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Transcriptional network dynamics in macrophage activation

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Abstract

Transcriptional regulatory networks govern cell differentiation and the cellular response to external stimuli. However, mammalian model systems have not yet been accessible for network analysis. Here, we present a genome-wide network analysis of the transcriptional regulation underlying the mouse macrophage response to bacterial lipopolysaccharide (LPS). Key to uncovering the network structure is our combination of time-series cap analysis of gene expression with in silico prediction of transcription factor binding sites. By integrating microarray and qPCR time-series expression data with a promoter analysis, we find dynamic subnetworks that describe how signaling pathways change dynamically during the progress of the macrophage LPS response, thus defining regulatory modules characteristic of the inflammatory response. In particular, our integrative analysis enabled us to suggest novel roles for the transcription factors ATF-3 and NRF-2 during the inflammatory response. We believe that our system approach presented here is applicable to understanding cellular differentiation in higher eukaryotes.

Keywords: System biology; Regulatory networks; Network dynamics; Complex systems; Transcriptional regulation; Genome; Macrophages; Innate immunity; inflammation

Macrophages are the classical two-edged sword of the innate immune system. Their ability to recognize and destroy microorganisms is essential to host defense, and they have many roles in development, wound healing, and homeostasis; yet their destructive potential and secretory products are central to the pathology of acute and chronic inflammatory disease in mammals [1,2]. The destructive potential of macrophages is stringently controlled. Recognition of conserved nonself molecules expressed by microorganisms is mediated by so-called pattern recognition receptors, many of which belong to the Toll-like receptor family. The most studied of these receptors is Toll-like receptor 4 (TLR4), which mediates signals generated by lipopolysaccharide (LPS), a major component of the cell walls of gram-negative microorganisms. In response to

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LPS, mouse macrophages undergo a major change in gene expression, in particular inducing the expression and release of numerous biologically active cytokines that orchestrate the inflammatory response. An extensive literature search revealed several hundred genes that were reported to be inducible in macrophages [3]. The LPS response in mouse macrophages, which is reflected in both morphology and gene expression patterns, has been analyzed on a number of different platforms [4,5]. Temporal profiling reveals a cascade of gene regulation, with many late-inducible genes responding to inducible transcription factors and/or inducible secreted regulators acting in an autocrine manner. This well-characterized, stereotypical response is ideal for identifying and understanding dynamic transcriptional networks [6,7]. Yet, the resolution of the networks that can be inferred directly from microarray data is limited, partly because transcription factors are often expressed below the detection limit of microarrays [8].

In this study, we have assembled a variety of data on the transcriptional response of murine bone marrow macrophages (BMMs) to LPS stimulation over time. BMMs actively proliferate in response to the lineage-specific growth factor macrophage colony-stimulating factor 1 (CSF-1). LPS down-regulates the CSF-1 receptor on the cells and causes growth arrest while at the same time promoting survival [9]. A substantial number of genes that are inducible by LPS in BMMs are actually induced by the inactivation of a repressive signal from the CSF-1 receptor. Hence, LPS signaling intersects with numerous fundamental biological events, such as proliferation, apoptosis, endocytosis, and secretion, common to most mammalian cells.

Data integration and perturbation are essential for recovering regulatory networks since, despite recent progress in identifying networks from genome-wide data in *Escherichia coli* [10,11] and yeast [6,12,13], it has not yet been possible to provide a reliable detailed map of the underlying regulatory transcriptional architecture. In addition, conventional clustering of coexpressed genes does not have sufficient resolution to detect regulatory interactions between genes. Several recent studies have demonstrated the advantage of integrating various data types obtained by high-throughput methods such as genome-wide expression profiling, genome-wide RNA interference, and chromatin immunoprecipitation complemented with promoter DNA microarray (ChIP-on-chip) [14]. In particular, Luscombe et al. [6] used transcription factor binding data from yeast to infer a "passive" network, whereas genome-wide expression data sampled from different states of the cell cycle defined the corresponding "active" subnetworks. However, their approach is limited to systems in which transcription factor binding experiments are feasible. This is not yet the case for mammalian systems, including the macrophage. Several studies have considered sequence-based promoter information, building on the belief that coexpressed genes are more likely to be coregulated by similar sets of transcription factors (TFs) [15,16]. However, genes in a coexpression cluster need not be coregulated by the same underlying mechanism. Two transcripts can have similar expression profiles and yet be regulated by different factors. An additional complication recognized by the FANTOM3 analysis of mouse promoters on a genome-wide scale is that the large majority of "genes" have more than one promoter with quite distinct regulation [17]. So, an arbitrary extraction of promoters based upon the sequence upstream of the longest known cDNA can combine distinct promoters with discordant regulation.

Here we instead design a method for discovering transcriptional networks active in a particular cell state, based on prediction of state-specific transcription factor binding sites (TFBSs), which are defined by experimentally validated transcription start sites. We present a novel algorithm for clustering transcripts based on similarity of promoter structure instead of coexpression. This analysis shows that genes within these clusters structures tend to be coexpressed and functionally related. Finally, we illustrate that our network inference method recovers many known features of the macrophage transcriptional response to LPS, and we discuss a number of novel findings. Although several studies attempted to link pairs of transcription factors to coregulation and coexpression in reconstruction of regulatory networks [12], we took the analysis one step further by determining coregulated genes based on similarities of promoter structures. Our approach can therefore account for complex combinatorial control by several transcription factors.

Results

Experimental system

We used LPS to activate murine BMMs. Similar to previous small-scale studies [5], we collected gene expression data (described below) monitoring the LPS response over a time course of 0 (before LPS stimulation), 2, 7, and 24 h.

Macrophage transcriptome analysis

To monitor whole-genome expression during the LPS response, we used RIKEN cDNA arrays [17] containing over 60,000 probes. Triplicate array hybridizations were used at each time point. In addition, we performed quantitative real-time PCR (qPCR) of 1559 known and predicted transcripts coding for TFs and other putative nuclear proteins, since these are often expressed in amounts below the detection limit of microarrays [8]. Their putative roles in regulatory networks have therefore not yet been examined directly on a global scale.

The qPCR analysis revealed that 43% (673/1559) of TFs were significantly expressed and regulated (Supplementary Table 1). The diversity of TFs detected in macrophages is rather striking. We will not review the data herein, but the list contains all of the factors previously identified based upon a literature survey of known macrophage-expressed and/or LPS-inducible TFs [18]. It is also interesting to consider the TFs that were not detected, including most members of known TF families involved in lineage determination and patterning in embryonic development (e.g., Hox, Sox, GATA, Tbx, Neurog, Nkx, Lhx, and Fox). That is, any TF of unknown function that is not present in macrophages in any state of activation is likely to be