Nonlinear differential equation model for quantification of transcriptional regulation applied to microarray data of *Saccharomyces cerevisiae*


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Outline

- Goal: Identification of transcriptional regulators that control cell cycle regulated genes of Saccharomyces cerevisiae using non linear model
- A new nonlinear differential equation model of gene expression
- Dynamic transcriptional control and optimization using least squares minimization
- Algorithm utilizes dynamic model of time continuous gene expression
- Method for determining correct regulators
- Trends in expression profiles for selected genes and their regulators
- Compared to linear model, nonlinear model gives better results in terms of correct identification of the regulator and better fit to gene expression profile of target.
Transcriptional regulation leads to changes in gene expression

- Transcriptional regulatory proteins recognize specific promoter sequences and allow binding of RNA polymerase and initiating transcription
- DNA microarrays allow for the visualization and recording of changes in gene expression over time
- Changes in gene expression throughout the cell cycle of *Saccharomyces cerevisiae* provides insight into regulator-target gene relationships and the network interactions that result
- Therefore, many studies focus on analyzing microarray data through clustering methods to identify cell cycle controlled genes
Previous Methods Identify Upstream Regulatory Genes through Generalized Linear Model

- Wolf and Wang: used fuzzy logic
- Nachman et al.: used dynamic Bayesian networks with a kinematic model
- Bar-Joseph: used gene expression analysis and genomic info alongside one another
- Wang et al. and Makita et al.: extending the work of Bar-Joseph, incorporated promoter sequence analysis into gene expression analysis
Alternative Method Replaces Linear Model with Non Linear Model

- 184 potential regulators chosen
- Set of 40 specific target genes within *S. cerevisiae* selected
- Genes from within set of potential regulators selected and applied to model in order to see if regulator fits target gene expression profile correctly
- Repeated for other target gene and potential regulator combos
- True regulators determined by identifying regulators that model the target gene profile correctly and are found in YEASTRACT database
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Dynamic Model for Transcriptional Control

- $g$: regulatory effect for a particular gene
- $w$: regulatory weights
- $y$: expression levels of regulators
- $b$: transcription initiation delay
- $q$: regulatory effects of non-target genes
- $k$: rate constants
- $z$: target gene expression
- $j$: regulators

\[
g \approx \sum_{j} w_{j}y_{j} - b, \quad 1
\]

\[
\rho = \frac{1}{1 + \exp\left(-\sum_{j=1}^{m} w_{j}y_{j} + b\right)}, \quad 2
\]

\[
\frac{dz}{dt} = k_{1} \frac{1}{1 + \exp\left(-\sum_{j=1}^{m} w_{j}y_{j} + b\right)} - k_{2}z. \quad 3
\]
Simplified Model for One Transcriptional Control

- Simplified version of equation three, focusing on the case of only one transcriptional factor
- Polynomial coefficients \([a_0, \ldots, a_n]\) are computed from the gene expression profile using least square minimization.
- Polynomial used as an approximation for ‘true’ expression profile with experimental errors.
Mean Square Error Function

- $Z\{z(t_\tau)\}$: Expression profiles of the target genes
- $Y\{y(t_\tau)\}$: Expression profiles of the regulator genes
- Time points $t = \tau$, $\tau = 1,2,\ldots,Q$
- Search for gene profiles: $Y \subseteq \{Y_i, 1,2,\ldots,m\}$ (the $m$ pool of regulators) that minimize $E$

\[
E = \frac{1}{Q} \sum_{\tau=1}^{Q} [z(t_\tau) - z^c(t_\tau)]^2,
\]

\[
\frac{dz}{dt} = d_0 + d_1y - d_2z,
\]
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Computational Algorithm

- Goal: select a set of potential regulators of a particular target gene by estimating its expression profile
- Method: find possible regulators using least squares minimization and the model equation 4 to minimize error function
- Degree of polynomial (equation 5) is chosen according to number of data points in the profile and the level of fluctuations for each experiment.
- Differential equation 4 is solved numerically and parameters are optimized in least squares loop until desired predefined number of iterations is obtained.
Computational Algorithm Continued

1. Use equation 5 to fit regulator genes with polynomial of degree \( n \)
2. Choose specific target gene
3. Choose possible regulator from large pool of potential ones
4. Use least squares minimization (equation 4) on target gene/ regulators & error function (equation 6)
5. Repeat starting at step 3 for all potential regulators
6. Choose best fit regulators that match criteria
7. Repeat for all the target genes starting at step 2
Dataset Selected Based on Previous Work

- Used eukaryotic cell cycle dataset (Spellman et al.) to evaluate model which includes:
  - gene expression changes at 18 time points over 2 cell cycle periods
  - 6178 open reading frames on microarray chip
  - identified 800 genes associated with cell cycle according to their expression
- However, number of regulators controlling cell cycle < 800, therefore pool of 184 chosen by researchers for this experiment based on YEASTRACT database and previous papers
- Chose same 40 target genes from Chen et al. paper to compare data
Inference of Regulators

- Data put into log base 2 of ratio [actual value of mRNA divided by value of a standard]
- Prior to use of algorithm, data squared and least squares minimization applied to target gene for potential regulator
  \[ \hat{\mathbf{g}} = \text{approximation of the unknown real profile of target gene} \]
  \[ z^p \approx c_0 + c_1 t + c_2 t^2 + \ldots + c_n t^n, \]
- \( z^p \) = approximation of the unknown real profile of target gene
- Approximation takes into account error due to experimentation and natural fluctuations by polynomial fit
Calculation of Deviation from Experimental Data Allows for Identification of Best Regulators

- In order to identify most probable regulator for target gene, must determine which regulator profile best models target gene profile (Equation 4) and minimizes error (Equation 6)
- Therefore, chose regulators with an $E$ less than or equal to the deviation $E_1$
- Best regulators= those that have a recognizably smaller $E$ than others

\[
E_1 = \frac{1}{Q} \sum_{\tau=1}^{Q} [z(t_{\tau}) - z^p(t_{\tau})]^2.
\]
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● **Dynamic transcriptional control and optimization using least squares minimization**
● **Algorithm** utilizes dynamic model of time continuous gene expression
● **Method for determining correct regulators**
● **Trends in expression profiles** for selected genes and their regulators
● **Compared to linear model**, nonlinear model gives better results in terms of correct identification of the regulator and better fit to gene expression profile of target.
## Comparison to YEASTRACT Database Determines Correct Regulators

### Table 1. Summary of identification of regulators for 40 selected yeast cell cycle regulated genes

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<th>$E \leq 1.1 * E_1$ m</th>
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As Criterion Decreases, Rate of False Positives Increases

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Vu, T. T., & Vohradsky, J. (2007)
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Repressor and Target Gene Display Opposite Trends

Vu, T. T., & Vohradsky, J. (2007)
Activator and Target Gene Display Similar Trends

Figure 1. Expression profiles of 12 cell cycle regulated genes and their predicted regulators. (A) repressors, (B) activators. Horizontal axis—time points, vertical axis—expression relative to time point zero. Gene names in captions are arranged as target/regulator, symbols—target gene profile, dotted line—target gene profile fitted using the model, solid line—profile of the best fitting regulator (the lines are interrupted at the positions where the original data points were missing).

Vu, T. T., & Vohradsky, J. (2007)
Non Linear Model Give Better Results Compared to Linear Model

Figure 2. Histogram of distribution of the order of correctly identified regulators in the sorted list of potential regulators [columns Min(m) and Min(m) In in Table 1], horizontal axis—the order in the sorted list. Regulators were sorted according to the error of approximation of the target gene expression profile (Equation 6). (A) Nonlinear model Equation 4, (B) linear model Equation 7.
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Summary

- Found that non linear model correctly identifies the regulators of target genes associated with the cell cycle in yeast and correctly determines their function (activator or repressor)
- Linear model gave lowest fit and lowest prediction ability when compared to both non linear model and model presented in Chen et. al (generalized linear model)
- When 3 models were compared (non linear, linear, Chen et. al) found that all 3 gave different results for the sets of genes
- The non linear model overall showed good accuracy and reasonable fit
- Since the model captures the behavior of transcriptional regulation/ provides info on influence of possible regulators and correctly predicts regulators, it can act as a useful tool in interpreting gene expression time series
- However, large scale network may require a large number of computations that may be unrealistic
Acknowledgments

• We would like to thank Dr. Dahlquist and Dr. Fitzpatrick
References