Chapter 1

Systems Biology via Redescription and Ontologies(II): A Tool for Discovery in Complex Systems

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A complex system creates a "whole that is larger than the sum of its parts," by coordinating many interacting simpler component processes. Yet, each of these processes is difficult to decipher as their visible signatures are only seen in a syntactic background, devoid of the context. Examples of such visible datasets are time-course description of gene-expression abundance levels, neural spike-trains, or click-streams for web pages. It has now become rather effortless to collect voluminous datasets of this nature; but how can we make sense of them and draw significant conclusions? For instance, in the case of time-course gene-expression datasets, rather than following small sets of known genes, can we develop a holistic approach that provides a view of the entire system as it evolves through time?

We have developed GOALIE (Gene-Ontology for Algorithmic Logic and Invariant Extraction) - a systems biology application that presents global and dynamic perspectives (e.g., invariants) inferred collectively over a gene-expression dataset. Such perspectives are important in order to obtain a process-level understanding of the

underlying cellular machinery; especially how cells react, respond, and recover from environmental changes. GOALIE uncovers formal temporal logic models of biological processes by redescribing time course microarray data into the vocabulary of biological processes and then piecing these redescriptions together into a Kripke structure. In such a model, possible worlds encode transcriptional states and are connected to future possible worlds by state transitions. An HKM (Hidden Kripke Model) constructed in this manner then supports various query, inference, and comparative assessment tasks, besides providing descriptive process-level summaries. The formal basis for GOALIE is a multi-attribute information bottleneck (IB) formulation, where we aim to retain the most relevant information about states and their transitions while at the same time compressing the number of syntactic signatures used for representing the data. We describe the mathematical formulation, software implementation, and a case study of the yeast (*S. cerevisiae*) cell cycle.

1.1 The problem of microarray analysis

Microarrays, which allow the measurement of expression levels for tens of thousands of genes at a time, are a useful technique for gathering biological data, but it can be difficult to make sense of the results they produce. Experiments can be repeated with varying conditions (such as starvation, heat shock, etc.) and with each microarray having upwards of 10,000 probes, and many time course experiments having over 10 time points, there is a vast amount of data being generated. One frequent method used to deal with this is to cluster[1] the data into groups that have similar properties. There are two common ways of doing this clustering: by expression patterns over the entire dataset, which fails to take into account variations of the data over time; and by function - using a known ontology of biological processes, which fails to find unknown groupings. What is needed is a method of modeling the data based on both biological function and temporal evolution and a way to relate it to other experiments.

1.2 Computation

Our computational methods are based on a temporal redescription, which takes genes and translates them into a controlled vocabulary, and then stitches those translations together to form a picture of the biological system as it evolves over time. To facilitate the construction of our model of the dataset, we begin by breaking the data into small overlapping (or non-overlapping) windows of time. Each window contains all of the genes in the data set, but with only their expression values for a specific interval of time. The initial cluster analysis, using the numerical gene expression data, is done on these windows, rather than the entire dataset. By doing this "windowing," we have simplified each computational step while also allowing for the fact that groups of genes may briefly act together but diverge across the whole dataset. Using this windowed approach we can make inferences such as "process A and process B act together beginning in hours 1-2 and continuing through hours 2-4. They are then joined by process

C during hours 4-6." Each cluster in each window of time is first redescribed into the vocabulary of biological processes, and then we redescribe the clusters again: in relation to each other. That is, we connect clusters across time windows by tracking their describing terms. The connection relationships can be both one-to-many and many-to-one, as their meaning is that the terms in the connection persist from the source cluster to the destination cluster. A more rigorous algorithm for constructing these clusters and cluster-connections has been developed using a generalization of Shannon-Kolmogorovs rate-distortion theory, called "information bottleneck approach:" in this setting, the redescription is simply viewed as a lossy-compression of all the temporal observations by a simpler finite automaton that introduces minimal distortion in terms of the known ontologies. This generalized algorithm appears elsewhere [2].

The basis for the computations we perform is the use of temporal logic in the form of a Kripke structure. Here, this is a directed graph (often acyclic, DAG), defined by its vertices, edges, and properties (V, E, P). Generalization to probabilistic versions of Kripke structures can be achieved by assigning probabilities of transitions to the edges. The vertices represent the reachable states of the system (clusters), edges are transitions between states (cluster connections across time windows), and properties (GO Categories) are used to annotate the states in which they are true. Terms within the Gene Ontology (GO)[4] have their own hierarchical structure, which is also incorporated into the model. In the case of our yeast (*S. cerevisiae*) data, describing terms would include "cell cycle", and more specifically "M phase" and "G1/S transition of mitotic cell cycle."

1.2.1 Computation in detail: HKM and IB

This section explains in detail, the methods used in GOALIE to derive a Kripke model in the form of a DAG from a given time series data set.

The Information Bottleneck Principle

The Information Bottleneck principle[14] is an information theoretic approach to clustering. Suppose each instance x_i of a random variable X is associated with an instance y_i of another random variable Y, and we desire a clustered representation T of X which preserves as much information about the relevant variable Y as possible. Typically X represents the features of the objects to be compressed and Y represents some relevant information about their classes. According to the IB principle the clustering scheme T is chosen to minimize the functional

$$L_{min} = I(T;X) - \beta I(T;Y) \tag{1.1}$$

where $I(T;X)$ represents the mutual information between T and X, $I(T;X) = \sum_{t,x} log\frac{p(t,x)}{p(t)p(x)}$, and β is the Lagrange parameter that controls the tradeoff between compression and preservation of relevant information. Lower values of β give more importance to compression while higher values give more importance to preserving relevant information. We can try to maximize the functional L in equation 1.2.1 as follows

$$L_{max} = I(T;Y) - \beta^{-1} I(T;X) \qquad (1.2)$$

This equation provides a metric to evaluate different clustering schemes of X.

The key steps in GOALIE are

1. Partitioning the given time series data into windows.
2. Clustering expression data in each window.
3. Connecting the clusters in neighboring windows.

Clustering within a window

Let G represent the random variable corresponding to the expression vectors in a given window W. Each expression vector g_i is an instantiation of G. Still *et al.*[12] show that k-means clustering algorithm can be derived from the information bottleneck method. Following equation 1.2.1, the IB formulation of k-means clustering C is as follows

$$L_{max} = I(C;G) - \beta^{-1} I(C;i) \qquad (1.3)$$

The clustering C compresses the data indices i while preserving similarity in expression space G.

Choosing the partitioning of windows

There are many ways to define an objective criterion for achieving a partitioning of the windows. One approach is as follows. For a given time series data set, we identify a tiling (overlapping or non-overlapping) of windows such that when each window is re-stated in terms of clusters, the cluster identities in one window are informative of cluster identities in the neighboring window. The sum of mutual information across all windows along with a penalty for overlaps can then be used to define the IB functional at this stage.

Connecting the clusters in neighboring windows

Given the set of windows that span across all the time points in the data set, our next task is to connect the clusters in neighboring windows to track the temporal relationships in the data. In each window we find the GO ids enriched using the Fisher-Exact test with Benjamini-Hochberg (or with an empirical Bayesian approach) correction. Two clusters C_i and C_j are said to be θ - *equivalent* if the Jaccards coefficient between the sets of GO ids enriched in C_i and C_j is $\geq \theta$. With these connections between the clusters, we can introduce a directed graph $G = (C, E)$ whose vertices C are the clusters and an edge from cluster C_i to C_j exists if they are in neighboring windows and the Jaccards coefficient of the GO ids in the clusters is at least θ. See [6] for additional details of the window-partitioning and neighbor-connecting algorithms.

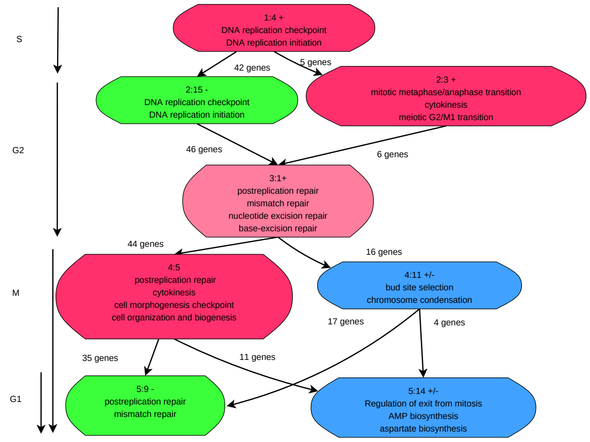
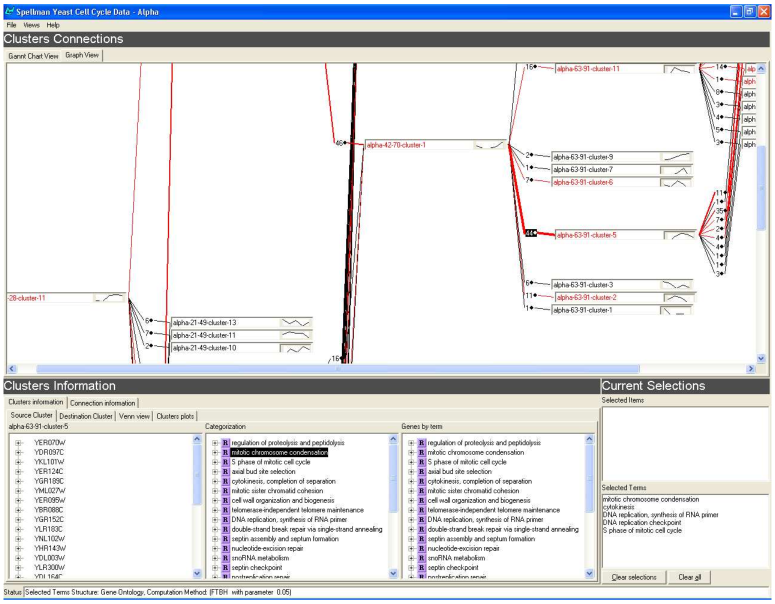


Figure 1.1: Diagram of GOALIE's output of the yeast cell cycle



alpha-63-91-cluster-5

YER070W
YDR097C
YKL101W
YER124C
YGR189C
YML027W
YER095W
YBR088C
YGR152C
YLR183C
YNL102W
YHR143W
YDL003W
YLR300W
YDL164C

Categorization

R regulation of proteolysis and peptidolysis
R mitotic chromosome condensation
R S phase of mitotic cell cycle
R axial bud site selection
R cytokinesis, completion of separation
R mitotic sister chromatid cohesion
R cell wall organization and biogenesis
R telomerase-independent telomere maintenance
R DNA replication, synthesis of RNA primer
R double-strand break repair via single-strand annealing
R septin assembly and septum formation
R nucleotide-excision repair
R snoRNA metabolism
R septin checkpoint
R postreplication repair

Genes by term

R regulation of proteolysis and peptidolysis
R mitotic chromosome condensation
R S phase of mitotic cell cycle
R axial bud site selection
R cytokinesis, completion of separation
R mitotic sister chromatid cohesion
R cell wall organization and biogenesis
R telomerase-independent telomere maintenance
R DNA replication, synthesis of RNA primer
R double-strand break repair via single-strand annealing
R septin assembly and septum formation
R nucleotide-excision repair
R snoRNA metabolism
R septin checkpoint
R postreplication repair

Selected Items

Selected Terms

mitotic chromosome condensation
cytokinesis
DNA replication, synthesis of RNA primer
DNA replication checkpoint
S phase of mitotic cell cycle

Status: Selected Terms Structure: Gene Ontology, Computation Method: (FTBH with parameter 0.05)

Figure 1.2: GOALIE output of the HKM as a graph of clusters

1.3 Causal Connections

Equipped with an HKM, as created by the methods described earlier, one could not only investigate what standard temporal logic invariants (e.g., reachability, safety, etc.) hold, but also seek out complex causal relationships that are not completely explicit. When interpreted in the context of gene expressions, causal relationships can refer to sets of processes, or genes, modulating one another to establish certain patterns of regulation. Attempts to determine these causal relationships using standard statistical approach have proven difficult[10], however, since we can reframe the problem in terms of model checking and use the pre-existing framework along with philosophical ideas of probabilistic causality[13], we have been able to infer these causal relationships from the HKM. The algorithm for this performs two key steps: namely, generate and represent causal relationships as a set of logical formulas; next, using multiple hypotheses testing, determine a subset of formulas satisfied by the model with a reasonably high confidence. We build our formulas from the representation of the following types of causes: prima facie (possible causes) - these cause raises the probability of the effect, spurious causes - those which seem to raise the probability of an effect but whose relationship may be explained by other factors, and genuine causes (non-spurious prima facie causes). This step results in three sets of temporal logical formulas (in PCTL), relating causes to their effects (which themselves may be logical formulas). See [7] for further details.

1.4 Software and Results

The main output of the software is a representation of the HKM as a directed graph. The graph presents clusters and their connections and allows exploration of the genes and terms comprising each. Integrated links to websites such as Entrez[3] and the Affymetrix database allow further study of the probes. We present a validation process for GOALIE that was tested primarily using the yeast (*S. cerevisiae*) cell cycle dataset collected by Spellman et al.[9]. There are three components to the Spellman yeast cell cycle data, following the yeast's behavior with α-factor, cdc15 and elutriation. This paper describes a case study using the alpha factor dataset. Further discussion of the visualization and software facilities in GOALIE is the subject of a forthcoming paper.

1.4.1 Cluster Graph

We began with time course gene expression data for 6178 genes at 18 time points. After filtering out genes with missing expression data, we ended up with 4489 genes left. The resulting data was partitioned into 5 time windows, with all but the last window having an overlap of two time points. Each window was divided into 15 clusters, yielding a total of 75 clusters. These clusters formed the initial input to GOALIE. They were re-described using the Fisher-Exact test with Benjamini-Hochberg correction and a p-value of 0.05. The redescription across time windows was computed with a Jacquard's coefficient $\theta = 0.8$. Using the notation W:C to denote cluster number C in window W, we describe a sampling of our results, as seen in figure 1.1.

1:4 to 2:15 and 2:3 DNA replication initiation as well as DNA replication checkpoint are initially up regulated, consistent with the S phase of the cell cycle. 1:4 then splits into two clusters, with opposite regulation patterns. In cluster 2:15, notice that the processes labeling the S portion of 1:4 are down regulated, while cytokinesis, meiotic G2/M1 transition and mitotic metaphase/anaphase transition are up regulated in 2:3.

3:1 At this time, genes from 2:15 and 2:3 which had been regulated separately converge, as G2 continues and processes associated with repairs are up regulated. As in the first window, this cluster again splits into two groups.

4:5 Cell organization and biogenesis, cell morphogenesis checkpoint, cytokinesis and postreplication repair are all up-regulated, signaling the beginning of the M phase. Genes from his cluster divide into oppositely regulated groups in the last window

4:11 Also part of M phase, but more sharply regulated, Bud site selection and chromosome condensation are first high and then go down in window 4. This cluster then separates into two groups, which overlap with genes from 4:5.

5:9 and 5:15 As The Mphase ends and G1 begins, 5:9 contains down regulated processes such as post replication repair and mismatch repair. In 5:14, there is a positive regulation of the exit from mitosis, AMP biosynthesis and aspartate biosynthesis as the cell readies for the G1 phase.

1.5 Conclusion and future directions

Many complex systems, whether natural or engineered, are amenable to GOALIEs semantic analysis within the kinds of logical frameworks it creates. Through the examples used here, such a logical framework has been seen to provide a new way of reasoning about complex biological systems as well as the interface that makes this information accessible to scientists. In the future, GOALIE is planned to provide support for other ontologies and controlled vocabularies, such as MeSH [8] and KEGG[5]. There are also several interesting technical questions to be answered: How does one select the optimal size of the Kripke model, mostly determined by window size and number of clusters[11], as those choices can strongly affect the rate (data-compression), distortion, and hence, the fidelity of the resulting models. Additionally, GOALIE will need to continually evolve to interface with the users from other fields through transparent representations such as the Gantt charts, which minimize the information loss and provide more background information on the genetic basis for the displayed terms. There is also the matter of determining the optimal window size and number of clusters[11], as those choices can strongly affect the resulting models. Additionally, we are continuing development on the Gantt charts, to minimize the information loss and provide more background on the genetic basis for the displayed terms.

1.6 Acknowledgements

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