Quantification of Genomic Response to Environmental Perturbation using a Modulus of Stability

Stephen J. Callister
Department of Civil and Environmental Engineering, Michigan State University, East Lansing, Michigan

February 27, 2003
Introduction

• The advancement of technology has dramatically increased our ability to study gene expression on a large scale.

• Current techniques used for analysis of gene expression in response to perturbation have proved useful for enlarging our knowledge about emergency response genes, transcriptional regulation, implied function etc...

• Common experiments performed using these technologies are perturbation response experiments.

• Relating gene response and perturbation could give insight when comparing:
  • Commonly expressed genes across different environmental perturbations.
  • Gene expression to different magnitudes of the same environmental perturbation.
  • The similar genes found on different genomes.
OBJECTIVE - Quantify the relationship between genomic response and perturbation using a *Modulus of Stability*.

Definitions:

**Stability** - The ability of a system to withstand a period of stress and return to equilibrium afterwards. (Source: Harrison, G.W. (1979) *American Naturalist* 113, 659-669)

**System** - Set of selected genes expressed either upwards or downwards in response to an environmental perturbation.

**Modulus of Stability** - Index that can be used to understand the stress (perturbation) strain (response) relationships of a genomic system.

1. Quantify the perturbation.
2. Quantify response - observed patterns of gene expression.
3. Relate the two
Quantifying the Perturbation

- Two typical perturbations - pulse and press (Bender et al., 1984; Yodzis, 1988).

Graphical Representation of Pulse and Press Perturbations
What are Typically Observed Patterns of Gene Expression?

Generalized Relative Gene Expression Patterns in Response to Perturbation
Quantification of Patterns of Gene Expression

• Stability parameters

  \textit{Resilience}- Rate at which an asymptotically stable system returns to equilibrium.

  \textit{Return time}- Time required for an asymptotically stable system to return to equilibrium. Inverse of resilience

  \textit{Reactivity}- Rate at which a system departs from equilibrium. Complimentary to resilience

  \textit{Resistance}- Maximum deviation from the pre-perturbed equilibrium.

\textit{Graphical Depiction of Stability Parameters}
Quantification of Patterns of Gene Expression

• Aggregate measure of stability parameters (Hashsham et al., 2000):

\[
\text{Moment of Area}, M = \int_{0}^{i} A_i \cdot t_i
\]

Graphical Depiction of the Moment of Area
Relating the Two

Perturbation as a function of the amplitude and duration. This represents the stress portion of the definition.

\[
\text{modulus of stability} \equiv \frac{\sum_{i=1}^{m} \sum_{j=1}^{n} k_{i,j} (r_{s_{i,j}}, r_{l_{i,j}} \ldots)}{p(a,d)}
\]

This is the strain (response in terms of gene expression) portion of the definition quantified in terms of an aggregate measure of the stated stability parameters where \( n \) is the number of one type or response for \( m \) number of characteristic expression pattern.

Comparatively smaller values indicate that less stress is required per resulting unit of strain (response).
Modulus of Stability: Application

1. Proof of Concept - Used previously published data and the Modulus of Stability to quantify genomic response to perturbation.

2. Current Research - Expand our understanding of the behavior of the Modulus of Stability to perturbation.
Proof of Concept: Introduction

- Raw data was obtained from the Stanford Microarray Database results were published by Gasch et al. (2000) *Molecular Biology of the Cell* 11, 4241-4257.
- Organism studied - *Saccharomyces cerevisiae*.
- Eight perturbations were used for proof of concept.

<table>
<thead>
<tr>
<th>Environmental Perturbation</th>
<th>Type of Cellular Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature from 25°C to 37°C</td>
<td>Temperature</td>
</tr>
<tr>
<td>Temperature from 37°C to 25°C</td>
<td>Temperature</td>
</tr>
<tr>
<td>Hyper-osmotic</td>
<td>Oxidative</td>
</tr>
<tr>
<td>Hypo-osmotic</td>
<td>Oxidative</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>Protein (chemical)</td>
</tr>
<tr>
<td>Menadione</td>
<td>Protein (chemical)</td>
</tr>
<tr>
<td>Diamide</td>
<td>Protein (chemical)</td>
</tr>
<tr>
<td>Dithiothreitol</td>
<td>Protein (chemical)</td>
</tr>
</tbody>
</table>
Proof of Concept: Calculating Modulus of Stability

Raw data downloaded to elucidate significantly expressed genes.

Excel

Expressed genes showing >2-fold expression across perturbations

GeneSpring

Cluster genes using K-means clustering and standard correlation.

Sort clustered genes into characteristic patterns of expression.

Mathcad

Calculate Moment of area

Moment of area used to calculate modulus of stability.
Comparison of Quantified Perturbations
### Proof of Concept: Results

<table>
<thead>
<tr>
<th>Stress Perturbation</th>
<th>Asymptotic Expression Patterns (Percent)</th>
<th>Non-asymptotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat Shock 25°C to 37°C</td>
<td>94%</td>
<td>4%</td>
</tr>
<tr>
<td>Heat Shock 37°C to 25°C</td>
<td>82%</td>
<td>12%</td>
</tr>
<tr>
<td>Hyper-osmotic Shock</td>
<td>94%</td>
<td>2%</td>
</tr>
<tr>
<td>Hypo-osmotic Shock</td>
<td>82%</td>
<td>9%</td>
</tr>
<tr>
<td>Hydrogen Peroxide Exposure</td>
<td>90%</td>
<td>4%</td>
</tr>
<tr>
<td>Oxidative Menadione Exposure</td>
<td>82%</td>
<td>14%</td>
</tr>
<tr>
<td>DTT Exposure</td>
<td>90%</td>
<td>6%</td>
</tr>
<tr>
<td>Protein (Chemical) Diamide Exposure</td>
<td>94%</td>
<td>4%</td>
</tr>
</tbody>
</table>
Proof of Concept: Results

[Graph showing log moment of area versus different conditions and treatments]

- Hyper-osmotic
- Hypo-osmotic
- Heat 37°C to 25°C
- Heat 25°C to 37°C
- Menadione
- Hydrogen peroxide
- Dithiothrietol (DTT)
- Diamide

Legend:
- 1 gene
- 5 genes
- 20 genes
- 79 genes
- 216 genes
- 433 genes
- All Genes
Proof of Concept: Results

General Categories -> Metabolism -> C Compound and Carbohydrate -> Utilization

ORF’s with the largest % contribution:
- YER070W
- YDL022W
- YDL124W
- YFL014W
- YKL009W
- YDR513W
## Proof of Concept: Results

<table>
<thead>
<tr>
<th>ORFs</th>
<th>Hyper-osmotic</th>
<th>Hypo-osmotic</th>
<th>Heat 37 to 25°C</th>
<th>Heat 25 to 37°C</th>
<th>Menadione</th>
<th>Hydrogen Peroxide</th>
<th>Diamide</th>
<th>DTT</th>
<th>Biological Process</th>
<th>Molecular Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>YDL022W</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Glycerol Accumulation</td>
<td>glycerol-3-phosphate dehydrogenase (NAD+)</td>
</tr>
<tr>
<td>YER070W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DNA Replication</td>
<td>ribonucleoside-diphosphate reductase</td>
</tr>
<tr>
<td>YDL124W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>Unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>YFL014W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>Heat, osmotic, oxidative stress</td>
<td>heat shock protein</td>
</tr>
<tr>
<td>YKL009W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>Unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>YDR513W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>Redox homeostasis, oxidative stress</td>
<td>thiol-disulfide exchange intermediate</td>
</tr>
</tbody>
</table>
Proof of Concept: Results

Log Modulus of Stability \( [\text{mol l}^{-1}] \times [\text{C (min)}^{-1}] \)

- Hyper-osmotic
- Hypo-osmotic
- *Heat 37° to 25° C
- *Heat 25° to 37° C
- Menadione
- Hydrogen peroxide
- Dithiothrietol (DTT)
- Diamide
Proof of Concept: Conclusions

• Modulus of stability can be applied to the study of gene expression to perturbation to give an understanding of the “stress” and “strain” of a genomic system.

• The genomic system can be made up of a few expressed gene to larger sets of genes representing a specific genomic feature, such as the environmental stress response.

• Dithiothrietol exposure resulted in the least stability of the environmental stress response.

• Environmental stress response exhibited the greatest stability to hypo-osmotic shock.

• Menadione, hydrogen peroxide, diamide, and DTT had the smallest modulus of stability values indicating that less stress (perturbation) is required per unit strain (gene expression response) compared to the osmotic and temperature perturbations.

• Further testing is needed, including statistical analysis, to give more validity to the concept.
Current Research: Introduction

• All living organisms have developed mechanisms to maintain water potential exerted on their cellular components due to natural changes in external osmolytes.

• *Saccharomyces cerevisiae* is one of a few organisms in which the effects of and response to sudden changes in water potential shock have been well studied.

Current Research: Introduction

**Graph Description:**
- **Time (min)**: The x-axis represents time measured in minutes, ranging from 0 to 400.
- **OD$_{600}$**: The y-axis represents optical density at 600 nm, ranging from 0 to 1.8.
- **Legend**:
  - **Control**: Represented by red diamonds.
  - **1.2M**: Represented by black squares.
  - **0.8M**: Represented by green triangles.
  - **0.4M**: Represented by blue squares.

**Key Events:**
- **Perturbation**: Indicated by a downward arrow at the 100-minute mark.
- **Resumption of Growth**: Indicated by upward arrows at the 200- and 300-minute marks.

**Observations:**
- The control group shows a gradual increase in OD$_{600}$ over time.
- The groups with perturbations (1.2M, 0.8M, 0.4M) show a temporary decrease followed by a resumption of growth.

**Implications:**
- This graph illustrates the response of different groups to a perturbation, highlighting the recovery and growth characteristics.

**Conclusion:**
- Further analysis is required to understand the underlying mechanisms and implications of these observations.
Current Research: Hypotheses

• Hypothesis 1  *A relationship exists between the stability of expressed genes to hyper-osmotic shock and the magnitude of the perturbation*

• Hypothesis 2  *The stability of expressed genes to hyper-osmotic shock increases with successive changes in the magnitude of the perturbation compared to non-successive changes.*
Current Research: Procedure

• Roughly 200 genes exhibiting significant expression are being studied.
• These genes make up five complete pathways. (Glycerol Lipid Metabolism, Cell Cycle, Glycolysis/Gluconeogenesis, MAPK signaling pathway)
• Real Time PCR using SYBR I Green will be used to measure gene expression.
• Saccharomyces cerevisiae will be subject to press perturbations of salt resulting in five (0.3 M, 0.6 M, 0.9 M, 1.2 M, and 1.4 M) final concentrations ranging from mild to severe hyper-osmotic shock.
• Selected Real Time PCR over Microarray for the following reasons:
  1. Real Time PCR is generally a more accurate measure of mRNAs.
  2. For proof of hypotheses a whole genome array is not necessary.
  3. Estimated that cost is be less using Real Time PCR for our purposes.
Summary

• The Modulus of Stability quantitatively relates the perturbation to the genomic response allowing for several types of comparisons to be made.

• The Moment of Area used to quantify gene expression response and is an aggregate measure of the stability of the genomic system.

• Validation of the Modulus of Stability, beyond proof of concept, is currently ongoing.

• As with all new concepts rigorous testing and criticism is required to ultimately determine the value of such a concept.
Acknowledgments

Syed Hashsham
Jim Tiedje
Bruce Dale
Susan Masten
Sean Spellman
Center for Microbial Ecology
Charlene Hardy

THE END