Dissecting the transcription networks of a cell using computational genomics

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A great challenge in understanding biological complexity in the post-genome era is to reconstruct the regulatory networks governing the patterns of gene expression. In the past few years, the rapid accumulation of genomic sequence and functional data has led to the development of computational approaches to systematically dissect transcriptional regulatory networks. Effective algorithms have been developed to predict *cis*-regulatory elements in a genome, to identify the target genes of transcription factors, to infer the conditions under which each transcription factor is either activated or deactivated, and to analyze combinatorial regulation by multiple transcription factors. Genomic approaches have profoundly changed the way biologists investigate transcriptional regulation, and global pictures of the transcription networks for several model organisms are beginning to emerge.

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Abbreviations

ChIP	chromatin immunoprecipitation
TF	transcription factor
TFPE	TF perturbation experiment

Introduction

A great challenge in the post-genome era is to understand gene regulation on a genomic scale. Organisms devote a significant fraction of their DNA to encoding *cis*-regulatory programs that both control and coordinate gene expression at the transcript level. The outputs of the *cis*-regulatory program depend on the cellular state and extra-cellular inputs. Typically, an external stimulus activates a signal transduction pathway, which leads to the modification of the activities of several transcription factors. These transcription factors then target a subset of genes in the genome, effecting regulation that is often combinatorial in nature. Figure 1 depicts a simplified picture of transcription regulation at a genomic scale. Dissecting the complexities of transcriptional networks is essential for understanding development, cellular responses to environmental and genetic perturbations, and the molecular basis of many diseases.

To form a comprehensive picture of the transcription networks, one needs to address the following challenges: first, identification of *cis*-regulatory elements in the genome; second, accurate identification of the direct regulatory targets of transcription factors (TFs); third, identification of the cellular and environmental context in which these TFs are either activated or deactivated; and fourth, analysis of how gene expression is tailored to different conditions through combinatorial control by multiple TFs. Here we review recent progresses in developing computational approaches to meet these challenges, driven by the rapid accumulation of sequence and functional genomics data.

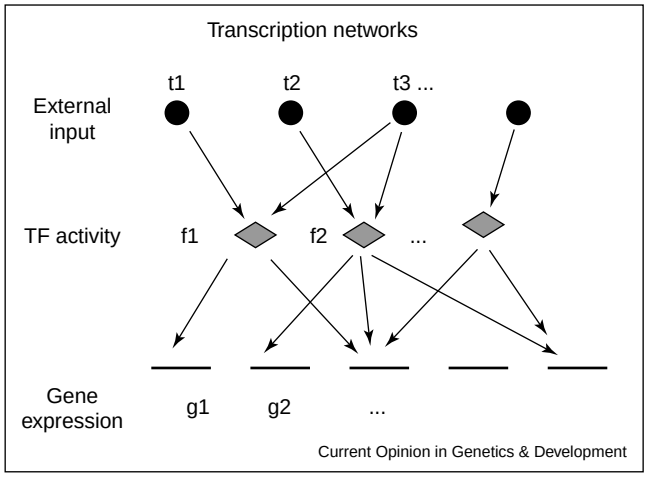
Identifying *cis*-regulatory elements in a genome

Until relatively recently, the identification of *cis*-regulatory elements in a genome has been difficult because these elements are typically short, degenerate, and obey few rules. The availability of large-scale gene expression data from DNA microarrays, complete genome sequences of many species for comparative analysis, and systematic ChIP–chip — chromatin immunoprecipitation followed by hybridization to DNA chip — experiments have led to the development of a large number of computational algorithms to identify *cis*-regulatory elements systematically. These algorithms generally fall into the following categories.

Combining sequence and expression data

A common approach for combining sequence and expression data is to first define groups of co-regulated genes on the basis of similarity in their expression profiles using clustering algorithms [1,2], then to search for enriched sequence patterns in the upstream regulatory regions of genes in a group. The underlying assumption is that genes with similar expression profiles are likely to be regulated by the same TFs. The search algorithms range from enumerating over-represented substrings or regular expression patterns [3–5] to local multiple sequence alignments [6–12]. (Some of these algorithms have been discussed in previous reviews [13,14].)

Figure 1



A diagram of transcription networks of a cell. The transcriptional response of the cell is determined by the cellular state and external input, as represented by the conditions t1, t2 (etc.) Elements f1, f2 (etc.) are transcription factors that are activated under specific conditions. Typically, transcription factors work together in a combinatorial fashion to control the expressions of genes g1, g2 (etc.).

The clustering-based approach has been quite successful in identifying regulatory elements but has its limitations. Clustering is far from an exact and objective process. Genes sharing the same motif may or may not cluster together depending on the expression measurement conditions. Partitioning genes into disjointed clusters may cause loss of information because groups of genes defined by a common motif may not be mutually exclusive, as a result of combinatorial regulation. In addition, clustering is not applicable in situations where only a single microarray measurement is available (e.g. a mutant/wild type comparison, or a ChIP–chip measurement). Several algorithms have been developed to extract regulatory elements without the need for clustering. Bussemaker, Li and Siggia developed the REDUCE (Regulatory Element Detection Using Correlation with Expression) algorithm that can identify combinatorial regulatory elements from a single microarray measurement, based on a linear regression model in which regulatory motifs contribute additively to the log of gene expression [15]. Liu *et al.* developed the MDscan algorithm which combines gene expression data with local multiple sequence alignment to identify TF binding sites from ChIP–chip data [16•]. Recently, Conlon *et al.* generalized the linear regression scheme used by the REDUCE algorithm to evaluate motifs described by position-specific weight matrices (which specify the probability of occurrence of the 4 nucleotides at each single base position) generated from the MDscan algorithm [17].

Single genome statistical analysis

Regulatory elements in a genome may be found on the basis of intra-genome statistics [18–20,21•]. A TF, in general, regulates more than one target and its binding site appears in many places in the genome — thus the binding site motif will be over-represented. Algorithms have been developed to identify putative regulatory elements using genome sequence information only. These algorithms search for over-represented motifs on the basis of certain 'background' models. One example is the Moby Dick algorithm developed by Bussemaker, Li and Siggia. This algorithm treats the genome as if it were a scrambled novel with 'words' representing putative regulatory elements. The algorithm reconstructs the lexicon by finding recurrent words using a probabilistic segmentation model [19,20]. When supplemented by specific knowledge of binding-site motifs, searches for over-represented motifs on the basis of genome-wide statistics can be very effective in finding regulatory elements. For example, using the observation that many DNA-binding proteins in bacteria bind to a bipartite motif with two short segments more conserved than the intervening region, Li *et al.* developed an algorithm that successfully identified about one-third of known regulatory motifs in the *Escherichia coli* genome and predicted many new ones [21•].

Comparative genome analysis

The availability of completely sequenced genomes of closely related species provides a great opportunity for delineating conserved regulatory elements. These elements are more conserved than general noncoding sequences because of functional constraints. Choosing species separated by appropriate evolutionary distances is essential for the success of this approach. The species have to be close enough to achieve sensible alignment of noncoding sequences, but sufficiently diverged such that conserved regulatory elements will stand out from the background.

Comparative genome analysis of regulatory sequences involves the identification of orthologous noncoding regions across species, followed by the search for conserved DNA segments. Some publicly available data sources and analysis tools are reviewed in [22]. The computational algorithms range from BLAST-like [23–26], to Hidden Markov model based [27,28], to local multiple sequence alignment [29]. Algorithms have also been developed where the statistical significance of alignment is evaluated under an appropriate background mutation model that takes into account the relatedness of the species [30]. The resolution at which the regulatory elements can be delineated depends on the type of sequence data available. Pair-wise alignment usually identifies highly conserved segments that are much longer (hundreds to thousands of bases) than the typical length of a TF binding site. This approach has been used by various groups, for example by Loots *et al.* [31] to identify regulatory sequences for interleukins in the human genome by comparison to mouse; by Waterston *et al.* [32•] to systematically analyze conserved noncoding

regions between human and mouse and to estimate the fraction of noncoding regions under selection; and by Kent and Zahler [28] to compare *Caenorhabditis elegans* and *C. briggisae*. When multiple species data are available, finer resolution can be achieved. For example, McCue *et al.* [29] used Gibbs sampler to identify regulatory motifs in the orthologous noncoding regions from several bacterial species [29]. The power of comparing several closely related species with appropriate evolutionary distance is clearly demonstrated by the recent sequencing and comparative analysis of several yeast species [33•,34•], where many known regulatory elements were identified by simply searching for bipartite patterns or oligonucleoties that are more conserved than expected by chance. There are also recent works where targeted genomic regions in multiple mammalian (e.g. [35]) and vertebrate (e.g. [36]) species were sequenced and novel regulatory sequences identified by comparative analysis. Although comparative analysis is quite successful, it is known that many regulatory elements lie outside the conserved regions, and thus will escape detection (E Emberly, N Rajewsky, E Siggia, personal communication).

Predicting *cis*-regulatory modules on the basis of clustering of binding sites

Identification of regulatory elements in metazoans (e.g. fly, mouse and human) is more difficult than in unicellular organisms (e.g. yeast). In contrast to yeast, where *cis*-regulatory elements are typically located a few hundred base pairs away from the translation start site, *cis*-regulatory elements in metazoans can be tens or even hundreds of kilobases away from the genes they regulate. In addition, the binding sites are, in general, not as sharply defined as in yeast. Thus, false positives occur frequently. Recently, notable progress has been made on the basis of the following simple observation. Analysis of the transcriptional program governing early fly embryo development revealed that the *cis*-regulatory elements organize into well separable modules, each defining a specific aspect of the spatio-temporal pattern [37,38]. Such a modular structure has also been revealed, for instance, in the studies of sea urchin development [39–41]. In an early study, Fickett and Wasserman [42] used a combination of muscle-specific TF binding sites to search for muscle-specific genes in the human genome. Recently, several groups [43–45,46•] developed algorithms to search for *cis*-regulatory modules responsible for early fly embryo patterning. Most of the algorithms are based on counting the number of matches of a certain minimum similarity to known motifs in a sequence window. Rajewsky *et al.* [46•] used known motif profiles and a statistical segmentation algorithm (discussed in [19,20]) to compute the likelihood ratio of a given sequence being 'module' versus 'background'. This algorithm circumvents the arbitrary cut-off on motif matches and potentially permits multiple weak motifs to contribute. Frith *et al.* [47,48] have developed an algorithm based on hidden Markov model to analyze clusters in the human genome and have made the tool available free online.

Identifying target genes of TFs

It remains a significant challenge to link predicted *cis*-regulatory elements to the TFs that recognize them. Typically, the potential functions of the predicted elements are evaluated by comparison with known TF binding sites and targets, or by functional analysis of the genes that contain the element. This approach was used, for example, by Kellis *et al.* [33•] and Cliften *et al.* [34•] to assign putative functions for *cis*-regulatory elements identified by comparative analyses.

One exciting development in the past few years has been the invention [49,50] and large-scale application [51•] of the ChIP–chip technology to identify the direct targets of a TF. Recently Lee *et al.* [51•] applied the technology systematically to yeast and published a dataset for 106 TFs, the most comprehensive dataset for TF binding in the yeast genome to date. The ChIP–chip technology is now used to study TF binding in mammalian cells [52–54]. Using DNA microarray containing the proximal promoters of ~5,000 well annotated genes, Li *et al.* systematically identified the targets of c-Myc in Burkitt's lymphoma cells [53]. The amount of ChIP–chip data are rapidly accumulating as various laboratories are using similar approaches to analyze TFs under various conditions. However, these data cannot be used blindly to define the target genes of a TF. It is important to have the ChIP–chip experiment done under the right conditions where the TF is activated. Apart from identifying target genes, it is also non-trivial to accurately locate the binding site of a TF, because ChIP–chip data only allows the identification of TF binding loci with a resolution of ~1 kb. One approach is to first identify a set of potential target genes on the basis of ChIP–chip data and then to search for common sequence patterns in their promoters using local sequence alignment algorithms [51•]. Other algorithms have been developed to identify binding sites ([16•]; W Wang *et al.*, unpublished data) and target genes of a TF (W Wang *et al.*, unpublished data) more effectively by combining ChIP–chip data and sequence information.

ChIP–chip experiments map the genomic location of a TF's binding site, but do not provide direct evidence for the regulation of the genes bound by the TF. A functional assay is a TF perturbation experiment (TFPE). In a TFPE, the expression profile of the wild type is compared to a mutant in which the TF has been perturbed (e.g. either deleted or overexpressed) under conditions where the TF plays a regulatory role. Identification of the binding sites and the direct targets of a TF using TFPE has received less attention because of concerns over the difficulty of distinguishing direct and indirect targets. However, Wang *et al.* recently demonstrated that the

binding site and target genes of a TF can be identified with high specificity by combining promoter sequence analysis with TFPE data ([55•]; W Wang *et al.*, unpublished data). Their work suggests that TFPEs for all the TFs in the genome may be a comprehensive and efficient way to map transcriptional networks on a genomic scale.

Identifying the cellular and environmental context in which a transcription factor is active

Although significant progress has been made in identifying *cis*-regulatory elements and mapping the links from TFs to their targets (the bottom portion of the network diagram in Figure 1), the development of tools to map the links from conditions to transcription factors (the top portion of the network) is still in its infancy. Identification of the cellular and environmental contexts in which each TF is either activated or deactivated is crucial for translating the static information encoded in the DNA sequence into an understanding of the dynamic regulatory network. At present, there is no high-throughput method to measure the activities of all the TFs in a genome directly. mRNA expression level, for example, is insufficient because the activity of TFs is often regulated by post-translational modifications. Several computational approaches have been developed to infer the activities of TFs from microarray expression data indirectly. Wang *et al.* [55•] have developed an inference scheme on the basis of 'local similarity' between the expression data from a TFPE experiment and that from a condition of interest, under the assumption that if the TF is activated under that condition, genes regulated by the TF should have responses similar to those in the TFPE. Barkai *et al.* [56] developed an algorithm to identify groups of genes that are coherently expressed under a subset of conditions. If genes in a group are known to be regulated by a TF, then the TF can be inferred to be active under those conditions. Algorithms have also been developed to search for TFs regulating a gene cluster on the basis of similarity between the expression profile of a TF and that of the cluster [57]. Segal *et al.* used a similar idea to infer potential condition specific regulators [58•]. This approach is limited, for instance by the fact that many TFs are not regulated at the transcript level, and by the difficulty of inferring causality from correlations.

Combinatorial regulation

Combinatorial regulation is known to be an essential feature of transcriptional regulation. Examples include combinatorial control for spatial temporal patterning during development [37–41], and the stress response in yeast [59]. An understanding of combinatorial regulation at a genomic scale is a major challenge, as the number of possible combinations is huge and the cooperation between TFs is context-dependent. With the rapid accumulation of data on gene expression, TFs, and their target genes, it is possible now to systematically analyze genes regulated by multiple TFs and to relate the complex transcriptional response of a gene to the combinations of TF binding sites. We expect that this will become one of the focuses in computational analysis of transcriptional regulation in the next few years.

One straightforward approach to identifying combinatorial regulation is to examine the overlaps between the target genes of different TFs ([51•]; W Wang *et al.*, unpublished data). This approach can be very powerful if TFPE or ChIP–chip data under the right activation condition is available for TFs involved in the regulation. Using ChIP–chip data in conjunction with expression data, Lee *et al.* identified genes bound by a common set of regulators as well as co-expressed throughout the cell cycle, and built a model of a transcriptional network for cell-cycle regulation [51•]. Wang *et al.* integrated TFPE, ChIP–chip and gene expression data to derive a mechanistic model for combinatorial regulation during sporulation (W Wang *et al.*, unpublished data). In a different approach, Pilpel *et al.* [60] screened for pairs of regulatory motifs which may function together on the basis of the assumption that genes sharing both motifs should be more tightly co-regulated. Segal *et al.* developed a scheme to infer a binary decision tree suggesting potential combinatorial regulation [58•]. Taking advantage of multiple yeast species sequence data, Chiang *et al.* [61] searched for potential combinatorial motifs by enumerating pairs of hexameric sequences that are jointly conserved and exhibit non-random spacing.

The context-dependent nature of combinatorial regulation poses a great challenge for reconstructing transcription networks. Because a TF can work together with different TFs to regulate different sets of genes depending on the conditions, context-dependent methods such as TFPE or ChIP–chip experiments (TF binding is also condition-dependent) are essential. On the other hand, because enumerating all different contexts is a daunting task, one needs to develop computational tools to assemble all the partial information into an integrated picture of the network. Context-independent approaches, such as those identifying all TF-binding sites and combinations of sites in the genome on the basis of sequence analysis only, will be indispensable for extending knowledge gained in specific contexts and for suggesting new contexts to be explored.

Conclusions

In the past few years, the availability of genomic sequence and functional data has led to the development of computational approaches to dissecting transcription networks at the system level. For simple model organisms such as yeast, global pictures of the network are beginning to emerge. In the future, there will be continuing efforts to collect increasing amounts of sequence and functional data and develop better theoretical models and computational algorithms to obtain a comprehensive picture of the network, both in uni-cellular and multi-cellular organisms.

We believe one step beyond reconstructing the network is to have a mechanistic understanding of how the network performs its regulatory function. In the long run, analyzing transcriptional networks by combining bioinformatic analysis with physical modeling is likely to yield insights into the basic constraints and underlying principles for how the transcription network and the *cis*-regulatory system of a genome is designed.

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