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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 various 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.

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
  (Edrman & Snyder 2001)

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)

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 its effect on growth. Lowest rate of response at 30°C with a strain dependent interaction causing a higher relative rate in YML45 at 35°C.
- Based on gene expression results, we expect higher concentration will increase response in both strains.

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.

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 10^6 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 every ~20 min. loaded onto a hemocytometer and the proportion of shmooing cells counted.

Discussion/ Conclusions

- Strain specific differences in 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 (Falccioni et al. 2011). Is cellular differentiation from a morphological standpoint a function of the number of transcription factor bindings or something else?
- Our original log odds model was a useful, 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.

Works Cited


Results

- Full Regression Model
  - Table 2
    - Strain
      - S288C
      - YML45
    - Temperature (°C)
      - 25
      - 30
      - 35
      - 12
      - 24
      - 50
      - 100
    - Concentration (nM)
      - 0
      - 50
      - 100
      - 500
    - Slope
      - 0.0124
      - 0.0042
      - 0.0026
      - 0.0087
      - 0.0029
      - 0.0046
      - 0.0029
    - Intercept
      - -3.30
      - -3.04
      - -3.01
      - -3.06
      - -3.03
      - -3.05
      - -3.02

- Table 1
  - Strain
    - S288C
    - YML45
  - Description
    - Lab
    - Clinical

- Table 3
  - Full Regression Model
    - Strain
      - S288C
      - YML45
    - Temperature (°C)
      - 25
      - 30
      - 35
      - 12
      - 24
      - 50
      - 100
    - Concentration (nM)
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      - -3.06
      - -3.03
      - -3.05
      - -3.02

- Figure 2: Diagram of the mating pheromone response pathway.
- Figure 3: Example plot of data points and corresponding best fit log odds model with Time and Temp* terms.
- 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 (lower negative intercept) but then a higher rate (slope) than YML45 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.

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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