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The past 15 years have seen the gene-specific approach to studying the regulation of gene expression joined by global-scale analysis, enabled by microarrays, whole-genome sequencing and RNA sequencing (RNA-seq). The Toll-like receptor 4 (TLR4)-mediated cascade of transcriptional activity, which is induced by lipopolysaccharide (LPS) or lipid A in the mouse innate immune system, has been well characterized from this global perspective. However, previous analyses have been limited to statistical trends and were biased toward genes differentially expressed at small magnitudes. Now, Tong *et al.* use a gene-centric analysis of LPS and lipid A-induced changes in gene expression to help identify unique molecular mechanisms regulating individual genes.

The team first focused their analysis on nascent, chromatin-associated transcripts from 226 strongly induced genes identified by RNA-seq following stimulation of mouse bone-marrow-derived macrophages. Within this set, the authors identified 'primary response genes' (PRGs) and a subset of 94 'secondary response genes' (SRGs). SRGs were further subdivided into type I interferon receptor (IFNAR)-dependent and -independent genes. Gene ontology analysis of the three gene sets revealed that PRGs were associated with blood cell function and regulation of inflammation, IFNAR-dependent SRGs had roles in antiviral responses, and the IFNAR-independent SRGs specifically regulated T cell responses.

To investigate PRGs in closer detail, the group examined expression kinetics in macrophages deficient for key players of the TLR4 signalling cascade (*Myd88*^{-/-}, *Trif*^{-/-}, *Myd88*^{-/-}*Trif*^{-/-} and *Irf3*^{-/-}), as well as wild-type macrophages treated with lipid A in the presence of ERK and p38 MAPK inhibitors, which attenuate inflammatory signalling. They identified 16 gene clusters whose expression and kinetics were either dependent or independent of the presence of each factor. Focusing on the role of NF-κB, by combining chromatin immunoprecipitation followed by sequencing (ChIP-seq) with binding-motif analysis, the authors observed that the 37 genes with the strongest induction combined strong ChIP-seq signals with strong motif strength. This set included genes that encode NF-κB or IκB family

members or regulators. Interestingly, a subset of genes showed strong NF-κB ChIP-seq peaks but weak binding motifs, which suggests potential mediation via third-party transcription factors or chromatin looping.

Turning to the five PRGs that combined NF-κB ChIP-seq signals, NF-κB motifs in their promoters and were dependent on IRF3 for their expression, the team observed delayed kinetics for *Ccl5* and *Ifnb1*. ChIP-seq, ATAC-seq (assay for transposase-accessible chromatin using sequencing) and ChIP-qPCR (quantitative PCR) indicated that nucleosome remodelling and coordination between NF-κB and IRF3 were important for the expression of these genes. Extending this analysis across their data sets, the authors could identify only six additional promoters that shared these features. These findings are in stark contrast to previous work that suggested hundreds of genes were regulated by the cooperative actions of NF-κB and IRF3. Finally, the team repeated their ChIP-seq signal and motif-binding analysis for serum response factor (SRF), revealing seven PRGs with strong signals in both parameters, all displaying rapid induction. Further analysis across all PRGs suggests that SRF and NF-κB can cooperate to achieve rapid induction of PRGs.

By combining global-level data sets and focusing on a gene-centric analysis, Tong *et al.* have been able to finely analyse the expression and kinetics of genes induced by inflammatory signalling. Their approach can distinguish the features marking rapidly induced genes from slow ones, and strongly induced genes from weak ones, thereby starting to map out the underlying logic of the transcriptional response. "Whereas many systems biology studies focus on fairly distant views of large clusters of genes that are roughly co-regulated, the stringent approach we employed allowed us to gain an appreciation for the highly unique strategies used to regulate individual genes," concludes senior author Stephen Smale (UCLA).

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