Hybrid analysis of gene dynamics predicts context-specific expression and offers regulatory insights

Justin D. Finkle and Neda Bagheri

Abstract

Motivation: To understand the regulatory pathways underlying diseases, studies often investigate the differential gene expression between genetically or chemically differing cell populations. Differential expression analysis identifies global changes in transcription and enables the inference of functional roles of applied perturbations. This approach has transformed the discovery of genetic drivers of disease and possible therapies. However, differential expression analysis does not provide quantitative predictions of gene expression in untested conditions. We present a hybrid approach, termed Differential Expression in Python (DiffExPy), that uniquely combines discrete, differential expression analysis with in silico differential equation simulations to yield accurate, quantitative predictions of gene expression from time-series data.

Results: To demonstrate the distinct insight provided by DiffExPy, we applied it to published, in vitro, time-series RNA-seq data from several genetic PI3K/PTEN variants of MCF10a cells stimulated with epidermal growth factor. DiffExPy proposed ensembles of several minimal differential equation systems for each differentially expressed gene. These systems provide quantitative models of expression for several previously uncharacterized genes and uncover new regulation by the PI3K/PTEN pathways. We validated model predictions on expression data from conditions that were not used for model training. Our discrete, differential expression analysis also identified SUZ12 and FOXA1 as possible regulators of specific groups of genes that exhibit late changes in expression. Our work reveals how DiffExPy generates quantitatively predictive models with testable, biological hypotheses from time-series expression data.

Availability and implementation: DiffExPy is available on GitHub (https://github.com/bagherilab/diffexpy).

Contact: n-bagheri@northwestern.edu

Supplementary information: Supplementary data are available at Bioinformatics online.
adjusted P-value. DEGs are often split into groups of genes that are overexpressed or underexpressed (Law et al., 2014; Pimentel et al., 2017). Finding enriched Gene Ontology (GO) terms or pathways associated with the DEGs can elucidate the functional role of the experimental condition (Consortium, 2016; Subramanian et al., 2005).

Measuring and analyzing the dynamics of gene expression are also critical to understanding responses involved in DNA repair, development and circadian rhythms (Hafner et al., 2017; Jiang et al., 2000, 2015). A typical time-series, gene expression experiment compares expression between experimental conditions over several time points (Hafner et al., 2017; Schulz et al., 2012). Many algorithms that identify DEGs from time-series data exist, but these algorithms focus on DEG identification for subsequent enrichment analyses (Spies et al., 2017). Other algorithms use time-series expression data to infer the structure of gene regulatory networks (Finkle et al., 2018; Madar et al., 2009; Zoppoli et al., 2010), or attempt to identify transcription factors (TFs) that explain changes in expression between conditions, by pairing the expression data with ChIP-seq data (Mina et al., 2015; Schulz et al., 2012).

A limitation of existing differential expression analyses—both of static and time-series data—is that they do not propose quantitative models of a gene’s expression that can be tested in new experimental conditions. For example, if a gene is overexpressed in cells treated with a particular drug (compared with untreated cells), existing analyses cannot predict if that gene will be overexpressed, unchanged or underexpressed when a different drug is applied. Researchers can only infer how the regulation might occur and qualitatively predict how expression will differ in untested contexts.

Distinct from statistical enrichment approaches, differential equation models aim to use mechanistic information to describe how species, such as genes or proteins, interact and are well-suited to quantitatively predict gene expression in untrained conditions. However, designing and fitting differential equation parameters requires sufficient data; therefore, such models only exist for a few well-studied systems (Gambin et al., 2013; Orton et al., 2005; Pappalardo et al., 2016; Sulaimanov et al., 2017). Genetic and sparse regression algorithms can generate differential equation models directly from data, but current gene expression technologies cannot produce the highly sampled, low-noise data that these algorithms require (François and Hakim, 2004; Mangan et al., 2016).

Data-driven methods to generate models that can quantitatively predict gene expression are currently limited. Network inference methods generate genome-scale models, however, to predict the expression of any one gene requires knowing the expression of several others (Bansal et al., 2006; Finkle et al., 2018; Geurts et al., 2018). Other methods explicitly fit expression to a time variable, which ignores the molecular contexts driving expression (Hafner et al., 2017). To fill this gap, we present Differential Expression in Python (DiffExPy), a framework that uses time-series expression data to create dynamical-systems models of gene expression.

DiffExPy first determines a discrete response from the expression of each gene in the time series based on the sign and significance of the gene’s LFC between conditions at each time point. Next, DiffExPy simulates time-series expression data from a library of minimal stochastic differential equation (SDE) systems that mimic the experimental conditions. Then, the discrete response of a gene is matched to models in the simulation library to train an ensemble model. This trained model can predict that gene’s expression in new conditions. DiffExPy also clusters genes by discrete response, and infers the timing of regulatory events by associating these gene groups with TFs and GO terms (Fig. 1).

We demonstrate the efficacy of DiffExPy on publicly available RNA-seq data from the GeneExpressionOmnibus (GEO) repository, accession number GSE69822 (Kiselev et al., 2015). Previous analysis of this dataset further elucidated the transcriptional roles of phosphoinositide 3-kinase (PI3K) and phosphatase and tensin homolog (PTEN), which respectively phosphorylate and dephosphorylate phosphatidylinositidol-3,4,5-trisphosphate (PIP3). PIP3 regulates many downstream pathways, most notably the AKT pathway (Kiselev et al., 2015). For our analyses, we use data from the wild-type (WT), PTEN knockout (PTEN KO), A66-treated cells and PI3K knockin
DiffExPy predicts gene expression

(PIK3CA H1047R) conditions. A66 inhibits the p110α PISK3CA and we refer to it as the inhibited condition (PISK3CA). The histidine-to-arginine substitution makes PISK3CA constitutively active, and we refer to it as the knockin (PISK3KI) condition. In the original study, expression was measured from MCF10a cells, a commonly used human breast epithelial cell line, stimulated with epidermal growth factor (EGF) using RNA-seq in three replicates at 0, 15, 40, 90, 180 and 300 min after EGF stimulation (Kiselev et al., 2015).

Using the differential-expression data between the PISK3Knth and WT conditions, we train ensemble models for several genes. We validate the expression predictions for each gene using the PISK3KI time-series data and provide a straightforward approach to rank the confidence of each trained ensemble. The ensembles vary in size and consist of minimal SDE systems with different connectivities. We highlight results of three genes known to interact with the PISK3 pathway that currently lack quantitative models for their expression. In doing so, we demonstrate how the trained ensemble models provide simple starting models for less-studied genes. We also use the discrete response calculated by DiffExPy to identify the timing of regulation by suppressor of zeste 12 (SUZ12) and forkhead box A1 (FOXA1) on their target genes.

DiffExPy is distinct from the status quo in generating dynamical system models de novo for many genes that were not previously characterized. Currently, DiffExPy is limited in that it constructs small models based on time-series data from individual perturbations. However, DiffExPy is readily extensible and can be adapted to other differential-expression packages, model assumptions and genomic data. For example, future improvements to DiffExPy could be made to incorporate multiple perturbations, additional omics data types and prior knowledge. Our work provides a foundation on which more complex models of gene expression can be developed.

2 Materials and methods

Using time-series RNA-seq data, DiffExPy sorts genes according to their discrete, dynamic, differential gene expression profiles (Fig. 1 and Supplementary Fig. S1). Each gene’s discrete profile is used to train an ensemble model of minimal SDE systems that predict expression in new conditions (Fig. 1). DiffExPy also associates GO terms with resulting groups, which suggest functional roles for the genes in each distinct cluster. Finally, DiffExPy associates TFs with genes that exhibit similar responses at specific times (Fig. 1). Overall, DiffExPy identifies (i) minimal dynamical systems models that accurately predict gene expression dynamics in untrained conditions, (ii) specific GO terms associated with classes of expression dynamics and (iii) specific TFs associated with genes with similar expression responses.

2.1 DiffExPy assigns discrete differential responses

To match gene expression responses to dynamical systems models, DiffExPy first calculates discrete responses from LFC contrasts generated by differential expression analysis (Supplementary Fig. S1) using the package limma (Ritchie et al., 2015). A contrast is defined as a comparison of expression between conditions, time points or both. The discrete response is derived from the LFC value for a contrast, which can be positive (+1), negative (−1) or not significant (0). We assign gene labels based on discretized LFC values (Fig. 1 and Supplementary Fig. S1) as DEGs, dynamic DEGs (dDEGs) and differentially responding genes (DRGs).

DEGs are genes that are differentially expressed between conditions at one or more time points after the treatment, based on an F-test. dDEGs define the subset of DEGs that exhibit dynamic, or variable, differential expression across time. For instance, an expression profile need not be differentially expressed at time 0, but it can become differentially expressed at a later time point. DRGs contain at least one time point in which the LFC is significantly different from the LFC either at the previous time point (i.e. LFCt ≢ LFCt−1) or from the time the treatment is applied (i.e. LFCt ≢ LFC0), where significance is determined using an F-test. Classification of a gene as a dDEG or DRG is not mutually exclusive, and by definition, a dDEG or DRG is also a DEG.

2.1.1 Definition of gene expression contrasts

A gene expression contrast compares the distribution of expression values of a gene between samples (Ritchie et al., 2015). Using time-series data, the basic set of values used in a contrast for gene i, given condition c, with R replicates and at time t is defined as:

\[ g_{i,t}^R = [g_{i,t}^1, g_{i,t}^2, \ldots, g_{i,t}^R] \]  

(1)

The LFC is calculated as the ratio of the mean log2 expression value between the conditions:

\[ l_i^t = \frac{g_{i,exp}^t}{g_{i,ctrl}^t} \]  

(2)

where exp is the experimental condition and ctrl is the control condition. By convention, the control condition is in the denominator, so positive LFC values correspond to overexpression in the experimental condition. For each contrast, a corresponding P-value is calculated. When multiple contrasts are made, an overall significance level is also calculated using an F-test. Significance levels are corrected for multiple hypothesis testing (Ritchie et al., 2015). Differential expression calculations between genes are linearly independent and are easily extended to matrix form.

DiffExPy departs from the status quo by using time-series data to create more complex contrasts. Pairwise (PW) contrasts compare expression between experimental conditions at each time points. Time-series (TS) contrasts compare expression between a time point and the previous time point. Autoregressive (AR) contrasts compare expression between a time point and the time point before the treatment was applied. A detailed description of these and other combinations of contrasts (PW-TS and PW-AR) is available in Supplementary Figure S1.

2.1.2 Discrete expression responses

To facilitate downstream analyses, DiffExPy calculates a discrete response for each gene based on the P-values and signs of LFC for the individual contrasts. If the P-value for a contrast is above the user-specified threshold, the LFC is not considered significant and is set to zero. The discrete response for gene i is defined as \( d_{i,x} = d(l) \forall l \in \hat{l}_{i,x} \), where x is one of the set (PW, TS, AR, PW-TS or PW-AR). The discrete values are calculated using the signs of the LFC values as follows:

\[ d(l) = \begin{cases} 1 & \text{sign}(l) > 0 \text{ and } p(l) < p_{\text{cut}} \\ 0 & p(l) \geq p_{\text{cut}} \\ -1 & \text{sign}(l) < 0 \text{ and } p(l) < p_{\text{cut}} \end{cases} \]  

(3)

where \( p(l) \) is the adjusted P-value of the LFC for the contrast and \( p_{\text{cut}} \) is the significance threshold. For a time series of T time points, each discrete response has \( 3^{T-1} \) possible clusters—except for \( d_{\text{PW}} \), which has \( 3^T \). We did not filter LFC values by magnitude, but the option is provided in DiffExPy. We used a P-value threshold of 0.05.
for all of our tests. A lower P-value cut-off could result in discrete responses with more zero values.

2.2 Training predictive models of gene expression

DiffExPy uses GeneNetWeaver (GNW) to generate minimal differential equation models for unique, three-node networks and to carry out stochastic simulations. GNW models include a protein and mRNA component (Schaffter et al., 2011). Each model is an abstract representation of the flow of information that might regulate a gene’s expression and should not be used to identify specific regulation between genes. To mimic the experimental data, we used DiffExPy to conduct three independent, stochastic runs of each model and sampled each model at the same time points as the RNA-seq data. Microarray-like measurement noise was added to the data, and values were normalized between 0 and 1. A complete description of model generation is available in Supplementary Material.

Simulations were conducted under three genetic conditions: WT, knockout and knockin. It is important to note that the identity of the perturbed gene does not need to be known to gain information from DiffExPy. If a treatment with an unknown target is applied, DiffExPy can still provide insight into possible motifs of which a gene is a part that results in the observed expression behavior.

2.2.1 Matching models to genes

After simulation, each SDE model had time-series data mimicking the experimental data. We conducted the same discrete clustering process on the simulated data. Each gene was matched to all SDE models with the same discrete response as the gene match $\{m \in M; l_i^t = l_m^t \forall t \in T\}$, where $M$ is the set of all models in the simulation library and $T$ is the number of time points in the discrete response.

2.3 Model predictions

Each SDE model matched by DiffExPy can then simulate time-series data under different conditions to generate predicted LFC values at each time point, represented as a $T$-length vector $l_k = [l_k^1, \ldots, l_k^T]$, where $k$ is the index of the matched SDE system and $T$ is the number of time points in the predicted time series. The time series of the control condition provides an internal control for predicting the response.

For our predictions, we applied a simulated knockin of PI3K to each of the trained SDE models as this matches the PI3K KI perturbation that was applied in the experimental data. Importantly, predictions to match different treatment strategies can also be made—such as targeting multiple nodes in the SDE models, inhibiting interactions between SDE nodes or changing the forcing function.

We found no feature of the individual models that correlated with their prediction error. We therefore created an ensemble prediction by using the median predicted LFC. For each gene, the predicted LFC is calculated as the median LFC of all matched SDE predictions for each time point:

$$l_i^t = \text{median}(l_m^t \forall m \in \text{match}_i).$$ (4)

The simulated model predictions in log2 expression space are $\hat{y}_i^t = b_i^t + l_i^t$, where $b_i^t$ is the log2 expression of the control condition at time $t$, $l_i^t$ is the model predicted LFC and $\hat{y}_i^t$ is the predicted log2 expression of the experimental condition.

2.3.1 Scoring prediction accuracy

We validated the accuracy of the quantitative predictions by calculating the error between PI3K KI expression of a gene and its corresponding model’s prediction. We define accuracy as the mean-squared error (MSE) between a model’s average LFC (of three stochastic runs) and the true LFC. MSE values range from 0 to $\infty$, where smaller values indicate that the prediction is closer to the true LFC value. We know of no existing, data-driven method that generates models capable of quantitative, time-series, gene expression prediction to provide an appropriate basis for comparison. Thus, we used the selection of a random model from our library as the null model comparison.

3 Results

3.1 Many previously uncharacterized genes are matched to ensemble models

We used the discrete response profiles to match each gene to an ensemble of three-node SDE models that each share similar dynamics upon simulation. Our library consists of 2172 uniquely structured SDE models. We trained the models using the PI3Kinh and WT data, and we used the PI3K KI data as test data to validate the predictions. Simulations for each network model were created to match the PI3K genetic condition and EGF stimulation. The simulated data are sampled at the same time points used in the experiments. Details of the library creation are provided in Supplementary Material.

As the PI3Kinh does not affect the expression of many genes (Kiselev et al., 2015), DiffExPy only identifies 223 dDEGs from the differential expression analysis of the PI3Kinh and WT data. There is a many-to-many match between the dDEGs and the possible three-node SDE systems. A total of 217 of these dDEGs were matched to at least one network model. Of the 217 matched genes, few are well-studied; there is sparse information about their functional role. We identified just nine genes whose paralogs were likely to exist in current computational models of well-studied signaling pathways. These include genes in the MAPK, JAK/STAT and PI3K/AKT/mTOR pathways (Gambin et al., 2013; Orton et al., 2005; Pappalardo et al., 2016; Sulaimanov et al., 2017).

3.2 Ensemble models highlight possible dynamical systems from which to build more detailed models

Each independent model in the ensemble suggests a possible SDE system whose simulations match the qualitative features of the experimentally measured expression. Specifically, each SDE system represents how the gene of interest (node $y$) might interact with the perturbed gene (node $x$) and the rest of the genome (node $z$). In this experiment, $G$ represents PI3K as it was the knocked-out, knocked-in or inhibited gene. Summaries of the models that match each gene and create the quantitative predictions reveal possible regulatory interactions that result in the observed dynamics (Fig. 2A). We highlight results of trained models for three genes that exhibit different discrete responses and predictive accuracy: cytoplasmic linker-associated protein 1 (CLASP1), regulator of cell cycle (RGCC) and retinoic acid receptor alpha (RARA). CLASP1 and RARA were previously shown to interact with components of the PI3K/AKT pathway (Lansbergen et al., 2006; Srinivas et al., 2006).

Models for CLASP1 and RGCC primarily contain inhibition by both $x$ and $G$, whereas $x$ and $G$ appear as activators of RARA. Furthermore, in almost all models matched to RARA, both $x$ and $G$ must be present to activate RARA. The RGCC models often contain activation of $G$ by RGCC. Conversely, models of CLASP1 and RARA often exhibit feedback on $G$ but are not consistently activating or inhibiting. Overall, these models suggest modes of regulation between PI3K and CLASP1, RGCC and RARA, and predict gene
responses to future conditions and treatments. These models also provide a basis from which more detailed models can be developed.

### 3.3 DiffExPy ranking sorts models by predictive accuracy

Because every ensemble model is not equally predictive of its respective gene, we searched for a metric to rank model predictions. We find that the mean absolute LFC between the PI3K KI and WT conditions correlates with improved model accuracy (Spearman’s $\rho = 0.636$, $P = 5.4e-26$, Supplementary Fig. S2). Ordering gene predictions by the mean absolute LFC places genes with lower MSE at the top of the list. Treating genes with positive MSE (i.e. lower error than random) as positive classifications, we can assess the area under the receiver operating characteristic (AUROC) and area under precision recall (AUPR) curves. The AUROC for this ranking is 0.76 and the AUPR is 0.78, both of which are significantly greater than expected from a random ordering (Fig. 2B and Supplementary Fig. S3).

Since future experiments will not always include validation data, we sought a proxy for model confidence. We find that the mean absolute LFC between the PI3K KI and WT correlates with the mean absolute LFC between the PI3K KI and WT (Spearman’s $\rho = 0.684$, $P = 2.7e-31$, Supplementary Fig. S4). The mean absolute LFC between the PI3K KI and WT also correlates with improved model accuracy (Spearman’s $\rho = 0.418$, $P = 1.4e-10$, Supplementary Fig. S5). This ranking yields an AUROC of 0.66 and an AUPR of 0.73, which are slightly lower, but still significantly better than random (Fig. 2B and Supplementary Fig. S3).

We believe this sorting is intuitive. A gene with a greater effect size in the transcriptional response provides more information during training, which results in better matched models. On average, a random model predicts no LFC between WT and another condition for a given gene, so a trained model prediction should be more accurate (Supplementary Fig. S6).

### 3.4 Top-ranked genes offer accurate predictions

Overall, the predictions for the dDEGs have a median MSE that is 0.027 ($P = 0.018$) lower than random (AMSE). However, after ranking genes by mean PI3K KI-WT LFC, we applied an elbow rule to select the top 40 genes. Our results indicate that the top-ranked genes have significantly more accurate predictions than random models. The top genes have a median AMSE of 0.523 ($P = 1.45e-6$) and a %MSE of 33.3% ($P = 1.74e-5$, Fig. 2C). The elbow rule gives an empirical threshold to select the top predictive genes (Supplementary Fig. S7).

### 3.5 Gene classifications from discrete responses associate with specific GO terms

To demonstrate the high-level biological insights gained from the discrete responses, we present results of classifying genes from their discrete responses comparing the PI3K KI to WT time-series expression data. We identify 8508 DEGs, of which 3961 are classified as dDEGs, 140 as DRGs and 283 as all three (Fig. 3B). We performed GO term enrichment analysis on each of these non-mutually exclusive gene classifications. Enriched GO terms were grouped by the exclusive GO term enrichment analysis on each of these non-mutually exclusive gene classifications. Enriched GO terms were grouped by the exclusive GO term enrichment analysis on each of these non-mutually exclusive gene classifications. Enriched GO terms were grouped by the exclusive GO term enrichment analysis on each of these non-mutually exclusive gene classifications. Enriched GO terms were grouped by the exclusive GO term enrichment analysis on each of these non-mutually exclusive gene classifications. Enriched GO terms were grouped by the exclusive GO term enrichment analysis on each of these non-mutually exclusive gene classifications. Enriched GO terms were grouped by the exclusive GO term enrichment analysis on each of these non-mutually exclusive gene classifications. Enriched GO terms were grouped by the exclusive GO term enrichment analysis on each of these non-mutually exclusive gene classifications. Enriched GO terms were grouped by the exclusive GO term enrichment analysis on each of these non-mutually exclusive gene classifications.
is used as proxy for term specificity. Even though no genes are categorized as direct regulators (PRC2). PRC2 has histone methylation activity, yet its regulatory role in cell fate is uncertain (Comet et al., 2016; Squazzo et al., 2006). FOXA1 is an important TF in breast and prostate cancers and is known to be a target of both MAPK and AKT (Bernardo et al., 2013; Potter et al., 2012).

Using the temporal information inherent to a time-series dataset, we can identify when, and how, the regulation by these factors occurs. For example, we identify the set of 41 genes that have lower LFC at 300 min than at 0 min, which are enriched for association with SUZ12 (Fig. 4A). A total of 13 of the 41 genes are known to be associated with SUZ12, and the enrichment suggests that changes in expression for this group are regulated by SUZ12. Interestingly, there is no identifiable change in SUZ12 expression (Fig. 4A), indicating that downstream gene regulation by SUZ12 might depend on post-transcriptional changes, such as sumoylation (Rising et al., 2008).

We also find FOXA1 to be associated with genes that show an increase in LFC between 90 and 180 min. A natural hypothesis is that this group of 79 genes all show the same change in expression at these later time points because they share a common regulator, FOXA1 (Fig. 4B). In contrast to SUZ12, FOXA1 exhibits a distinct differential response beginning 90 min after the EGF stimulus. Several of the genes that have the described behavior and are associated with FOXA1 show a similar qualitative differential response to EGF as FOXA1. These results suggest that FOXA1 might regulate the expression of these genes, as well as others in the set, in response to EGF stimulation. Additionally, each of these genes, including FOXA1, is classified as a DRG, further supporting the hypothesis that PTEN is required for proper expression of these genes in response to EGF stimulation.

SUZ12 and FOXA1 are not the only TFs associated with discrete response clusters. Instead, these examples demonstrate two possible

---

**Fig. 3.** Summary of gene classifications comparing PTEN KO to WT. (A) Heatmap of row normalized LFC for all DEGs. Genes that are classified as dDEG or DRG are also labeled. (B) Overlap of gene sets that are classified as DEG, dDEG, DRG or some combination. By definition, all dDEGs and DRGs must also be DEGs. (C) Comparison of distributions of GO term depths uniquely associated with intersections of gene sets. Even though no genes are classified as only dDEG or DRG, these gene sets associate with unique, specific GO terms not found in the dDEG set. The number of unique terms associated with each set, , is shown. -values were calculated using a discrete KS test. *P < 0.05, **P < 0.001.

All terms were called from the same directed acyclic graph (DAG). Term depth quantifies the level in the GO hierarchy, and it is used as proxy for term specificity. Even though no genes are categorized exclusively as dDEGs or DRGs, there are very specific terms associated only with these groups (Fig. 3C). Results of the gene classifications for the PI3K and PI3K pathways compared with WT are provided in Supplementary Figures S8 and S9. Specific gene clusters with similar discrete responses may also be used for GO enrichment analysis, but we next focus on using them for TF enrichment.

**3.6 TF enrichment suggests regulators of gene expression**

Similar to GO term enrichment analysis, we calculate TF enrichment for gene clusters. A group of genes enriched for association with a particular TF may indicate that the TF is responsible for the observed change in expression. Existing methods, such as weighted gene co-expression network analysis (WGCNA) and dynamic regulatory events miner (DREM), perform clustering of gene profiles for subsequent GO and TF enrichment analysis (Langfelder and Horvath, 2008; Schulz et al., 2012; Wise and Bar-Joseph, 2015). In contrast, DiffExPy uses the discrete LFC values (0, 1, –1) to generate default clusters. This discretization enables grouping genes in various ways that suggest different types of coregulation by a shared TF.

For example, the set of all DRGs is enriched for association with 52 TFs (adjusted P < 0.05). This set includes suppressor of zeste 12 (SUZ12) and forkhead box A1 (FOXA1), which were not identified in the original study (Kiselev et al., 2015). SUZ12 is a zinc finger protein and a component of the polycomb repressive complex 2 (PRC2). PRC2 has histone methylation activity, yet its regulatory
ways the discrete analysis might identify enriched regulators for groups with different response behaviors. The timing of the expression behavior creates strong, testable hypotheses for the inferred regulators. Additional enrichment results, and a complete discussion of the enrichment methods, are provided in Supplementary Material.

4 Discussion

The characterization of less-studied genes is fundamental to understanding cellular responses in diverse environmental contexts (Stoeger et al., 2018). In this study, we presented DiffExPy, an analytical framework that calculates discrete differential expression responses and trains dynamical systems models for many quantitatively uncharacterized genes.

We demonstrated how, for each matched gene, DiffExPy prototypes quantitative models that offer accurate predictions of gene expression in untrained conditions. We also validated the DiffExPy model predictions of each gene’s expression in the untrained PI3K KI condition (Fig. 2). Our results suggest that PI3K inhibits expression of both CLASP1 and RGCC, often in conjunction with additional factors. These de novo results are supported by previous experiments. CLASP1 was shown to interact with proteins affected by PI3K activity (Lansbergen et al., 2006). RGCC was also demonstrated to have several regulatory roles in the PI3K pathway (Tegla et al., 2015). Discrepancies with other data can refine the models and our understanding of each gene’s regulation. For example, the summary of RARA suggests that for the observed response, PI3K is a positive regulator of RARA. This result is surprising because PI3K activates AKT, which was shown to subsequently inhibit RARA (Srinivas et al., 2006). One explanation is that an unknown activating path between PI3K and RARA exists.

We also demonstrated how DiffExPy associates groups of similar, discrete gene expression responses with TFs, such as SUZ12 and FOXA1 (Fig. 4). Though SUZ12 expression does not differ between the PI3K null and WT conditions, several known and possibly new targets of SUZ12 exhibit a differential response to EGF stimulus. These observations might suggest that regulation by SUZ12 results from post-translational modification (Rising et al., 2008). Conversely, FOXA1 and many of its targets exhibit a differential response, which is consistent with previously studied interactions between FOXA1 and the AKT pathway (Bernardo et al., 2013; Potter et al., 2012). Finally, we show how each gene classification associates with GO terms that enable a unique understanding of regulatory functions.

DiffExPy was formulated to create predictive models for many genes with varied dynamic expression responses. We limited our model library to three-node gene regulatory networks without self-edges (Supplementary Fig. S10). As such, a suitable model match might not exist in the library for each gene. In our analysis, a small fraction of genes (6 of the 223 dDEGs, or less than 3%) did not match to a suitable model. The absence of matches might be attributable to the limited scope of SDE models in the library, limiting possible gene expression dynamics. The library could be expanded to include four-node networks, which might be capable of simulating more qualitatively diverse expression dynamics. Expanding the library might be computationally expensive and require optimizing the library generation step, simulation and matching. Additionally, the current SDE models could be simulated with different kinetic assumptions, though the accuracy of these assumptions would need to be validated. Finally, because our training and testing perturbation affect the same gene, the ranking of the results might not hold for all predictions. We also focused only on comparisons between pairs of experimental conditions (i.e. only one node is perturbed). Integrating multiple experimental conditions into model training might yield more predictive models, but also make training the models more difficult.

A complete understanding of cellular regulation cannot be gained only from transcriptomics. Epigenomic data from ChIP-seq, ATAC-seq, etc., can provide additional information to increase the mechanistic specificity of the models by identifying direct regulators and chromatin accessibility. Currently, TF enrichment is calculated using associations derived from ENCODE (Schulz et al., 2012). Direct measurements of TF association or chromatin accessibility, during the same time course, could be directly integrated into the existing framework. Unfortunately, this data was not available for our analysis and might currently be cost prohibitive.

Overall, few genes have detailed biochemical models that quantitatively predict their behavior in diverse conditions. Characterizing how less-studied genes are regulated in multiple contexts will improve our understanding and treatment of disease. The models generated by DiffExPy provide systematic, reliable starting points for quantitative models of regulation based on time-series, differential-expression data.

Funding

This research was supported, in part, by a Nicholson Fellowship and Biotechnology Training Program Fellowship T32 GM008449 (to J.D.F.); NSF CAREER Award [CBET-1653315 to N.B.]; NIH NU-CCNE (U54 CA199091-03); and the McCormick School of Engineering.

Conflict of Interest: none declared.

References

Bansal,M. et al. (2006) Inference of gene regulatory networks and compound mode of action from time course gene expression profiles. Bioinformatics, 22, 815–822.

Bernardo,G.M. et al. (2013) Foxa1 represses the molecular phenotype of basal breast cancer cells. Oncogene, 32, 534.

Comet,I. et al. (2016) Maintaining cell identity: PRC2-mediated regulation of transcription and cancer. Nat Rev. Cancer, 16, 803.

Consortium,G.O. (2016) Expansion of the gene ontology knowledgebase and resources. Nucleic Acids Res., 45, D331–D338.

Emilsson,V. et al. (2008) Genetics of gene expression and its effect on disease. Nature, 452, 423.

Finkle,J.D. et al. (2018) Windowed granger causal inference strategy improves discovery of gene regulatory networks. Proc. Natl. Acad. Sci. USA, 115, 2252–2257.

Francois,P. and Hakim,V. (2004) Design of genetic networks with specified functions by evolution in silico. Proc. Natl. Acad. Sci. USA, 101, 580–585.

Gambin,A. et al. (2013) Computational models of the JAK1/2-STAT1 signaling. JAKSTAT, 2, e24672.

Geurts,P. et al. (2018) dynGENIE3: dynamical GENIE3 for the inference of gene networks from time series expression data. Sci. Rep., 8, 3384.

Hafner,A. et al. (2017) p53 pulses lead to distinct patterns of gene expression albeit similar DNA-binding dynamics. Nat. Struct. Mol. Biol., 24, 840.

Jiang,Y.-J. et al. (2006) p53 pulses lead to distinct patterns of gene expression albeit similar DNA-binding dynamics. Nat. Struct. Mol. Biol., 24, 840.

Jiang,P. et al. (2013) A systems approach identifies networks and genes linking sleep and stress: implications for neuropsychiatric disorders. Cell Rep., 11, 835–848.

Jiang,Y.-J. et al. (2008) Maintaining cell identity: PRC2-mediated regulation of transcription and cancer. Nat Rev. Cancer, 16, 803.

Kiselev,V.Y. et al. (2015) Perturbations of PIP3 signalling trigger a global remodelling of mRNA landscape and reveal a transcriptional feedback loop. Nucleic Acids Res., 43, 9663–9679.
Langfelder, P. and Horvath, S. (2008) WGCNA: an r package for weighted cor-
relation network analysis. BMC Bioinformatics, 9, 559.
Lansbergen, G. et al. (2006) Clasps attach microtubule plus ends to the cell cor-
tex through a complex with If5b. Dev. Cell, 11, 21–32.
Law, C.W. et al. (2014) voom: Precision weights unlock linear model analysis
tools for RNA-seq read counts. Genome Biol., 15, R29.
Madar, A. et al. (2009) The inferelator 2.0: a scalable framework for recon-
struction of dynamic regulatory network models. Conf. Proc. IEEE Eng.
Med. Biol. Soc., 31, 5448–5451.
Mangan, N.M. et al. (2016) Inferring biological networks by sparse identifica-
tion of nonlinear dynamics. IEEE Trans. Mol. Biol. Multi-Scale Commun.,
2, 52–63.
Mina, M. et al. (2015) Promoter-level expression clustering identifies time de-
velopment of transcriptional regulatory cascades initiated by ErbB receptors
in breast cancer cells. Sci. Rep., 5, 11999.
Orton, R.J. et al. (2005) Computational modelling of the receptor-tyrosine-
kinase-activated MAPK pathway. Biochem. J., 392, 249–261.
Pappalardo, F. et al. (2016) Computational modeling of PI3K/AKT and MAPK
signaling pathways in melanoma cancer. PLoS One, 11, e0152104.
Pimentel, H. et al. (2017) Differential analysis of RNA-seq incorporating quan-
tification uncertainty. Nat. Methods, 14, 687.
Potter, A.S. et al. (2012) Forkhead box A1 (FOXA1) is a key mediator of
insulin-like growth factor I (IGF-I) activity. J. Cell. Biochem., 113, 110–121.
Rising, E.M. et al. (2008) The polycomb repressive complex 2 is a potential
target of sumo modifications. PLoS One, 3, e2704.
Ritchie, M.E. et al. (2015) limma powers differential expression analyses for
RNA-sequencing and microarray studies. Nucleic Acids Res., 43, e47.
Schaffer, T. et al. (2011) Genenetweaver: in silico benchmark generation and
performance profiling of network inference methods. Bioinformatics, 27,
2263–2270.
Schulz, M.H. et al. (2012) DREM 2.0: improved reconstruction of
dynamic regulatory networks from time-series expression data. BMC Syst.
Biol., 6, 104.
Spies, D. et al. (2017) Comparative analysis of differential gene expression
tools for RNA sequencing time course data. Brief. Bioinform., 20,
288–298.
Squazzo, S.L. et al. (2006) Suz12 binds to silenced regions of the genome in a
cell-type-specific manner. Genome Res., 16, 890–900.
Srinivas, H. et al. (2006) Akt phosphorylates and suppresses the transactiva-
tion of retinoic acid receptor alpha. Biochem. J., 395, 653–662.
Stoeger, T. et al. (2018) Large-scale investigation of the reasons why potential-
ly important genes are ignored. PLoS Biol., 16, e2006643.
Subramanian, A. et al. (2005) Gene set enrichment analysis: a
knowledge-based approach for interpreting genome-wide expression pro-
files. Proc. Natl. Acad. Sci. USA, 102, 15545–15550.
Sulaimanov, N. et al. (2017) Understanding the mTOR signaling pathway via
mathematical modeling. Wiley Interdiscip. Rev. Syst. Biol. Med., 9, e1379.
Tegla, C.A. et al. (2015) RGC-32 is a novel regulator of the t-lymphocyte cell
cycle. Exp. Mol. Pathol., 98, 328–337.
Wise, A. and Bar-Joseph, Z. (2015) cDREM: inferring dynamic combinatorial
gene regulation. J. Comput. Biol., 22, 324–333.
Zoppoli, P. et al. (2010) TimeDelay-ARACNE: reverse engineering of gene net-
works from time-course data by an information theoretic approach. BMC
Bioinformatics, 11, 154.