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Using Network Component Analysis to Dissect Regulatory Networks Mediated by Transcription Factors in Yeast

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Abstract

Understanding the relationship between genetic variation and gene expression is a central question in genetics. With the availability of data from high-throughput technologies such as ChIP-Chip, expression, and genotyping arrays, we can begin to not only identify associations but to understand how genetic variations perturb the underlying transcription regulatory networks to induce differential gene expression. In this study, we describe a simple model of transcription regulation where the expression of a gene is completely characterized by two properties: the concentrations and promoter affinities of active transcription factors. We devise a method that extends Network Component Analysis (NCA) to determine how genetic variations in the form of single nucleotide polymorphisms (SNPs) perturb these two properties. Applying our method to a segregating population of Saccharomyces cerevisiae, we found statistically significant examples of trans-acting SNPs located in regulatory hotspots that perturb transcription factor concentrations and affinities for target promoters to cause global differential expression and cis-acting genetic variations that perturb the promoter affinities of transcription factors on a single gene to cause local differential expression. Although many genetic variations linked to gene expressions have been identified, it is not clear how they perturb the underlying regulatory networks that govern gene expression. Our work begins to fill this void by showing that many genetic variations affect the concentrations of active transcription factors in a cell and their affinities for target promoters. Understanding the effects of these perturbations can help us to paint a more complete picture of the complex landscape of transcription regulation. The software package implementing the algorithms discussed in this work is available as a MATLAB package upon request.

Introduction

With advances in whole genome high-throughput technologies such as ChIP-Chip, expression, and genotyping arrays, it is now possible to integrate data from these sources together to decipher the complex regulatory networks that govern transcription. In addition to serving as powerful models for how basic cellular function is achieved, these regulatory networks can also help shed light on how certain disease phenotypes are manifested. At the heart of these networks are a few regulator genes such as transcription factors (TFs), miRNAs and histones whose activity govern the behavior of many other genes. Among these regulators, transcription factors that bind the promoter regions of genes are by far the most well understood. The process of TFs activating or repressing transcription at initiation is believed to be the primary mechanism of gene regulation. A central question in genetics is how genetic variations perturb this underlying regulatory mechanism to give rise to differential gene expression and ultimately complex phenotypes.

The simplest analysis one can perform to address this question is expression quantitative trait loci (eQTL) mapping, which identifies genetic variations such as SNPs in the form of linkages and associations that are correlated with gene expression. Such studies have been carried out in a variety of organisms including yeast [1,2] Arabidopsis [3], mouse [4,5] and human [6–8]. These studies have identified many linkages between SNPs and genes in close proximity suggesting potential local regulatory mechanisms mediated by regulators such as transcription factors and miRNAs. These studies have also identified a few SNPs linked to the expressions of many genes suggesting a global regulatory mechanism mediated by master regulators such as transcription factors and histones. Unfortunately, beyond nominating candidate genes either as targets or regulators, these studies give little insight into how SNPs perturb the underlying transcription regulatory networks that control gene expression.

To gain a better understanding of the mechanisms of transcription regulation, several systems biology based methods have been proposed including clustering of co-regulated genes [9], multipoint linkage analysis [10,11], pathway enrichment analysis [12–16], prediction of regulatory modules [17,18] and the prediction of causal regulatory relationships [19–23]. Many of these advanced methods aim to tease out both the nodes
Author Summary

One of the fundamental challenges in biology in the post-genomics era is understanding the complex regulatory mechanisms that govern how genes are turned on and off. In a single organism where the functions of individual genes in a population do not differ much, many of the differences between individuals including physical phenotypes, susceptibility to disease, and response to drugs can be attributed to how genes are regulated. Previous studies have largely focused on identifying regulator and target genes whose expressions are linked to genetic variations in a population. We present work that focuses on considering a specific set of regulators called transcription factors whose targets can be verified from experiments and whose interactions with those targets have been well studied and modeled. In this setting, we can begin to understand how genetic variations perturb the concentrations and promoter affinities of active transcription factors to induce differential expression of the targets. Understanding the effects of these perturbations is important to understanding the fundamental biology of gene regulation and can help us to design and assess therapeutics and treatments for complex diseases.

(regulators and targets) as well as the topology (mapping of edges) in a transcription regulatory network from only considering gene expression profiles. Although these methods have predicted some interesting relationships, there are at least two aspects of transcription regulation that go unaddressed when we use them to study transcription factors and their targets. First, most previous methods rely on probabilistic models that do not provide much insight into the hidden dynamics between the activity of transcription factors and the expression of their targets. Second, the relationships inferred by these methods from the expression profiles alone can be misleading because the in vivo activity of a transcription factor does not always correlate with its expression levels [24,25].

To overcome these problems, we adopt a framework from network component analysis (NCA) [26] that considers a simple bipartite network model of transcription regulation involving only transcription factors and their targets. In this model, the expression of a target gene is completely captured by two properties of the network, the concentrations and promoter affinities of transcription factors. In general, inferring these two quantities from the expression profiles alone is difficult. But by leveraging protein-DNA binding data from ChIP-Chip experiments [27,28], a partial topology of the network can be constructed and one can make the inference given certain constraints [26].

The NCA method as described by liao et al. has been successfully applied to several gene expression datasets to understand transcription regulation in a temporal setting [26] and in the context of gene knockouts [29]. In this study, we extended NCA to study transcription regulation over a population gradient by modeling three mechanisms by which genetic variations perturb the concentrations and promoter affinities of active transcription factors to induce differential expression. Figure 1 gives a simple example that illustrates the original NCA model and our extensions. Imagine we have a small experiment constructed and one can make the inference given certain constraints [26].

Because the inclusion of genetic variation creates additional parameters in each of our three models compared to the original NCA model, we expected them to always fit the data better. To effectively evaluate our models, we devised a likelihood ratio statistic and a permutation scheme to assess the statistical significance of our improvements. We then applied our method to study an expression data collected over 112 segregants of Saccharomyces cerevisiae yeast and two separate ChIP-Chip datasets generated by Harbison et al. and Lee et al.. We identified several interesting global regulatory networks perturbed by SNPs located in regulatory hotspots. Some of these networks have one property perturbed (transcription factor concentration or promoter affinity) while others have both properties perturbed suggesting a complex mechanism of global regulation.

An interesting method proposed by Sun et al. also used the NCA framework to infer the concentrations of active transcription factors from gene expression data collected over the same yeast strains. Their method was designed to detect linkages between the inferred concentrations and genetic variations and used conditional independence tests to find modules of genes controlled by the same causal regulator. Compared to this method, we expect to
find similar networks of genes and transcription factors but our method does not allow us to infer additional causal relationships using statistical tests. Instead, we focus on identifying different mechanisms by which genetic variations can perturb the regulatory networks by directly modeling the effects of these perturbations into the NCA framework. We do not attempt to make rigorous causal claims but use the causal information inherent in genotyping and ChIP-Chip experiments to suggest possible mechanisms of transcription regulation.

Results

Inferring Concentrations and Promoter Affinities of Active Transcription Factors over a Population Gradient

The NCA framework is a natural model for describing how transcription factors regulate gene expression. At the heart of the model is a log-linear equation that relates the expression levels of genes collected over a gradient (E) to the concentrations (C) and promoter affinities (PA) of active transcription factors. Such a model is well supported by known kinetic properties of protein-DNA interactions [30]. In linear model terms, the transcription factor concentrations are the regressors, the gene expression levels are the response variables and the promoter affinities are the coefficients that relate the two. Figure 2B shows the log-linear equations describing the graph shown in Figure 1B. The goal of NCA is to infer the matrices of concentrations [C] and promoter affinities [PA] from the matrix of gene expressions [E] under some restrictions in the least squares sense.

Treating genetic differences between individuals as a gradient, we applied this model to infer the matrices of concentrations [C] and promoter affinities [PA] from gene expressions collected from a population of yeast strains, [E]. For the inference to have been possible, we removed a number of transcription factors and target genes to construct a network from the original ChIP-Chip data that met certain constraints [26]. After preprocessing the Lee et al. ChIP-Chip dataset, we were left with a network with 100 transcription factors and 2,294 target genes. Similarly, preprocessing the Harbison et al. ChIP-Chip dataset left 158 transcription factors and 2,779 target genes. Using a two step optimization algorithm developed by Liao et al., we inferred the concentration profile for each transcription factor over the genetic gradient and compared it to the corresponding TF expression profile by computing Pearson’s correlations (r). Figure S3 shows that these

| Individual | Genotypes | Concentrations | Expressions |
|------------|-----------|----------------|-------------|
|            | SNP1  | SNP2  | SNP3  | TF1 | TF2 | G1 | G2 | G3 | G4 |
| 1          | A     | G     | A     | 4   | 1   | 8  | 2  | 12 | -2 |
| 2          | C     | G     | T     | 1   | 1   | 2  | 2  | -2 | -2 |
| 3          | C     | T     | T     | 1   | 1   | 2  | -6 | -2 | 6  |

A Genotypes, TF Concentrations and Gene Expressions

B Original NCA, no perturbations

C SNP1 perturbs the concentrations of transcription factor

D SNP2 perturbs the promoter affinities of transcription factors globally

E SNP3 perturbs the promoter affinities to a gene locally

Figure 1. Graphical illustration of NCA and extension of NCA to include genetic perturbations. (A) A small toy example of three individuals with known genotyping and expression levels and inferred concentrations of active transcription factors. Each row corresponds to the genotypes, gene expressions and inferred transcription factor concentrations collected in one individual. (B) NCA regulatory network model when the network is unperturbed and the expression levels of G1, G2, G3 and G4 are determined by the concentrations of TF1, TF2 and the corresponding promoter affinities. (C) Between individuals with the A allele (1) and C allele (2,3) at SNP1, the concentrations of TF1 is perturbed by SNP1 causing differential expression of G1 and G3. (D) Between individuals with the G allele (1,2) and T allele (3) at SNP2, the promoter affinities of TF2 are perturbed globally by SNP2 (i.e. edges from TF2 are perturbed) to cause differential expression in all of TF2’s targets G2, G3, and G4. (E) Between individuals with the A allele (1) and T allele (2,3) at SNP3, the affinities of TF1 and TF2 for the G3 promoter is perturbed locally by SNP3 to cause differential expression of G3.

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quantities were not well correlated with average correlation coefficients of $r^2 \approx 0.085$ and $r^2 \approx 0.077$ using the Lee et al. and Harbison et al. datasets respectively. The stability of the inferred TF concentrations were however robust when we compared results from the two ChIP-Chip datasets with a correlation coefficient of $r^2 \approx 0.472$ (Figure S4). The robustness was also verified by bootstrapping experiments [31] (Results not shown).

Identifying Regulatory Hotspots

We next applied our method to study the mechanisms by which regulatory hotspots, genomic locations in yeast shown to be linked to the expression of many genes, perturb the underlying transcription regulatory networks. Although several transcription factors have been known to act as master regulators in yeast, it has been surprisingly shown in previous eQTL studies that only a few regulatory hotspots are located close to transcription factors. We hypothesized that although complex regulatory mechanisms upstream of transcription regulation such as signaling pathways exist, transcription factors ultimately mediate the global regulation of gene expressions. Using our framework, we tested our hypothesis by determining whether a regulatory hotspot is linked to the concentrations or promoter affinities of active transcription factors to achieving this regulation.

To identify the regulatory hotspots, we performed simple linkage analysis on only a subset of genes that were NCA compliant (see Methods). Similar to previous reports, only a few hotspots were located cis to any known transcription factors [1,2]. For example, a hotspot located on chromosome 12 spanning basepairs 600,000 to 680,000 was cis to $HAP1$ while another hotspot located on chromosome 3 spanning basepairs 60,000 to 100,000 was cis to $LEU3$. Several approaches [20,23] have identified additional putative causal regulators, many of which are not transcription factors, corresponding to these regulatory hotspots.

Regulatory Hotspots Perturbed the Concentration of Active Transcription Factors To Cause Global Differential Expression

We first considered SNPs located in regulatory hotspots that perturbed the concentrations of active transcription factors to cause global differential expression. Extending the NCA model to incorporate SNPs as perturbations did not require changing the optimization procedure. As shown in Figure 2C, we first decomposed the inferred transcription factor concentration matrix from applying the original NCA algorithm, $C_{138}$, into two matrices $C_{z,138}$ and $C_{\{z,138}$ segregated by a SNP. Next, we identified those transcription factors whose concentrations were linked to the SNP using a simple $t$-test, an example is shown in bold in Figure 2C, and assessed the significance of the linkage by a permutation scheme (see Methods).
Using both the Harbison et al. and Lee et al. ChIP-Chip binding data, we found many transcription factors whose concentrations were linked to at least one SNP. Table 1 lists those linkages occurring at regulatory hotspots and the corresponding transcription factors. In addition to having a strong linkage, we also required the transcription factors in the table to have at least 6 (Lee et al) or 7 (Harbison et al) downstream targets whose expression levels were significantly linked to the regulatory hotspot. A number of transcription factors known to act as global regulators were identified. Of particular note, we found HAP1 to be the mediator of hotspot 6 located on chromosome 12, spanning basepairs 600,000 to 680,000 using the Harbison et al. dataset; and YAP1 and LEU3 to be mediators of hotspot 3 located on chromosome 3 spanning basepairs 60,000 to 100,000. Gcn4 was also identified as a mediator of this hotspot using the Lee et al. dataset but it was only marginally significant using the Harbison et al. dataset (Result not shown). These results are concordant with previous findings [2,23]. In particular, LEU2 has been previously implicated to be linked to hotspot 3 where an engineered deletion of the gene occurs. Figure 4 are heatmaps showing the strong correlations between concentration levels of transcription factors, HAP1 and LEU3 respectively, and the expression levels of their downstream targets linked to the respective regulatory hotspots.

We next examined hotspot 2, a hotspot that has been previously identified by brem et al.to regulate budding and daughter cell separation through the causal regulator AMN1 [9]. We identified four transcription factors, ACE2, MBP1, SKN7 and SWI4, whose active concentrations were significantly linked to hotspot 2 in both datasets. Five other transcription factors responsible for cell cycle transitions, ABF1, FKH1, OAF1, RAP1 and SWI5 were also found to be significant in the Harbison et al. dataset. Some of these transcription factors are known to interact with each other and have similar profiles such as ACE2 and SWI5; and MBP1, SKN7 and RAP1. Figure 3A and Figure 3B are heatmaps showing the strong correlation between the concentrations of transcription factors (ACE2 and SWI4) and the expression levels of their direct targets linked to the hotspot. Our results are consistent with previous findings that suggest ACE2 as a causal transcription factor mediating the global regulation of the mitotic-exit network (MEN) by AMN1 [23] even though ACE2’s direct targets were not overrepresented for any GO biological processes or functional groups. This is probably because many downstream transcripts of the MEN were not considered in this analysis because there’s no direct ChIP-Chip evidence of binding between these transcripts and ACE2.

Another interesting regulatory hotspot, occurring at chromosome 12 basepairs 1,040,000 to 1,060,000, was found by brem et al. to regulate subtelomERICally encoded helicases through the causal regulator SIR3. We found two transcription factors, GAT3 and YAP5, whose concentrations were linked to this hotspot using the Harbison et al. data. YAP5 was also significant using the Lee et al. data. Figure 3D and Figure 3C show the strong correlations between GAT3 and YAP5 concentrations and the expression profiles of their targets. Unlike the previous example, the targets of YAP5 were enriched for helicases (p < 4.099 × 10^{-4}) and consisted of many genes with unknown function as represented by a significant enrichment for the GO annotation of “biological process unknown” (p < 4.121 × 10^{-5}). These results suggest a potential novel mechanism for the regulation of subtelomERICally encoded helicases mediated by YAP5 and GAT3.

### Table 1. Regulatory hotspots and the transcription factors whose active concentrations are perturbed to achieve global regulation.

| Hotspot Location | # Linkages | Significant TFs |
|------------------|------------|-----------------|
| Chr Begin End    | Lee Habrison | Lee Harbison Shared |
| 1 2 360000 380000 | 24 29 | None None FHL1 |
| 2 2 480000 580000 | 103 142 | None None ABF1, FKH1, OAF1 RAP1, SWI5 |
| 3 3 60000 100000 | 89 113 | Gcn4, MCM1, MET4 MET32 LEU3, YAP1 |
| 4 5 340000 440000 | 34 48 | None SUT1 None |
| 5 8 80000 120000 | 36 51 | None None DIG1 |
| 6 12 60000 680000 | 54 91 | None HAP1 None |
| 7 12 1040000 1060000 | 8 12 | None GAT3 YAP5 |
| 8 13 40000 60000 | 20 27 | None None BAS1 |
| 9 14 44000 50000 | 130 179 | None None None |
| 10 15 140000 200000 | 76 117 | HAL9, RAP1, SWI5 FKH2, NDD1 None |
| 11 15 560000 580000 | 21 26 | None None HAP4 |

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We next considered SNPs located in regulatory hotspots that perturbed the promoter affinities of transcription factors to cause global differential expression. Modeling these perturbations required an extension to the NCA model. As shown in Figure 2D, in addition to decomposing the transcription factor concentration and gene expression matrices, we also decomposed the promoter affinities matrix, [PA] into [PA^+] and [PA^-] where the only difference between the two is the column corresponding to the global promoter affinities of the transcription factor of interest as shown in bold. We identified perturbed networks of genes and transcription factors by deriving a likelihood ratio statistic that compared the extended model to the original NCA model. Since the extended model included additional parameters, namely different promoter affinities between populations, we expected it to always fit the data better. Thus to assess significance,
we used a permutation scheme that randomized the decomposition of individuals while preserving the topology of the bipartite graph (see Methods).

We revisited the regulatory hotspots discussed in the previous section. We speculated that transcription factors whose promoter affinities were perturbed by a regulatory hotspot must interact with other transcription factors whose concentrations were perturbed by the same hotspot to induce global differential expression of the targets. The intuition being if the \textit{in vivo} concentrations of a transcription factor is relatively stable, then it could still regulate gene expression by differentially binding to other transcription factors to form a complex. A transcription factor’s binding affinity for promoters is then in part determined by the concentrations of its partnering transcription factors. This is exactly what we observed in

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{heatmap.png}
\caption{Correlations between concentrations of transcription factors and the expressions of their targets. Heatmap showing the correlations between concentrations of transcription factors and the expressions of their downstream targets linked to hotspot 2 on chromosome 2 (A) \textit{ACE2} and (B) \textit{SWI4} and hotspot 7 on chromosome 12 (C) \textit{YAP5} and (D) \textit{GAT3}). The bar above each heatmap designates the concentration profile of each transcription factor.}
\end{figure}
our results. For example, we found that hotspot 6 which was shown to be linked to the concentrations of HAP1 was also linked to the promoter affinities of HAP4. HAP1 and HAP4 are known to interact in a complex to regulate global respiratory gene expression. Similarly, hotspot 8 was linked to the concentrations of DIG1 and the promoter affinities of STE12. DIG1 has previously been shown to code for an inhibitor of STE12, a transcription factor involved in pheromone induction and invasive growth [32–34].

We next examined how two hotspots discussed in the previous section also perturbed promoter affinities of transcription factors. Figure 4 and Table 2 show that hotspot 2 was linked to the promoter affinities of ACE2, SWI4 and UME6. Hotspot 2 was also shown in the previous section to be linked to the concentrations of ACE2 and SWI4 but not UME6, see Figure S2 for the expression profiles of the downstream targets of UME6. Consistent with our speculation, UME6 has been shown to interact with SWI4 and SWI4 has been shown to interact with itself. Furthermore, we see that AMN1 is a target of ACE2 suggesting that the regulation of the mitotic-exit network might be feedback in nature.

Figure 4 also shows a similar network consisting of the two transcription factors whose concentrations linked to hotspot 7, GAT3 and YAP5. Notice that while YAP5’s promoter affinities were linked to the hotspot also (thick edges), GAT3’s were not (thin edges). Consistent with previous results, YAP5 has been shown to interact with itself to modulate gene expression. These results suggest that in some transcription factors, particularly those that interact with themselves, both promoter affinities and concentrations of the transcription factor could be perturbed by a regulatory

Figure 4. Networks perturbed by regulatory hotspots. Eleven hotspots and the networks of transcription factors and target genes perturbed. Large circular nodes represent transcription factors and square nodes represent target genes. The thickness of an edge represents how much a hotspot perturbs the promoter affinity. Red edges designate a change of a transcription factor from an activator to a repressor or vice versa. Notice that some perturbed networks share transcription factors. We show two hotspots and the corresponding networks in detail. Hotspot 2 in addition to affecting the promoter affinities of ACE2 and SWI4, also affects the promoter affinities of several other transcription factors, including UME6 which is known to interact with ACE2. Hotspot 7 affects the promoter affinities of YAP5 (thick edges) but its affect on GAT3 promoter affinities is not statistically significant (thin edges). Figure was generated using the Cytoscape software [42].

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Table 2. Regulatory hotspots and the transcription factors whose promoter affinities are perturbed to achieve global regulation.

| Chr | Begin | End   | # Linkages | Significant Transcription Factors | Lee     | Harbison | Shared |
|-----|-------|-------|------------|-----------------------------------|---------|----------|--------|
| 1   | 2     | 360000| 380000     | 24 29                             | None    | None     | None   |
| 2   | 2     | 480000| 580000     | 103 142                           | None    | SW4, ACE2, UME6 | None |
| 3   | 3     | 60000 | 100000     | 89 113                            | MET31   | AFB1     | None   |
| 4   | 5     | 340000| 440000     | 34 48                             | None    | None     | None   |
| 5   | 8     | 80000 | 120000     | 36 51                             | None    | STE12    | None   |
| 6   | 12    | 60000 | 680000     | 54 91                             | None    | HAP4     | None   |
| 7   | 12    | 104000| 106000     | 8 12                              | MSN4    | None     | YAP5   |
| 8   | 13    | 40000 | 60000      | 20 27                             | None    | None     | None   |
| 9   | 14    | 440000| 500000     | 130 179                           | ABF1    | None     | FKH1   |
| 10  | 15    | 140000| 200000     | 76 117                            | HAP1, SKN7 | SW4, CIN5 | None |
| 11  | 15    | 560000| 580000     | 21 26                             | None    | None     | None   |

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Most cis SNPs Perturbed the Local Promoter Affinities of Target Genes

Previous eQTL analyses have shown that the most significant linkages occur cis to genes [1,2] and often located or in LD with SNPs located in the promoter regions of genes harboring transcription factor binding sites [35]. Our model allowed us to determine if differences in expression of a single gene could be attributed to cis genetic variations perturbing the local affinities of transcription factors on the promoter.

There is a direct similarity between these perturbations and those that affect global promoter affinities. As shown in Figure 2E, SNP3 perturbs the local affinities of transcription factors for the promoter of G3. We modeled this affect by decomposing the [PA] matrix into [PA]+ and [PA]− where the only difference between the decomposed matrices was the row corresponding to G3, as shown in bold. We used a likelihood ratio statistic to choose between two different models and assessed the significance based on permuting the genotypes of the individuals.

Of the small subset of genes examined, 2294 from using the Lee et al. dataset and 2779 from using the Harbison et al. dataset, we found ≈45% of the transcripts (972/2294 Lee, 1315/2779 Harbison) linked to at least one SNP at a FDR of q < 0.05 with π0 = 1 using a standard t-test. Out of these linkages, ≈30% were cis (257/972 Lee, 331/1315 Harbison). These proportions are consistent with what has been reported [10].

We postulated that many cis linked loci found by previous analyses and confirmed by our analysis are in LD with causal SNPs located in promoter regions. We further postulated that such a causal SNP corresponds to a variation in the primary sequence of a transcription factor binding site that affects the promoter affinity of a transcription factor or a complex of transcription factors. This model is consistent with the idea that a genetic variation at regulatory regions of the genome can give rise to observed subtle differences in gene expression across populations. We identified a total of 138 and 174 genes which have their local promoter affinities affected by a SNP with a FDR of q < 0.05.

Figure 5A shows that there is high concordance between those genes with significant cis linkages and those whose promoter affinities were perturbed. We did not expect all cis linkages to perturb promoter affinities. There are potentially other regulatory machinery that operate on intronic 3′UTRs and 5′UTRs. Next we compared the perturbed genes found using the Lee et al. dataset versus those found using the Harbison et al. dataset (Figure 5B). At a FDR of q < 0.05, 72 significant genes were shared between the datasets and 168 genes were not. We suspected that the different results obtained from these two datasets can be attributed to differences in network topology. The two binding datasets often reported genes with different sets of bound transcription factors and transcription factors with different sets of targets making the estimates of certain quantities inconsistent. Additional discrepancies arose from different sets of genes having been eliminated from each analysis due to the criteria placed on the network topology.

Discussion

Although there is a growing wealth of literature identifying putative causal regulators in yeast and mouse using statistical approaches, some of which integrate different sources of information, it is not clear by what mechanism genetic variations perturb the underlying regulatory networks to give rise to global differential expression. We have presented an integrated framework based on network component analysis that directly models how genetic variations perturb the concentrations and promoter affinities of transcription factors to cause the differential expression of their targets. Such a model differs from current eQTL analyses in that a direct, testable mechanism of transcription regulation is specifically considered. Although these networks are limiting, both in terms of the amount of biology they explain as well as the dependence on experimental data for their inference, a substantial set of genes (~1/3) was still considered. In our analysis, we show that many genes with cis linkages are likely to be regulated by transcription factors binding differentially to their promoter regions. We also show two representative examples of the complex mechanism of achieving global differential expression of a large number of transcripts, where the regulation of transcription factors involve two distinct processes and maybe feedback in nature.

Our approach specifically uses one variation of the NCA algorithm to infer the concentrations and promoter affinities of...
transcription factors. The key aspect of our approach is that we treat genetic variations as perturbations to an underlying regulatory network whose structure is already known. In theory, any NCA like approach [36–38] where a network is inferred from known data such as ChIP-Chip experiments, protein-protein interaction experiments or literature can be extended to take into account genetic variation.

There are also some natural extensions to the framework we have presented. First, one is not limited to considering only genetic variation as a perturbation. Other forms of perturbation such as media condition and disease pathogenesis can as well be applied in this approach to identify the corresponding effect on the networks. Second, our method considers the perturbation of only one SNP. Although several approaches have been proposed to investigate the statistical interaction of multiple SNPs on a phenotype [11,39], it would be interesting to study the mechanistic interactions of multiple perturbations on a transcription regulatory network.

Methods

Strains, Expression Measurements, and Genotyping

We used the expression measurements (6,216 transcripts) and genotyping data (2,956 SNPs) collected over 112 segregants of yeast derived from two parental strains BY4716 and RM11-1a originally described by Brem et al. The gene expression data is available at GEO (http://www.ncbi.nlm.nih.gov/projects/geo/) with the accession number GSE1990.

Constructing Transcription Regulatory Networks from ChIP-Chip Data

ChIP-Chip data from two datasets [27,28] were used to generate two different transcription regulatory networks at a \( p \)-value cutoff of 0.001. Consistency was checked in each case by comparing the networks generated from using a \( p \)-value cutoff of 0.01 and 0.001.

We next checked for NCA compliance as outlined [31]. We were left with a sub-network of 2,294 transcripts and 100 transcription factors after processing the Lee et al. dataset and 2,779 transcripts and 158 transcription factors after processing the Harbison et al. dataset.

Computing Genetic Linkage and Identifying Regulatory Hotspots

We first performed a standard \( t \)-test to compare the population means between the segregated expression profiles of a single gene by a given SNP. We assessed the significance of our linkages by performing a permutation test as described [40].

We then identified regulatory hotspots by dividing the yeast genome into 493 20 kb bins and counted the number of significant \( \text{trans} \) linkages to unique gene expression levels each bin contained from the standard \( t \)-test. We found a total of 430 significant \( \text{trans} \) linkages using the Harbison et al. data and 290 using the Lee et al. data. Assuming a Poisson process where the rare event of a \( \text{trans} \) linkage occurs at a rate of 0.87 (430/493 Harbison) and 0.60 (290/493 Lee), the probability of observing \( \geq 7 \) linkages in the largest bin using the harbison_transcriptional_2004 data is \( p < 0.02 \) and the probability of observing \( > 6 \) linkages in the largest bin using the Lee et al. data is \( p < 0.02 \). Because of the differences in the set of genes used in the different datasets, we constructed a set of 11 hotspots shared between the two.

Application of NCA to Gene Expression Data Collected over a Population

NCA was originally developed to analyze time series based gene expression data but can be easily adapted to analyze whole

Figure 5. Concordances between applying different statistical tests and using different protein-DNA binding datasets. (A) Percent of top genes with promoter affinities perturbed detected by likelihood ratio test concordant with those with \( \text{cis} \) linkages detected by \( t \)-test (red: Harbison dataset, blue: Lee dataset, gray: random). (B) Percent of top genes concordant between Lee and Harbison datasets using different tests (red: \( t \)-test for \( \text{cis} \) linkages, blue: likelihood ratio test for perturbed promoter affinities, gray: random).

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expression levels of the target genes. This inference is made given a matrix \( E \) where we have decomposed \([E^+] \) into \([E^+]\) and \([E^-]\), and \([C]\) into \([C^+]\) and \([C^-]\) representing the expression levels and the inferred concentrations of transcription factors in two different populations segregated by a genetic variation. \([PA^+]\) and \([PA^-]\) are the corresponding promoter affinity matrices of the two populations. \( \Gamma \) is again the residual.

Computing the linkage between transcription factor concentrations and genetic variations. If a genetic variation affects the concentrations of the transcription factors to induce differential expression, we can model the effect by decomposing the originally inferred \([C]\) matrix into \([C^+]\) and \([C^-]\) that differ in the row corresponding to the transcription of interest. For each transcription factor, we can then apply a simple \( t \)-test treating the concentration as a quantitative trait segregated by the genetic variation. We assess the significance of the statistic by shuffling the genotypes of the individuals 1000 times [40] and computing the false discovery rate (FDR) [41].

Computing the linkage between promoter activities and genetic variation using a likelihood ratio based statistic. Notice that if a genetic variation perturbs the promoter affinities either globally or locally, we can simply compare the \([PA^+]\) and \([PA^-]\) matrices. Instead, we can use model selection techniques to compare our more complex model with the simpler NCA model. Specifically, we define the optimization problem similar to Equation 3:

\[
\min \| [E - [PA][C]] \|^2
\]

We can approximate the solution to Equation 5 by running the original NCA algorithm and fixing the \([C]\) matrix and re-estimating the \([PA]\) matrix.

To test the validity of our model, we define the null and alternative hypotheses corresponding to the two models as:

Hypothesis \( H_1 \): The expression levels \([E]\) can be decomposed into \([E^+]\) and \([E^-]\) for those individuals with the major and minor alleles respectively, and approximated by a log-linear models characterized by the parameters \( \Theta \):

- \([PA^+]\): A \( N \times L \) matrix representing the promoter affinities of transcription factors in individuals with the major allele
- \([PA^-]\): A \( N \times L \) matrix representing the promoter affinities of transcription factors in individuals with the minor allele
- \([C]\): A \( L \times M \) matrix that can be decomposed into \([C^+]\) and \([C^-]\) representing the concentrations of active transcription factors in the \( M^+ \) individuals with the major allele and \( M^- \) individuals with the minor allele respectively.

Hypothesis \( H_0 \): The expression levels \([E]\) can be approximated by a log-linear model characterized by the parameters \( \Theta_0 \):

\( [PA]: A N \times L \) matrix representing the promoter affinities of transcription factors in all individuals (i.e., \([PA^+] = [PA^-]\)).
[C]: A \(L \times M\) matrix representing the concentrations of transcription factors in all individuals.

When a genetic variation perturbs the promoter affinities to one gene locally, the difference in the number of parameters is equal to the number of regulators of the target gene. If we are re-estimating the promoter affinities globally for one transcription factor, the difference in the number of parameters is equal to the number of targets of the transcription factor. In both cases, we can compare our alternative model against the null model using a likelihood ratio statistic remembering that the \(e_i\)s are independent.

\[
-2 \log \Lambda(E) = 2\sup\{\{PA, C, \Gamma|E\}\} - 2\sup\{\{PA^+, C_, \Gamma^+|E\}\}
\]

(6)

\[
\approx \frac{RSS_0 - RSS_1}{\sigma^2}
\]

(7)

\[
= \log \sum_{ij} \hat{e}_{0ij}^2 - \log \sum_{ij} \hat{e}_{1ij}^2
\]

(8)

where \(RSS_0 = \sum_{ij} \hat{e}_{0ij}^2\) and \(RSS_1 = \sum_{ij} \hat{e}_{1ij}^2\) are the residual sum of squares from solving the least squares equations for \(H_0\) and \(H_1\) respectively. We estimate the two error variances \(\sigma_0^2\) and \(\sigma_1^2\) from the residual sum of squares of the larger model:

\[
\sigma^2 = \frac{RSS_1}{n - df_1}
\]

(9)

where \(df_1\) is the degrees of freedom of the model.

The above statistic follows the \(\chi^2\) distribution asymptotically. However, since we are not re-estimating the full model in our extension, we perform permutations by rearranging the genotype labels of the individuals [40] 1000 times. We further estimated the significance of the permuted \(p\)-values by computing the false discovery rate [41].

**References**

1. Brem RB, Kruglyak L (2005) The landscape of genetic complexity across 5,700 gene expression traits in yeast. Proc Natl Acad Sci U S A 102(3): 1572–1577.
2. Brem RB, Yvert G, Clinton R, Kruglyak L (2002) Genetic dissection of transcriptional regulation in budding yeast. Science 296(5568): 752–755.
3. Keurentjes JJ, Fu J, Terpstra IR, Garcia JM, van den Ackerveken G, et al. (2007) Regulatory network construction in arabidopsis by using genomewide gene expression quantitative trait loci. Proc Natl Acad Sci U S A 104: 1708–1713.
4. Bystrynk L, Weissing E, Donije B, Sutton S, Petchor MT, et al. (2005) Uncovering regulatory pathways that affect hematopoietic stem cell function using ‘genetical genomics’. Nat Genet 37(3): 225–232.
5. Chester EJ, Lu L, Shou S, Qu Y, Lu J, et al. (2005) Complex trait analysis of gene expression uncovers polygenic and pleiotropic networks that modulate nervous system function. Nat Genet 37(3): 233–243.
6. Cheung VG, Spielman RS, Esens KG, Weber TM, Morley M, et al. (2005) Mapping determinants of human gene expression by regional and genome-wide association. Nature 437: 1365–1369.
7. Stranger BE, Forrest MS, Dunning M, Ingle CE, Beazley C, et al. (2007) Relative impact of nucleotide and copy number variation on gene expression phenotypes. Science 315: 848–853.
8. Stranger BE, Nica AC, Forrest MS, Dimas A, Bird CP, et al. (2007) Relative impact of nucleotide and copy number variation on gene expression phenotypes. Science 315: 848–853.
9. Yvert G, Brem RB, Whitle J, Akey JM, Foss E, et al. (2003) Transcript regulatory variation in Saccharomyces cerevisiae and the role of transcription factors. Nat Genet 35(1): 57–64.
10. Brem RB, Storey JD, Whitle J, Kruglyak L (2003) Genetic interactions between polymorphisms that affect gene expression in yeast. Nature 436(7051): 701–703.
11. Storey JD, Akey JM, Kruglyak L (2005) Multiple locus linkage analysis of genomewide expression in yeast. PLoS Biol 3: e267. doi:10.1371/journal.phb.0030267.

**Supporting Information**

**Figure S1** Heatmaps showing correlations between concentrations of known transcription factors and the expressions of their targets. This figure shows heatmaps of the concentration levels of (A) HAPI and (B) LEU3, two transcription factors known to mediate global regulation, correlated with the expression levels of their downstream targets. Found at: doi:10.1371/journal.pcbi.1000311.s001 (0.40 MB TIF)

**Figure S2** Heatmap showing lack of correlation between UME6 concentrations and the expressions of its targets. This figure shows that UME6's concentrations are not perturbed by regulatory hotspot 2 but the expression levels of its targets are. Found at: doi:10.1371/journal.pcbi.1000311.s002 (0.58 MB TIF)

**Figure S3** Heatmaps showing correlation between transcription factor concentrations and expression levels. The heatmaps show the correlation between expression levels and concentrations of transcription factors for (A) 158 transcription factors in the Harbison dataset and (B) 100 transcription factors in the Lee dataset. Found at: doi:10.1371/journal.pcbi.1000311.s003 (0.68 MB TIF)

**Figure S4** Heatmaps showing correlation of transcription factor concentrations between two datasets. A heatmap that shows the correlation of inferred transcription factor concentrations between the Harbison and Lee datasets. Found at: doi:10.1371/journal.pcbi.1000311.s004 (0.68 MB TIF)

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**Author Contributions**

Conceived and designed the experiments: CY SJG EE. Performed the experiments: CY. Analyzed the data: CY. Contributed reagents/materials/analysis tools: CY SJG JCL. Wrote the paper: CY SJG JCL EE.

12. Efron B, Tibshirani R (2007) On testing the significance of sets of genes. Ann Stat 1: 107–129.
13. Mootha VK, Lindgren CM, Eriksson K, Subramanian A, Sihag S, et al. (2003) PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 34(5): 267–273.
14. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, et al. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 102: 15545–15550.
15. Tian L, Greenberg SA, Kong SW, Allschuler J, Kohane IS, et al. (2005) Discovering statistically significant pathways in expression profiling studies. Proc Natl Acad Sci U S A 102: 13444–13449.
16. Ye C, Ekin E (2007) Discovering tightly regulated and differentially expressed gene sets in whole genome expression data. Bioinformatics 23: e84–e90.
17. Ghazalpour A, Doss S, Zhang B, Wang S, Plaisier C, et al. (2006) Integrating genetic and network analysis to characterize genes related to mouse weight. PLoS Genet 2(8): e130. doi:10.1371/journal.pgen.0020130.
18. Lee S, Pe’er D, Dudley AM, Church GM, Koller D (2006) Identifying regulatory mechanisms using individual variation reveals key role for chromatin modification. Proc Natl Acad Sci U S A 103: 14602–14607.
19. Bing N, Hoeschele I (2005) Genetical genomics analysis of a yeast segregant population for transcription network inference. Genetics 170(2): 533–542.
20. Chen L, Emmert-Streib F, Storey J (2007) Harnessing naturally randomized transcription to infer regulatory relationships among genes. Genome Biol 8(10): R219.
21. Kulip D, Jagalur M (2006) Causal inference of regulator-target pairs by gene mapping of expression phenotypes. BMC Genomics 7: 123.
22. Li R, Tsaih S, Shockley K, Stylianou IM, Wergedal J, et al. (2006) Structural model analysis of multiple quantitative traits. PLoS Genet 2: e114. doi:10.1371/journal.pgen.0020114.
24. Rustici G, Mata J, Kivinen K, Lio P, Pinkert CJ, et al. (2004) Periodic gene expression program of the fission yeast cell cycle. Nat Genet 36: 699–717.

25. Vliegell MM, Bos R, Buerger H, van der Groep P, Saramaki OR, et al. (2004) No amplifications of hypoxia-inducible factor-1α gene in invasive breast cancer: a tissue microarray study. Cell Oncol 26: 347–351.

26. Liao JC, Boscolo R, Yang Y, Tran LM, Sabatti C, et al. (2003) Network component analysis: reconstruction of regulatory signals in biological systems. Proc Natl Acad Sci U S A 100(26): 15522–15527.

27. Harbison CT, Gordon DB, Lee TI, Rinaldi NJ, Macisaac KD, et al. (2004) Transcriptional regulatory code of a eukaryotic genome. Nature 431(7004): 91–100.

28. Lee TI, Rinaldi NJ, Robert F, Odom DT, Bar-Joseph Z, et al. (2002) Transcriptional regulatory networks in Saccharomyces cerevisiae. Science 298(5594): 799–804.

29. Tran L, Brynildsen M, Kao K, Suen J, Liao J (2005) gNCA: a framework for determining transcription factor activity based on transcriptome: identifiability and numerical implementation. Metab Eng 7: 128–141.

30. Ronen M, Rosenberg R, Shraiman BI, Alon U (2002) Assigning numbers to the arrows: parameterizing a gene regulation network by using accurate expression kinetics. Proc Natl Acad Sci U S A 99: 10555–10560.

31. Galbraith SJ, Tran LM, Liao JC (2006) Transcriptome network component analysis with limited microarray data. Bioinformatics 22(13): 1896–1894.

32. Bardwell L, Cook KG, Zhu-Shimoni JX, Voora D, Thorner J (1998) Differential regulation of transcription: repression by unactivated mitogen-activated protein kinase ksl1 requires the dig1 and dig2 proteins. Proc Natl Acad Sci U S A 95(26): 15460–15465.

33. Smith EN, Kruglyak L (2008) Gene–environment interaction in yeast gene expression. PLoS Biol 6(4): e1000083. doi:10.1371/journal.pbio.0060083.

34. Tedford K, Kim S, Sa D, Stevens K, Tyers M (1997) Regulation of the mating pheromone and invasive growth responses in yeast by two MAP kinase substrates. Curr Biol 7(4): 228–238.

35. GuhaThakurta D, Xie T, Anand M, Edwards S, Li G, et al. (2006) Cis-regulatory variations: a study of SNPs around genes showing cis-linkage in segregating mouse populations. BMC Genomics 7: 235.

36. Bouzdeau A, Strimmer K (2005) Predicting transcription factor activities from combined analysis of microarray and ChIP data: a partial least squares approach. Theor Biol Med Model 2(1): 23.

37. Gao F, Foo B, Bussemaker H (2004) Defining transcriptional networks through integrative modeling of mRNA expression and transcription factor binding data. BMC Bioinformatics 5(1): 51.

38. Sabatti C, James G (2006) Bayesian sparse hidden components analysis for transcription regulation networks. Bioinformatics 22: 739–746.

39. Zapala MA, Schork NJ (2006) Multivariate regression analysis of distance matrices for testing associations between gene expression patterns and related variables. Proc Natl Acad Sci U S A 103(51): 19430–19435.

40. Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138(3): 963–971.

41. Storey JD, Tibshirani R (2005) Statistical significance for genomewide studies. Proc Natl Acad Sci U S A 100(2): 9440–9445.

42. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, et al. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13(11): 2498–2504.