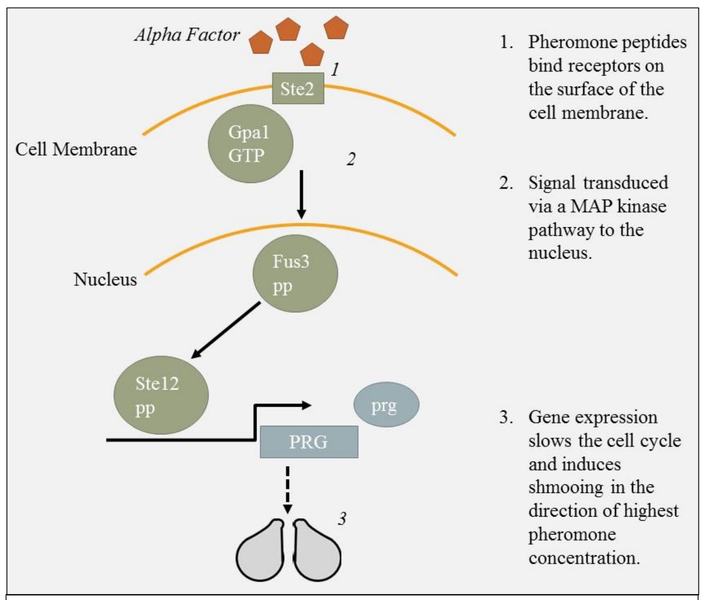


Figure 2: Diagram of the mating pheromone response pathway.

Temperature

- Normal growth temp around 25-30° C
- Some clinical strains have been found to grow at abnormally high temperatures

(Steinmetz et al. 2002)

Concentration

- Higher concentrations of pheromone found to increase expression of a mating specific reporter construct in S288c

(Falconnet et al. 2011)

Mathematical Models

- Quantitative models have proven to be extremely useful and sometimes essential in describing and predicting how processes or organisms will react in different situations.
- Models in growth and development process have thus far been largely qualitative.

(Umulis 2015, Gilbert 2006)

Strains

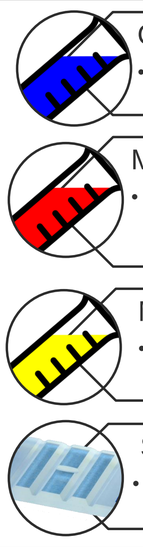
- Response at the molecular level has been shown to vary between strains

(Pollard et al. 2016, Zheng et al. 2010)

Table 1	Strain	Description
Lab	S288c	A common haploid laboratory strain originally obtained from a fig. Carries an allele known to increase mating efficiency. (Lang et al. 2009)
Clinical	YJM145	A homozygous diploid strain derived from the lung of an AIDS patient. Known to have the high temperature growth phenotype.

Methods

- Trials at different temperatures and concentrations randomized into pairs and one pair performed on a single day.
- Proportion of cells transformed using a log odds fit.



Overnight Cultures
- Cells from a single colony are suspended in YPD media and incubated overnight at 30°C.

Morning Cultures
- A sample from the overnight culture is added to YPD media to make 10mL cultures of equivalent concentrations and incubated at 30°C until culture has doubled twice.

Mating Pheromone Added
- Mating pheromone added to each morning culture at the specified concentration and incubated at specified temperature.

Sample Taken
- A sample is taken from each morning culture ever ~20 min, loaded onto a hemocytometer and the proportion of shmooing cells counted.

Results

Sample Regression Model

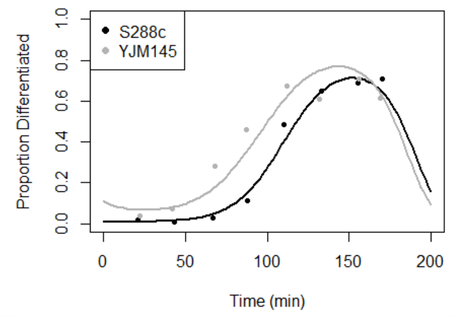


Figure 3: Example plot of data points and corresponding best fit log odds model with Time² and Time³ terms.

Full Regression Model

Table 2	Strain		Temperature (°C)			Concentration (nM)			
	S288c	YJM145	25	30	35	12	24	50	100
Slope	0.0114*	0.0044*	0.0055	0.0078	0.0078	0.0085	0.0099	0.0079	NA
Intercept	-4.30*	-3.01*	-3.88	-3.66	-3.43	-3.61	-3.64	-3.66	NA

* Indicates a significant value, p<0.01

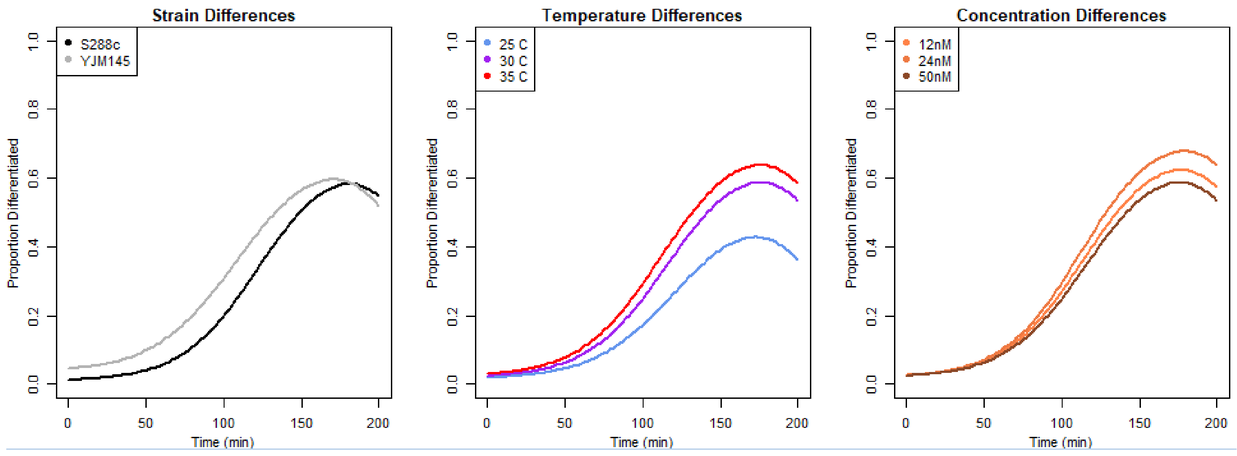


Figure 4: Plots of full regression model showing differences between conditions listed and averaged over all other variables.

- Only strain specific differences were found to be significant with S288c showing more of a response delay (more negative intercept) but then a higher rate (slope) than YJM145 on average.
- Temperature shows trends towards higher temperatures eliciting a higher rate of response: both a less negative intercept and greater slope as temperature increases.
- Concentration shows no clear trends.

Discussion/ Conclusions

- Strain specific differences on rate of differentiation fit with our prediction, though our data lacks the power to see how strain differences relate to changes in other conditions.
- Temperature shows trends towards a faster response rate as temperature increases. This partially supports our hypothesis, but is inconsistent with the prediction that response would peak at 30° C. The effect may become clearer with more data allowing us to separate temperature effects between strains.
- The lack of a trend with concentration is unexpected based on published gene expression results (Falconnet et al 2011). Is cellular differentiation from a morphological standpoint insensitive to large scale variation in gene expression responses to mating pheromone?
- Our original log odds model was a useable, but poor fit to the data and did not capture the reversal or provide a way to estimate maximum proportion. Adding terms with powers of time seems to correct that problem and more accurately capture the trends shown in the data.
- Maximum proportion magnitude and timing vary across strains and conditions motivating further investigation into more accurate estimation and the biological implications.

What's Next?

- Expand dataset with more replicates to increase the power.
- Explore the use of additional interaction terms to improve the model.
- Improve estimates of magnitude and timing of maximum response proportion across strains and conditions.
- Test novel conditions to see if the results fit with the model's predictions.

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