A critical step in understanding how a genome functions is determining which transcription factors (TFs) regulate each gene. Accordingly, extensive effort has been devoted to mapping TF networks. In *Saccharomyces cerevisiae*, protein–DNA interactions have been identified for most TFs by ChIP-chip, and expression profiling has been done on strains deleted for most TFs. These studies revealed that there is little overlap between the genes whose promoters are bound by a TF and those whose expression changes when the TF is deleted, leaving us without a definitive TF network for any eukaryote and without an efficient method for mapping functional TF networks. This paper describes NetProphet, a novel algorithm that improves the efficiency of network mapping from gene expression data. NetProphet exploits a fundamental observation about the nature of TF networks: The response to disrupting or overexpressing a TF is strongest on its direct targets and dissipates rapidly as it propagates through the network. Using *S. cerevisiae* data, we show that NetProphet can predict thousands of direct, functional regulatory interactions, using only gene expression data. The targets that NetProphet predicts for a TF are at least as likely to have sites matching the TF's binding specificity as the targets implicated by ChIP. Unlike most ChIP targets, the NetProphet targets also show evidence of functional regulation. This suggests a surprising conclusion: The best way to begin mapping direct, functional TF-promoter interactions may not be by measuring binding. We also show that NetProphet yields new insights into the functions of several yeast TFs, including a well-studied TF, Cbf1, and a completely unstudied TF, Edf1.

[Supplemental material is available for this article.]
case, coexpression analysis can only make predictions for TFs whose expression varies sufficiently in the available expression profiles. Algorithms that exploit DE typically construct networks with regulatory edges from each TF to all the genes that are differentially expressed when the TF is deleted (Hu et al. 2007; Pinna et al. 2010), overexpressed, or otherwise perturbed. Some edges in this network reflect direct binding of the TF to the target (the intended result), while others reflect indirect effects of perturbing the TF (which are considered false positives). In order to reduce the number of edges that reflect indirect effects, edges are often removed until there is only one path from each TF to each target that is affected by perturbing the TF.

Coexpression and DE have complementary strengths and weaknesses. Coexpression can identify targets of TFs that have not been individually perturbed. This greatly increases the range of expression profiling data that can be used and opens up the possibility of analyzing all TFs, regardless of the data set. DE creates variation in the expression of a TF by direct experimental intervention, rather than relying on chance, and it does not risk confusing correlation with causation.

NetProphet capitalizes on the complementarity of the coexpression and DE strategies by combining them. The coexpression component enables NetProphet to exploit any expression data source, including environmental perturbations that affect many TFs simultaneously, and to predict the targets of TFs that have not been individually perturbed in the available expression profiles. The DE component enables NetProphet to exploit the power of targeted TF perturbations (see Results). For every TF and every possible target gene, NetProphet computes a confidence score that combines a number representing its coexpression analysis with a number representing its DE analysis. The coexpression number is a LASSO regression coefficient reflecting the degree to which coexpression patterns allow the level of the putative target to be predicted from that of the TF. This is similar to the calculation done by Inferelator (Bonneau et al. 2006), a method that has performed very well in DREAM assessments (Greenfield et al. 2010; Madar et al. 2010) and that we compare to NetProphet below (see Methods for differences). The DE number is the log odds that the putative target is differentially expressed when the TF is perturbed, given the available replicate expression profiles (see Methods). NetProphet then ranks all possible TF-target interactions by a confidence score that is, roughly speaking, a weighted sum of the coexpression number, the DE number, and their product. This method of integrating coexpression and DE is both simple and, empirically, very effective.

A surprising insight that we gained from these studies is that the most efficient way to start mapping a network of functional TF-DNA interactions may not be by measuring binding directly with methods like ChIP. Our results suggest that an expression-based approach like NetProphet can identify thousands of direct TF-DNA interactions with accuracy at least as good as that of existing ChIP-chip data. Whereas ChIP-chip reveals nonfunctional as well as functional binding, the NetProphet approach reveals only functional binding. Furthermore, the NetProphet approach is easier to scale up than in vivo binding experiments, and it requires less specialized expertise. Binding studies are a valuable complement that make the map more complete, but the most efficient path to a network map may start with the NetProphet approach.

Another surprising finding from this study is that the strength of evidence supporting a gene’s response to perturbation of a TF is a good predictor of whether the gene is a direct target of the TF (see Methods). This reveals something about the fundamental nature of signal propagation in TF networks: The response to a TF perturbation tends to dissipate rapidly. This rapid dissipation of the effects of variations in TF level may help explain how cells tolerate noise in the expression of TFs.

Results

We ran NetProphet using a comprehensive collection of microarray expression profiling data on yeast TF deletion mutants (Hu et al. 2007) and analyzed the results as described below (the complete NetProphet output can be found in Supplemental Table S1).

Top NetProphet predictions identify direct binding potential better than ChIP data does

The top 4000 regulatory links predicted by NetProphet comprise 219 distinct TFs regulating 1744 distinct targets. We evaluated these links in a two-stage process. First, we evaluated the potential of each TF to bind each of its predicted targets by using a position weight matrix (PWM) model of its binding specificity. Second, we made the same calculation for all regulatory links implicated by ChIP experiments, regardless of NetProphet score. We then set high-, medium-, and low-stringency thresholds for support by each PWM. At each stringency level, the percentage of ChIP-implicated targets supported by PWMs serves to calibrate the NetProphet results, allowing us to determine what should count as a good outcome for NetProphet. Of the top 4000 regulatory links predicted by NetProphet, 1408 involved a regulator that had been studied by ChIP-chip or ChIP-seq (Lee et al. 2002; Harbison et al. 2004; Balaji et al. 2006; Abdulrehman et al. 2011) and for which a PWM was available in the UNIPROBE database (Gordan et al. 2011; Robasky and Bulyk 2011). These PWMs are based on in vitro data obtained using protein-binding microarrays (PBMs), so they are not influenced by either gene expression data or ChIP data. We calculated the percentage of these predictions that are supported by high binding potential using each of the three stringency levels for PWM support (Fig. 1). A TF-target relationship was counted as
been subjected to ChIP.

adds value for predicting direct targets of TFs that have already
ChIP hits in general (Fig. 1), indicating that NetProphet analysis
binding directly. ChIP hits that are also NetProphet predictions are
only gene expression data and knows nothing about binding,
plemental Fig. S2). This is remarkable, given that NetProphet uses
richment for both PWM support and ChIP support in all blocks of
significant enrichment in either PWM-supported interactions or
better than chance, we tested each block of 4000 predictions for
To determine the lowest rank at which NetProphet predictions are
The top 40,000 NetProphet predictions are enriched for PWM
and ChIP support.

NetProphet rank is predictive of support by binding potential
and by ChIP results

We wanted to investigate the degree to which the rank assigned
to a potential TF-target link by NetProphet corresponds to its
likelihood of being supported by independent evidence. Thus,
we calculated PWM support and ChIP support as a function of
NetProphet rank. Using the high-stringency threshold for PWM
support, we calculated the fraction of predictions supported for
the top 4000 NetProphet predictions, the next 4000, and so on
(Fig. 2A, solid green line). These are incremental evaluations
of predictions in a given range of NetProphet ranks, not cumulative
evaluations of all predictions above a given rank. There is a clear
trend in Figure 2A: potential links that are ranked more highly by
NetProphet are more likely to be supported by PWM evidence.
The analysis of ChIP support as a function of NetProphet rank
shows the same trend (Fig. 2B). Thus, NetProphet rank is a good
predictor of PWM and ChIP support.

The top 40,000 NetProphet predictions are enriched for PWM
and ChIP support

To determine the lowest rank at which NetProphet predictions are
better than chance, we tested each block of 4000 predictions for
significant enrichment in either PWM-supported interactions or
ChIP-supported interactions. The results showed significant en-
richment for both PWM support and ChIP support in all blocks of
edges down to rank 40,000, below which we did not test (Fig. 2).

For TFs that have been subjected to both ChIP and PBM studies, 9%
of the targets in the top 40,000 NetProphet predictions are sup-
ported by PBM-derived PWMs. For the same TFs, 9% of the targets
in the top 29,946 ChIP-implicated interactions are supported by
PBM-derived PWMs. Thus, the complete NetProphet network may
contain as many direct interactions as the complete ChIP-implic-
cated network.

Additional data improves lower ranked predictions

Previous studies have shown that TF overexpression data can be
complimentary to deletion data and can help to identify direct
targets (Chua et al. 2006; Sopko et al. 2006), so we added profiling
data from cells grown in YPGal and overexpressing each of 55 TFs
(Chua et al. 2006). Since many genes are only expressed in stress
conditions, we also added profiling data from time courses of re-
sponses to multiple stressors (Gasch et al. 2000). Combined, these
additional data sets resulted in a marginal improvement in the
fraction of the top 4000 predictions supported by PWMs and ChIP
(Supplemental Fig. S3). Predictions ranked 24,000–40,000 showed
the greatest improvement.

To investigate the effects of having smaller data sets, we ran
NetProphet on subsamples of the data from Hu et al. (2007), com-
prising expression profiles from deletions of one-fourth, one-half,
or three-fourths of the TFs. For each input data set, we considered
TFs whose deletion profiles were or were not included in the data
separately (Supplemental Methods). For TFs whose deletion profiles
were included in the input data, adding more data comprising
deletion profiles for other TFs did not improve their predicted tar-
gets (not shown). For TFs whose deletion profiles were not included
in the input data, target prediction was less accurate overall but
adding more data improved prediction accuracy (as assessed by
PWM support) at all NetProphet ranks (Supplemental Fig. S4); ChIP
support did not change (data not shown).

To gain further insight into the interplay between data-set size
and NetProphet rank, we evaluated NetProphet’s LASSO regression
and DE components separately. The results showed that DE per-
forms substantially better than LASSO in terms of its top 4000 picks,
slightly better on ranks 4001–20,000 (at least by PWM support),
and about the same on 20,001–40,000 (Supplemental Fig. S5). By
combining DE and LASSO. NetProphet performs somewhat better than DE on the top 4000 and on 20,001–40,000, and about the same as DE on 4001–20,000. Thus, LASSO regression enhances NetProphet accuracy in some rank ranges more than others, and the extent of its contribution depends on how much data is available.

**NetProphet rank predicts PWM and ChIP support better than other methods tested**

We compared the top 4000 NetProphet predictions to the top 4000 predictions from two other publicly available methods that have won DREAM competitions, Inferelator (Bonneau et al. 2006; Greenfield et al. 2010; Madar et al. 2010) and Genie3 (Huynh-Thu et al. 2010). The metrics used were percentage of interactions supported by high-stringency PWM evidence, ChIP evidence, or both (Fig. 3A). The NetProphet predictions received substantially more support by all three metrics. To determine whether this good performance was broadly distributed across TFs, we considered the five most highly ranked targets of each TF and asked whether at least one of the five was supported by PWM evidence, ChIP evidence, or both. NetProphet was able to identify at least one correct target for many more TFs than either of the other methods tested (Fig. 3B). We found similar results when comparing NetProphet to Inferelator and Genie3 on simulated data from the DREAM4 evaluation (Supplemental Fig. S6).

**NetProphet predictions identify novel TF functions that cannot be found by ChIP**

After we validated NetProphet by both PWM and ChIP support, we used it to predict TF functions that could not be identified using existing ChIP data. For each TF, we performed Gene Ontology (GO) enrichment analysis on all its targets in the top 10,000 interactions ranked by NetProphet (see Methods and Supplemental Table S2). We then removed any GO terms that were enriched among the targets implicated by existing ChIP data. Since there can be many overlapping GO terms for a gene set, some of which are extremely broad, we chose a single, specific GO term to represent sets of redundant terms for the same genes. The results include 44 functional annotations on 42 TFs.

Of the 44 functional annotations that could not be derived from existing ChIP data, 29 (66%) were supported in the literature by non-ChIP evidence, such as mutant phenotypes and protein-protein interactions (Supplemental Table S3). Examples include involvement of *GCR1* and *GCR2* in regulating hexose catabolism (Clifton et al. 1978), *DIG1* in pheromone response (Tedford et al. 1997), *PHO2* in polyphosphate metabolism and dephosphorylation (Ogawa et al. 2000), and *SIP1* in glycolysis (Cipollina et al. 2008). In two cases, we could find evidence supporting the function in other species but not in *S. cerevisiae* (Table 1). For example, NetProphet predicts that *RIM101* regulates iron metabolism in *cerevisiae*. The orthologous TF in *Cryptococcus neoformans*, a pathogenic fungus of phylum Basidiomycota, is known to regulate iron metabolism (O’Meara et al. 2010). Thirteen of the functional predictions are completely novel (Table 1), including regulation of cytokinesis by *HAP2*, *HAP4*, and *HSF1*, response to nitrogen starvation by *SNF6*, and lysine biosynthesis by *EDS1* (discussed below).

**NetProphet identifies a novel biological function for Cbf1**

NetProphet predicts that centromere binding factor 1 (Cbf1) activates genes involved in phosphate acquisition, including all three repressible acid phosphatases (*PHO5*, *PHO11*, *PHO12*) and a repressible alkaline phosphatase (*PHO8*). Cbf1 has long been known to regulate sulfate metabolism (Kuras et al. 1996) but not phosphate metabolism, and the Cbf1 ChIP studies in the curated databases used for our computational analyses detected no significant binding of Cbf1 to these phosphatase promoters. Recently, it was found that Cbf1 binds the promoters of genes in the Pho4 regulon and regulates their expression (Zhou and O’Shea 2011). However, Zhou and O’Shea found that Cbf1 represses acid phosphatases while NetProphet predicts that it activates them. Following up on this apparent discrepancy, we noticed that Zhou and O’Shea’s experiments involved cultures grown in synthetic complete medium with 10 mM inorganic phosphate (SC+10 mM Pi), whereas the NetProphet predictions were based on microarray data from cultures grown in YPD (Hu et al. 2007). YPD has ample organic phosphate that can be liberated by phosphatases but relatively little free (i.e., inorganic) phosphate. To validate the predicted novel function of Cbf1 as an activator of phosphatases, we assayed the expression of acid phosphatases from cultures of the same strains used by Zhou and O’Shea, grown in either SC+10 mM Pi or YPD (see Methods). The results showed that Cbf1 is a repressor of acid phosphatases in SC+10 mM Pi, as reported by Zhou and O’Shea (2011), but an activator of acid phosphatases in YPD, as predicted by NetProphet (Fig. 4). Thus, rather than simply serving as a repressor, Cbf1 serves to amplify the effect of medium on expression of phosphatases. The activating role of Cbf1 explains the fact that the Cbf1 deletion mutant grows slowly in conditions that derepress phosphatases but not in conditions that repress...
Table 1. Novel TF functions predicted by NetProphet but not by existing ChiP data

<table>
<thead>
<tr>
<th>Name</th>
<th>NP #</th>
<th>GO #</th>
<th>Overlap</th>
<th>P-value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDS1</td>
<td>18</td>
<td>10</td>
<td>7</td>
<td>$1 \times 10^{-16}$</td>
<td>Lysine biosynthesis</td>
</tr>
<tr>
<td>CS76</td>
<td>595</td>
<td>167</td>
<td>49</td>
<td>$1 \times 10^{-11}$</td>
<td>Cytoplasmic translation</td>
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<tr>
<td>STL1</td>
<td>18</td>
<td>9</td>
<td>4</td>
<td>$1 \times 10^{-8}$</td>
<td>Siderophore transport</td>
</tr>
<tr>
<td>SWI4</td>
<td>40</td>
<td>250</td>
<td>11</td>
<td>$9 \times 10^{-7}$</td>
<td>Cell wall organization/biogenesis</td>
</tr>
<tr>
<td>RIM101</td>
<td>62</td>
<td>80</td>
<td>8</td>
<td>$2 \times 10^{-6}$</td>
<td>Metal ion transport</td>
</tr>
<tr>
<td>SNF6</td>
<td>351</td>
<td>6</td>
<td>5</td>
<td>$6 \times 10^{-6}$</td>
<td>Cellular response to nitrogen starvation</td>
</tr>
<tr>
<td>HSFI1</td>
<td>66</td>
<td>11</td>
<td>4</td>
<td>$6 \times 10^{-5}$</td>
<td>Cytokinesis, completion of separation</td>
</tr>
<tr>
<td>STP1</td>
<td>27</td>
<td>80</td>
<td>5</td>
<td>$3 \times 10^{-5}$</td>
<td>Metal ion transport</td>
</tr>
<tr>
<td>SNF2</td>
<td>245</td>
<td>6</td>
<td>4</td>
<td>$5 \times 10^{-5}$</td>
<td>Cellular response to nitrogen starvation</td>
</tr>
<tr>
<td>RPN4</td>
<td>60</td>
<td>98</td>
<td>7</td>
<td>$8 \times 10^{-5}$</td>
<td>Response to pheromone</td>
</tr>
<tr>
<td>SIN4</td>
<td>505</td>
<td>34</td>
<td>11</td>
<td>$1 \times 10^{-4}$</td>
<td>Glycolysis</td>
</tr>
<tr>
<td>HAP2</td>
<td>67</td>
<td>114</td>
<td>7</td>
<td>$4 \times 10^{-4}$</td>
<td>Cytokinesis</td>
</tr>
<tr>
<td>ASH1</td>
<td>9</td>
<td>98</td>
<td>3</td>
<td>$4 \times 10^{-4}$</td>
<td>Response to pheromone</td>
</tr>
<tr>
<td>AFT1</td>
<td>112</td>
<td>8</td>
<td>3</td>
<td>$4 \times 10^{-3}$</td>
<td>Lysine biosynthesis</td>
</tr>
<tr>
<td>HAP4</td>
<td>18</td>
<td>11</td>
<td>2</td>
<td>$5 \times 10^{-3}$</td>
<td>Cytokinesis, completion of separation</td>
</tr>
</tbody>
</table>

(NP #) Number of targets predicted by NetProphet; (GO #) number of yeast genes in the indicated Gene Ontology (GO) Biological Process category; (Overlap) number of genes in both the NetProphet target set and the GO category; (P-value) probability of such a large overlap occurring by chance; (Description) description of the GO category.

Figure 4. Effect of Cbf1 on expression of acetyl phosphatases in cells grown in synthetic complete medium with 10 mM inorganic phosphate (red) or on cells grown in YPD (blue). (A) Expression of PHO5. (B) Combined expression of PHO11 and PHO12, which have much sequence similarity that we were not able to distinguish their transcripts. Error bars representing one standard error of the mean of two technical replicates were too small to be seen in the figure.
2. Most of the TF binding sites revealed by in vivo binding experiments show no evidence of affecting transcription rates. Even after binding experiments have been carried out, TF perturbation and expression profiling are required for determining which of the genes that are bound by a TF are also regulated by it. This does not mean that only direct targets are differentially expressed or even that the majority of the differentially expressed targets are differentially expressed or even that the majority of the differentially expressed genes are direct targets. Indeed, the typical approach to DE analysis—setting a threshold for significance and treating all significant targets as equal—is not very useful for network mapping. However, the direct targets show a stronger differential expression effect, on average, than indirect targets. Because it exploits the strength of differential expression, NetProphet does not need an explicit mechanism for dealing with the fact that many indirect targets are differentially expressed at statistically significant levels. Algorithms based on a strength-of-DE ranking have been tried on simulated data (Greenfield et al. 2010; Pinna et al. 2010; Yip et al. 2010), but the studies presented here are the first to demonstrate that this approach is useful for inferring real TF networks from real gene expression data.

3. Whereas binding data contain limited information about functional regulation, expression data carry substantial information about binding. For example, the top 4000 interactions predicted by NetProphet were supported by PWM models of binding specificity more often than the interactions indicated by significant ChIP hits were (Fig. 1). Even the top 40,000, taken as a group, were supported by PWM models at the same rate as the complete set of just under 30,000 ChIP-supported interactions in the TNET and YEASTRACT databases.

Building a more comprehensive network

Once an initial map has been built by the NetProphet approach, binding studies can be used to make it more comprehensive. Several algorithms have been developed for inferring networks by combining multiple data types. For example, Marbach et al. (2012) and Beyer et al. (2006) both combined coexpression data with in vivo binding data from ChIP, evolutionary conservation, and PWM models of binding specificity. The latter can be derived by several experimental methods, including protein binding arrays (Zhu et al. 2009), Selex (Jolma et al. 2010), and yeast one-hybrid assays (Reece-Hoyes et al. 2011). Interestingly, the analysis by Beyer et al. (2006), which included a comprehensive yeast ChIP data set (Harbison et al. 2004), identified only 5245 TF-target relationships that they considered “high confidence.” Neither this analysis nor that of Marbach et al. (2012) used differential expression data, so adding NetProphet scores to the inputs they combine has the potential to enhance their results substantially.

Key insight behind NetProphet

To determine whether the method of analyzing expression profiles from TF perturbation studies matters, we applied NetProphet and two highly regarded methods (Inferelator and Genie3) to the same expression data set. The results showed a substantial accuracy advantage for NetProphet (Fig. 3). Much of this advantage is due to NetProphet’s global ranking of interactions by the likelihood that the target is differentially expressed when the TF is perturbed. The key insight is that the effect of deleting a TF is strongest on its direct targets and diminishes rapidly as it propagates through the network. This does not mean that only direct targets are differentially expressed or even that the majority of the differentially expressed genes are direct targets. Indeed, the typical approach to DE analysis—setting a threshold for significance and treating all significant targets as equal—is not very useful for network mapping. However, the direct targets show a stronger differential expression effect, on average, than indirect targets. Because it exploits the strength of differential expression, NetProphet does not need an explicit mechanism for dealing with the fact that many indirect targets are differentially expressed at statistically significant levels. Algorithms based on a strength-of-DE ranking have been tried on simulated data (Greenfield et al. 2010; Pinna et al. 2010; Yip et al. 2010), but the studies presented here are the first to demonstrate that this approach is useful for inferring real TF networks from real gene expression data.

The effects of data-set size and the importance of combining DE with regression

We carried out a number of experiments to answer two intertwined questions: “How important is it to have expression profiles for a lot...
of TF perturbations?” and “How important is it to use regression as well as global ranking by probability of DE?” The results indicate that NetProphet effectively predicts the targets of TFs whose deletions have been profiled even if the number of such TFs is small. When the number of TF-deletion profiles is small, as it may be in many focused studies of organisms other than yeast, NetProphet’s effectiveness derives primarily from its DE-based ranking algorithm. Adding deletion profiles of additional TFs to a data set improves accuracy in two ways. First, it greatly improves the accuracy of target prediction for the TFs deleted in the new data. Second, it improves the accuracy of target prediction for all TFs for which a deletion profile is not available. The latter effect is due entirely to NetProphet’s LASSO regression algorithm. In Supplemental Table S1, which shows NetProphet predictions based on all available deletion profiles, there are many ChIP-supported predictions for TFs whose deletion profiles are not available. Such predictions could not be made by DE alone. The regression component also makes it possible to improve accuracy by including expression profiles that do not involve single-TF perturbations, such as profiles of strains grown in stress conditions.

Identifying novel TF functions

One of the applications of genome scale network mapping is discovering the functions of TFs based on Gene Ontology (GO) terms that are significantly overrepresented in the annotations of their predicted targets. (In less-well-studied organisms, many target genes can be confidently assigned GO terms by orthology.) Applying this approach to NetProphet predictions produced significant enrichment of biological process terms for 239 of the 263 S. cerevisiae TFs (Supplemental Table S2). Even when we excluded GO terms that were significant in ChIP-implicated targets of each TF, we were left with 44 functions assigned to 42 TFs, of which 66% were supported by mutant phenotypes or protein-protein interactions and 34% were novel. This suggests that the majority of such inferences are correct and highlights the fact that NetProphet analysis provides valuable information even after most TFs have been ChIPed.

One novel prediction is that Cbf1 would activate expression of certain phosphatases when cells are grown in YPD. We verified this experimentally and showed that Cbf1 can best be understood as amplifying the effect of inorganic phosphate availability on the expression of phosphatases, rather than simply repressing or activating them in response to phosphate availability. Another novel prediction is that, Eds1, a TF with no known function, is a highly specific repressor of genes involved in lysine biosynthesis (including one that encodes an enzyme in the TCA cycle). We identified strong binding sites for Eds1 in the promoters of five of these genes, and verified experimentally that deletion of ED51 results in substantial up-regulation of these five and complementation of the ED51 deletion restores wild-type expression levels. Eds1 is the closest homolog of Rgt1, also a repressor, which regulates glucose transporters. The promoter of ED51 contains a significant binding site for Mig1/Mig2, major enforcers of glucose repression whose targets also include glucose transporters, and ED51 is differentially expressed in the mig1 mig2 double mutant but not in either single mutant (Westholm et al. 2008). Thus, Eds1 may link lysine biosynthesis to glucose availability and the fermentative/oxidative balance.

Testing other applications of network models

By comparing NetProphet predictions to ChIP data and to PWM models of binding potential, we have demonstrated the application of network models to predicting TF binding. Other important applications for future studies are predicting gene expression and phenotype in response to new TF perturbations. A good benchmark for predictive power is the ability to predict gene expression and phenotype in double TF mutants using only data from single mutants. Ideally, a network map should predict epistatic interactions and thereby make it possible to predict expression in a double mutant more accurately than the average of the expression profiles of the two single mutants. Developing systematic network mapping methods that reliably achieve this will require innovations in both mapping and quantitative modeling. In the meantime, network maps produced by the current version of NetProphet correctly predict functional TF-promoter interactions and overall TF functions using only gene expression data that can be generated by scalable, commodity methods within modest budgets.

Methods

Materials

All chemicals were from Sigma-Aldrich. All kits were used according to manufacturer recommendations unless otherwise specified.

Strains and growth conditions

All strains used in the CBF1 study, including cbf1Δ and the wild-type parent strain, have been described (Zhou and O’Shea 2011) and were kindly provided by the O’Shea lab.

Yeast microarray normalization and quantification

The microarray data used to map the yeast transcriptional network was originally published in Hu et al. (2007) and later reanalyzed in Reimand et al. (2010). We downloaded the raw GenePix files for each of the S88 microarrays from the Longhorn Microarray Database (Killion et al. 2003) and normalized this data following the scheme described by Reimand et al. with minor modifications (see Supplemental Methods).

ChIP data curation

A list of protein-DNA interactions in S. cerevisiae was compiled from two sources: TNET (Babu et al. 2004) and YEASTRACT (Abdulrehman et al. 2011). Taken together, TNET and YEASTRACT include the results of several major ChIP studies (Svetlov and Cooper 1995; Horak et al. 2002; Lee et al. 2002; Harbison et al. 2004; Luscombe et al. 2004; Teichmann and Babu 2004) as well as many smaller studies. YEASTRACT is regularly updated to include new ChIP data as it becomes available. TNET contains 12,873 interactions over 157 TFs and 4410 target genes. YEASTRACT contains 28,145 ChIP-supported interactions over 160 TFs and 5683 target genes. There was good agreement between these two sources, with 11,073 interactions in common. We used the union of TNET and YEASTRACT, which contained 29,945 interactions covering 184 TFs and 5790 target genes. This union is referred to below as the interactions in curated ChIP studies.

Estimating binding potential with position weight matrices

To establish binding site evidence for an interaction, the curated PWMs from the UNIPROBE database (Giordan et al. 2011; Robasky and Bulyk 2011) were scanned over the yeast promoters using FIMO (Grant et al. 2011). A gene’s promoter region was defined as extending from 800 bases upstream of the transcription start site.
(excluding sequence from neighboring open reading frames) to 200 bases downstream from the transcription start site. Binding sites that were identified by HIMO at a P-value < 0.005 were considered in subsequent analyses. Two models of binding were considered. For each TF, the strong site model ranks promoters containing one or more significant binding sites according to the negative log P-value of the most significant site. The weak site model ranks promoters containing one or more significant binding sites by the sum of the negative log P-values for all significant sites in a promoter. If a promoter is ranked in the top K promoters for one or both binding models, it is considered to be supported by binding potential evidence. Reasonable values of K are determined on a TF by TF basis. For each TF, K is set such that a certain fraction of ChIP interactions for that TF are recovered in the top K ranked promoters. Three different binding potential stringencies were considered using this formulation: high (~10% ChIP interactions recovered), medium (~33% ChIP interactions recovered), and low (~50% ChIP interactions recovered). At the high, medium, and low stringencies, the random baselines (percentage of random TF-gene pairs supported by binding potential) are 6.4%, 22.1%, and 36.8%, respectively. This process was repeated using the curated PWMs from the ScetTF database (Spivak and Stormo 2012), for which the random baselines at the high, medium, and low stringencies are 5.7%, 20.4%, and 35.9%, respectively.

**NetProphet**

The NetProphet method for inferring a transcriptional network from a gene expression data set combines the scores from two independent analyses. The first analysis is a sparse linear model that predicts the expression of each gene as a function of the expression of one or more transcriptional regulators. The second analysis assesses differential expression on each expression profile in the data set in which a specific regulatory gene has been perturbed (via knockout, knockdown, or overexpression) compared to wild-type strain, encoding a TF.) LASSO chooses the influence coefficients that minimize the sum of the squared prediction errors, by a parameter $t$. When $t$ is 0, the optimization of $B$ is equivalent to ordinary least squares regression. As $t$ grows, components of $B$ are forced to zero, yielding a sparser solution. In this application, we disallow autoregulation by prohibiting $B_{ij}$ from becoming nonzero when regulator $i$ is encoded by gene $j$. Unlike the typical LASSO regression carried out by Inferelator, NetProphet uses a single, global weighting parameter $t$ for all target genes. This reduces the number of learned parameters by more than 5000, thereby reducing the risk of overfitting the data. Parameter $t$ is determined using 10-fold cross-validation, minimizing predictive error. We handle gene perturbations in the regression by omitting measurements in which gene $j$ has been perturbed when fitting the coefficients $B_{ij}$.

DE analysis is used to rank potential regulator-target interactions based on the estimated probability that each gene changes expression in response to the regulator deletion according to a log-odds statistic:

$$L_D(j) = \log \left( \frac{\Pr(F_{\Delta j} \neq 0)}{\Pr(F_{\Delta j} = 0)} \right),$$

where $F_{\Delta j}$ is the true log2-fold change of gene $j$ in the wild type relative to a $\Delta$ background. We compute $L_D(j)$ using LIMMA, which estimates the posterior log odds score by way of a moderated t-statistic in which gene specific variances are shrunk toward a common value as described (Smyth 2004, 2005). A signed confidence score $D_{ij}$ is assigned to each potential regulator-target interaction as follows:

$$D_{ij} = \begin{cases} L_D(j) \cdot \text{sgn}(Y_{\Delta j}(j)) & \text{if } L_D(j) > 0 \\ 0 & \text{otherwise} \end{cases}.$$

$D_{ij}$ represents the signed confidence score that regulator $i$ directly regulates gene $j$, and $Y_{\Delta j}(j)$ is the observed log2-fold change of gene $j$ in the wild type relative to a $\Delta$ background. The sign of $D_{ij}$ indicates whether regulator $i$ is repressing or activating gene $j$. When it is more likely that gene $j$’s expression is unchanged in the $\Delta$ background (i.e., $L_D(j) < 0$), the interaction is assigned a confidence score of 0. If the gene expression compendium contains no measurements for the $\Delta$ strain, $D_{ij}$ is set to zero for all $j$.

LASSO regression and DE analyses are combined using a model averaging scheme. Before combining the score matrices $B$ (from regression) and $D$ (from differential expression), each matrix is normalized such that its values lie on the interval $[-1, 1]$. After normalization the combined scores, $M_{ij}$, are computed as follows:

$$M_{ij} = (|B_{ij}| + c_b) \cdot (|D_{ij}| + c_d) \cdot \omega_{R(B_{ij}, D_{ij})},$$

where $c_b$ and $c_d$ are constants that prevent $M_{ij}$ from becoming zero when only one of the two individual scores is zero. The product of the two analyses is scaled by $\omega$, which is a six-component vector that weighs the average differently according to the signs of $B_{ij}$ and $D_{ij}$ which indicate the predicted regulatory influence (activating, repressing, or no influence). $\omega$ is indexed as a function of $B_{ij}$ and $D_{ij}$ as follows:

$$R(B_{ij}, D_{ij}) = \begin{cases} I & B_{ij} > 0; D_{ij} > 0 \\ H & B_{ij} < 0; D_{ij} > 0 \\ II & B_{ij} < 0; D_{ij} < 0 \\ IV & B_{ij} > 0; D_{ij} = 0 \\ B & B_{ij} = 0; D_{ij} < 0 \\ D & B_{ij} = 0; D_{ij} = 0 \end{cases}$$

In the absence of training data, $c_b$ and $c_d$ are set to 0.01 and $\omega$ is set to 1. Otherwise, the offset coefficients $c_b$ and $c_d$ and the weight vector $\omega$ are learned by cross-validation on the training data (labeled interactions), maximizing the area under the precision recall curve.
GO enrichment analysis to detect novel TF function
GO process enrichment was performed using R Bioconductor package GOstats (Falcon and Gentleman 2007). GO process enrichment was performed for each TF’s targets as predicted by NetProphet, using the top 10,000 predicted targets. GO process enrichment was also performed over the set of targets identified for each TF by curated ChIP studies. Processes that received a P-value of 0.01 or less by NetProphet predictions and were not enriched (P-value > 0.05) by ChIP data were considered to be novel relative to ChIP. The novel processes identified for each TF were manually curated to remove redundant and generic processes.

Expression profiling of \( \Delta cbf1 \) strains
The wild-type and \( \Delta cbf1 \) strains (see Methods) were assessed for acid phosphatase expression using a QuantiGene assay (Affymetrix). Briefly, 4-mL YPD overnight cultures were inoculated from single colonies. The following day, cells were washed and transferred to 50 mL of media containing low or high levels of inorganic phosphate (YPD or SC + 10 mM Pi, respectively). The high phosphate media, SC + 10 mM Pi, was made according to the recipe described in Zhou and O’Shea (2011). Cultures were inoculated at a density selected to achieve OD between 0.5 and 0.6 the following day, based on the doubling times determined for each strain/media pairing. Cells were harvested the following day, and selected RNAs were quantified using QuantiGene (Affymetrix) (see Supplemental Methods).

Raw probe counts were background-normalized according to the negative control probes (Affymetrix), and the background corrected counts of \( PHO11 + PHO12 \) (which could not be distinguished) and \( PHO5 \) were normalized by the geometric mean of the background-corrected counts of three housekeeping genes: \( TFC1 \), \( UBC6 \), and \( PDA1 \). The results of technical duplicates were averaged.

Expression profiling of \( \Delta eds1 \)
Four-milliliter YPD overnight cultures of wild type (BY4741), \( eds1 \Delta \) (YBR033W), and \( eds1 \Delta \) complemented with \( ED1 \) under the control of the tetO3 promoter were inoculated from single colonies. The following day, cells were washed and transferred to 30 mL SC + 2% glucose, grown overnight, synchronized to OD 0.5, and grown for 4 h to approximately OD 0.8. After harvesting by centrifugation, the cells were lysed. Crude lysate was quantified using QuantiGene (Affymetrix) (see Supplemental Methods).

Background correction and normalization of \( ED1 \), \( LYS4 \), \( LYS9 \), \( CTP1 \), \( ACO2 \), and \( LYS12 \) were as described above.

Data access
No data suitable for submission to databases were produced; however, the full QuantiGene data sets from which the data in Figures 4 and 5B were extracted are provided as supplemental spreadsheets.

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