Title: Waves of chromatin modifications in mouse dendritic cells in response to LPS stimulation

Authors: Alexis Vandenbon[1$]*, Yutaro Kumagai[2$], Mengjie Lin[3], Yutaka Suzuki[3], Kenta Nakai[4]*

[1] Immuno-Genomics Research Unit, Immunology Frontier Research Center (IFReC), Osaka University, Suita, 565-0871, Japan

[2] Quantitative Immunology Research Unit, Immunology Frontier Research Center (IFReC), Osaka University, Suita, 565-0871, Japan

[3]Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa 277-8561, Japan

[4] Laboratory of Functional Analysis in silico, The Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo, 108-8639, Japan

$ Equal contribution

* To whom correspondence should be addressed. Email: alexisvdb@ifrec.osaka-u.ac.jp, knakai@ims.u-tokyo.ac.jp

Abstract

Background: The importance of transcription factors (TFs) and epigenetic modifications in the control of gene expression is widely accepted. However, causal relationships between changes in TF binding, histone modifications, and gene expression during the response to extracellular stimuli are not well understood. Here, we analyzed the ordering of these events on a genome-wide scale in dendritic cells (DCs) in response to lipopolysaccharide (LPS) stimulation.

Results: Using a ChIP-seq time series dataset, we found that the timing of H3K27ac accumulation at promoters coincided with their transcriptional induction. However, in contrast, the LPS-induced

accumulation of several other histone modifications at promoters and enhancers occurred in "waves" within specific time frames after stimulation, independent of the timing of transcriptional induction. Integrative analysis with TF binding data revealed potential links between the timing of TF activation and accumulation of histone modifications. Especially, binding by STAT1/2 coincided with induction of H3K9K14ac, and was followed by increases in H3K4me3. In a subset of LPS-induced genes the induction of these modifications was found to be TRIF-, IRF3-, and IFNR-dependent, further supporting a role for STAT1/2 in the regulation of these histone modifications.

Conclusions: The timing of several stimulus-induced, short-term changes in histone modifications appears to be relatively independent of dynamics in activity of regulatory regions. This suggests a lack of a direct causal relationship. Changes in these modifications more likely reflect the activation of stimulus-dependent TFs and their interactions with chromatin modifiers.

Running title: Waves of chromatin modification in immune response

Keywords: histone modifications, dendritic cells, epigenetics, transcription factors, ChIP-seq, time series

Background

Epigenetic features, such as covalent post-translational modifications of histone proteins and DNA methylation, are thought to play a crucial role in controlling the accessibility of DNA to RNA polymerases. Associations have been found between histone modifications and both long-term and short-term cellular processes, including development, heritability of cell type identity, DNA repair, and transcriptional control [1,2]. For cells of the hematopoietic lineage, cell type-defining enhancers are thought to be established during the process of differentiation from stem cells by priming with the H3K4me1 marker [3,4]. On the other hand, in differentiated cells, extracellular stimuli are accompanied by relatively short-term or transient changes in histone modifications reflecting the changes in activity of enhancers and promoters [5–7].

TFs are key regulators in the control of epigenetic changes [8,9]. During the long-term process of development, closed chromatin is first bound by pioneer TFs, which results in structural changes that make it accessible to other TFs and RNA polymerase II (Pol2) [7,10]. Similarly, more short-term changes in gene expression following stimulation of immune cells are regulated by TFs. This regulation is thought to involve TF binding, induction of changes in histone modifications, and recruitment of Pol2 [11–14]. However, details of the temporal ordering and causal relationships between these events remain poorly understood [15,16]. Especially, it is unclear whether certain histone modifications are a requirement for, or a result of, TF binding and transcription [17–19].

As sentinel cells of the innate immune system, DCs are well equipped for detecting the presence of pathogens. Lipopolysaccharide (LPS), a component of the cell wall of Gram negative bacteria, is recognized by DCs through the membrane-bound Toll-like receptor 4 (TLR4), resulting in the activation of two downstream signaling pathways [20]. One pathway is dependent on the adaptor protein MyD88, and leads to the activation of the TF NF-κB, which induces expression of proinflammatory cytokines. The other pathway involves the receptor protein TRIF, whose activation induces phosphorylation of the TF IRF3 by TBK1 kinase. The activated IRF3 induces expression of

63 type I interferon, which in turn activates the JAK-STAT signaling pathway, by binding to the type I IFN
64 receptor (IFNR) [21].

65 Here, we present a large-scale study of short-term changes in histone modifications in mouse DCs
66 during the response to LPS. We focused on the timing of accumulation of histone modifications at
67 promoters and enhancers, relative to the induction of transcription and to TF binding events. We
68 observed that LPS stimulation induced increased levels of H3K9K14ac, H3K27ac, H3K4me3 and
69 H3K36me3 at LPS-induced promoters and enhancers. However, surprisingly, we found that the
70 accumulation of H3K9K14ac (between 0.5 and 3 hours) and H3K4me3 (between 2 and 4 hours)
71 occurred within specific time frames after stimulation, independent of the timing of transcriptional
72 induction of nearby genes. This finding suggests a lack of a direct causal relation between increases
73 in these markers and induction of gene expression. H3K36me3 in LPS-induced genes spreads from
74 the 3' end of gene bodies towards the 5' end, reaching promoters at later time points (between 8
75 and 24 hours). Integrated analysis of induction times of histone modifications with genome-wide
76 binding data for 24 TFs revealed possible associations between increases in H3K9K14ac and
77 H3K4me3 and binding by Rela, Irf1, and especially STAT1/2. For STAT1/2, this association was further
78 supported using independent ChIP-qPCR experiments in $TRIF^{-/-}$, $IRF3^{-/-}$, and $IFNR^{-/-}$ cells, and in wild
79 type (WT) and $TRIF^{-/-}$ cells stimulated with IFN-β. Together, these results suggest that accumulation
80 of histone modification within specific time windows reflects the timing of activation of stimulus-
81 dependent TFs, and that the accumulation of H3K9K14ac and H3K4me3 is neither an absolute
82 requirement for, nor a direct result of, stimulus-dependent transcriptional induction.

Results

Genome-wide Measurement of Histone Modifications at Promoter and Enhancer Regions

To elucidate the temporal ordering of stimulus-induced changes in transcription and chromatin structure, we performed chromatin immunoprecipitation experiments followed by high-throughput sequencing (ChIP-seq) for the following histone modifications in mouse DCs before and after LPS stimulation: H3K4me1, H3K4me3, H3K9K14ac, H3K9me3, H3K27ac, H3K27me3, H3K36me3, and similarly for Pol2 (Fig. S1), for ten time points (0h, 0.5h, 1h, 2h, 3h, 4h, 6h, 8h, 16h, 24h). We integrated this data with publicly available whole-genome transcription start site (TSS) data (TSS-seq) [22]. All data originated from the same cell type, treated with the same stimulus, and samples taken at the same time points.

Using this data collection, we defined 24,416 promoters (based on TSS-seq data and Refseq annotations) and 34,079 enhancers (using $H3K4me1^{high}/H3K4me3^{low}$ signals) (see Methods). For this genome-wide set of promoters and enhancers, we estimated the levels of histone modifications, Pol2 binding, and RNA reads over time (see Methods).

Epigenetic Changes at Inducible Promoters and their Enhancers

Recent studies using the same cell type and stimulus showed that most changes in gene expression patterns were controlled at the transcriptional level, without widespread changes in RNA degradation rates [23,24]. We therefore defined 1,413 LPS-induced promoters based on increases in TSS-seq reads after LPS stimulation, and inferred their transcriptional induction time (see Methods). Fig. 1A shows changes in transcriptional activity of LPS-induced promoters. In addition to a relatively large number of immediate-early induced promoters (0.5h, 259 promoters) and promoters with very late induction (24h, 299 promoters), at each time point we observed between 85 to 180 induced

106 promoters. In addition, we defined a set of 772 promoters with highly stable activity over the entire
107 time course (see Methods).

108 A previous study suggested only limited dynamics of histone modifications at stimulus-induced
109 promoters in mouse DCs, based on data taken at a few time points [25]. Our dataset, however,
110 allows the analysis of the timing of changes over an extended time period after stimulation.

111 For both promoters and enhancers, we defined significant increases in histone modifications and
112 Pol2 binding by comparison to pre-stimulation levels (see Methods). Our analysis suggested that
113 changes were in general rare; only 0.7 to 5.3 % of all promoters (Fig. 1B) and 0.2 to 11.0 % of all
114 enhancers (Fig. 1C) experienced significant increases in histone modifications and Pol2 binding.
115 Changes at the promoters and enhancers of stably active promoters were similarly rare (not shown).
116 However, changes were found relatively frequently at LPS-induced promoters, especially for markers
117 of activity such as Pol2 binding, H3K4me3, H3K27ac, and H3K9K14ac, as well as for H3K36me3 (Fig.
118 1B). For example, while only 957 promoters (out of a total of 24,416 promoters; 3.9%) experienced
119 significant increases in H3K9K14ac, this included 27.6% of the LPS-induced promoters (390 out of
120 1,413 promoters). To a lesser extent, we observed the same tendency at associated enhancers (Fig.
121 1C). The smaller differences at enhancers are likely to be caused by imperfect assignments of
122 enhancers to LPS-induced promoters (i.e. we naively assigned enhancers to their most proximal
123 promoter).

124 LPS-induced promoters were less frequently associated with CpG islands (57%) than stably
125 expressed promoters (87%, Fig. S2A) [26]. Non-CpG promoters more frequently had lower basal
126 levels (i.e. levels at 0h, before stimulation) of activation-associated histone modifications, such as
127 H3K27ac, H3K9K14ac, H3K4me3, and similarly lower levels of Pol2 binding and pre-stimulation gene
128 expression (Fig. S2B). This partly explains the higher frequency of significant increases in histone
129 modifications at LPS-induced promoters (Fig. S2B), and the higher fold-induction of genes associated
130 with non-CpG promoters (Fig. S2C).

Previous studies have reported only limited combinatorial complexity between histone modifications, i.e. subsets of modifications are highly correlated in their occurrence [27,28]. In our data too, basal levels of activation markers at promoters and, to a lesser degree at enhancers, were highly correlation (Fig. S3). Stimulus-induced accumulations of histone modifications and Pol2 binding at promoters and enhancers further support this view. For example, increases in H3K9K14ac, H3K4me3, H3K36me3, H3K27ac, Pol2 binding, and transcription often occurred at the same promoters (Fig. 1D). Similarly, increases in H3K9K14ac, H3K27ac, Pol2 binding, and transcription often coincided at enhancer regions (Fig. 1E). In general, activated regions experienced increases in several activation markers.

Several Histone Modifications are Induced at a Specific Time after Stimulation

Focusing on the set of induced promoters, we analyzed the ordering of induction times of different features (transcription activity, Pol2 binding, histone modifications). We defined the "induction time" of a feature *X* in a genomic locus as the first time point (if any) where *X* was increased significantly compared to its basal level in that loci (i.e. at 0h; see Methods). Defining induction times in individual genomic loci is not straightforward, due to the noisy nature of biological data. Therefore, we here focused on genome-wide trends by analyzing induction times in sets of promoters and enhancers.

As a proof of concept and a positive control, we observed that RNA reads (based on RNA-seq) mapped to promoter regions were generally induced at the same time and in the same order of the induction of transcription initiation (based on TSS-seq, independent of RNA-seq data; Fig. 2A (top) and Fig. S4). E.g. at promoters with early induction of transcription initiation (TSS-seq) there was an early induction of mapped RNA reads, while those with later induction have later induction of mapped RNA reads. Plotting the same data using cumulative plots, we again observed that increases in RNA-seq reads roughly follow the same order as transcription induction times (Fig. 2A, bottom).

Promoters of stably expressed genes lack induction of mapped RNA reads at their promoter. Similar observations were made for induction of Pol2 binding (Fig. 2B).

However, in striking contrast, induction times of H3K9K14ac, H3K4me3, and H3K36me3 at LPS-induced promoters were concentrated within specific time windows (Fig. 2D-F), and, moreover, the time of induction of these markers appeared to be largely independent of transcriptional induction times. Induction of H3K9K14ac was in general concentrated between 0.5h and 3h after stimulation (Fig. 2D), although promoters with early induction of transcription (0.5h, 1h, 2h) tended to have early increases in H3K9K14ac (at 0.5h). Even genes with transcriptional induction at 3, 4h (and to a lesser extend 6 and 8h) had induction of H3K9K14ac mostly before 3h after stimulation. Therefore, the induction of acetylation for these promoters *preceded* induction of transcription. Very few promoters showed significant increases later than 3 hours after stimulation. Finally, at promoters with late induction (16h, 24h) or at stably active promoters, increases in H3K9K14ac were rare (not shown in Fig. 2 in the interest of clarity).

In contrast with H3K9K14ac, no significant induction of H3K4me3 was observed at time points 0.5h and 1h (Fig. 2E). Induction of H3K4me3 at LPS-induced promoters was concentrated between 2 and 4 hours after stimulation, regardless of their transcriptional induction times. While induction of H3K4me3 was rare at immediate-early promoters, between 20 to 45% of promoters induced at 1h, 2h, 3h, 4h, 6h, and 8h had significant increases of this modification. These results suggest that stimulus-induced accumulation of H3K4me3 is not necessarily a prerequisite nor a direct consequence of transcription induction, since it can both *precede* or *follow* induction of transcription (see also Discussion).

Finally, H3K36me3 was only induced at later time points, especially at 16h and 24h, regardless of transcriptional induction times of promoters (Fig. 2F). In contrast with H3K9K14ac and H3K4me3, H3K36me3 is located within gene bodies and peaks towards their 3' end (Fig. S5) [29]. Upon stimulation, H3K36me3 gradually accumulated within the gene bodies of LPS-induced genes,

spreading towards the 5' end, and reached the promoter region at the later time points in our time series (Fig. S5A). Stably expressed genes had on average high basal levels of H3K36me3, with only limited changes over time. However, interestingly, at time points 16-24h, an accumulation of H3K36me3 was observed towards their 5' end (Fig. S5B), resulting in a relatively high fraction of stably expressed promoters having an induction of H3K36me3 (6.9%). Induction of H3K9me3 was rare at promoters, but this too had a tendency to occur at later time points (16h and 24h; Fig. S6A).

Remarkably, the induction times of H3K9K14ac, H3K4me3, and H3K36me3 at promoters did not change depending on their basal levels (Fig. S7); regardless of their pre-stimulus levels, increases in H3K9K14ac were early, followed by H3K4me3, and H3K36me3 accumulation was late. This might indicate that a common mechanism is regulating these accumulations, regardless of basal levels.

The tendencies described above were confirmed using RT-qPCR and ChIP-qPCR measuring RNA, H3K9K14ac, H3K4me3 (see WT data in Fig. 7), and H3K36me3 (Fig. S8) at the promoters of 9 LPS-induced genes. In general, accumulation of H3K9K14ac in WT occurred before that of H3K4me3, and accumulation of H3K36me3 was late.

Significant increases in H3K27ac appeared to be relatively rare at promoters (Fig. 2C), and were somewhat enriched at earlier time points (0.5h, 1h, and 2h). However, promoters with later induction of transcription experienced later accumulation of H3K27ac. The pattern for H3K27ac therefore appeared to be an intermediate between that of H3K9K14ac (early increases), and that of RNA or Pol2 (increases follow transcription induction times). Very few inductions were observed for H3K27me3 and H3K4me1 (Fig. S6B-C).

Increases in histone modifications were more frequent at non-CpG promoters than at CpG island-associated promoters (Fig. S9). This can be explained by the lower basal levels of most activation-associated histone modifications at non-CpG promoters (Fig. S2B). However, no differences in the accumulation times were observed between both types of promoters.

Similarities and Differences between Enhancer and Promoter Induction Patterns

Interactions between enhancers and promoters could allow active histone modifiers at promoters to also affect modifications at enhancer regions, and vice versa. We therefore analyzed induction of features at enhancers in function of induction of transcription at promoters.

To some degree, we found similar tendencies at enhancers assigned to induced promoters. We found that a small fraction of RNA-seq reads was aligned to enhancers, indicating that polyA-tailed enhancer-associated transcripts were transcribed from these regions. Induction of enhancer-associated transcripts to some extent followed the order of transcription induction at nearby promoters, though they seemed to somewhat precede transcription induction at promoters, and increases were relatively frequent at early time points (0.5 and 1h; Fig. 3A). These observations fit with those reported in a recent study showing that transcription at enhancers precedes that of promoters in cells treated with various stimuli [30]. We observed a somewhat similar, though weaker, pattern for Pol2 binding at enhancers (Fig. 3B).

For H3K9K14ac, the induction pattern at enhancers was similar to that observed for promoters (Fig. 3C); induction of H3K9K14ac was mainly concentrated at time points 2h and 3h after stimulation. Although in general a lower percentage of enhancers experienced increases of H3K9K14ac than promoters (5-10% vs 20-50%), typically 20-40% of induced promoters had at least one assigned enhancer at which an induction of H3K9K14ac occurred (Fig. S10). Increases in H3K27ac at enhancers appeared to follow to some degree the order of transcription induction of nearby promoters (Fig. 3D), in a pattern that was more similar to that of Pol2 and RNA-seq reads.

Finally, for other modifications, there are discrepancies between promoters and enhancers. We observed a gradual increase in H3K4me1 markers at enhancers over the time course (Fig. S11A), and limited increases in H3K36me3 (Fig. S11B), again independent of timing of transcriptional induction. H3K4me3, H3K9me3, and H3K27me3 signals were in general low, and no increases were observed (Fig. S11C-E).

Correlation between LPS-induced TF Binding and Increases in Epigenetic Features

Next, to reveal potential regulatory mechanisms underlying the epigenetic changes induced by LPS, we performed an integrative analysis of our histone modification data with TF binding data. For this we used a publicly available ChIP-seq dataset for 24 TFs with high expression in mouse DCs [25], before and after treatment with LPS (typical time points include 0h, 0.5h, 1h, and 2h, see Methods).

As reported in the original study, we observed widespread pre-stimulation binding of both stably-expressed and LPS-induced promoters by PU.1 and C/EBPβ, and to a lesser degree by IRF4, JUNB, and ATF3 [25] (Fig. S12A). The known association between H3K4me1 and binding by PU.1 and C/EBPβ was also successfully recapitulated (Fig. S13A,B) [10,13]. Binding by TFs controlling the response to LPS, such as NF-κB (subunits NFKB1, REL, and RELA) and STAT family members, was relatively frequent at LPS-induced promoters (Fig. S12B).

Focusing on the overlap between LPS-induced TF binding at promoters and enhancers, and induction of epigenetic features, we found that new binding of promoters by RelA, IRF1, STAT1, and STAT2 was especially associated with increases in H3K9K14ac, H3K4me3, H3K36me3, transcription, and to a lesser degree Pol2 binding and H3K27ac (Fig. 4; Fisher's exact test). For example, of the 418 promoter regions that become newly bound by STAT1 after stimulation, 223 (53.3%) experience increases in H3K9K14ac (vs 3.0% of promoters not bound by STAT1; p: 8.3E-205). LPS-induced binding by the same four TFs was also strongly associated with increases in H3K9K14ac and H3K27ac at enhancers (Fig. 4). Combinations of these four TFs often bind to the same promoter and enhancer regions (Fig. S12C,D), and STAT1 functions both as a homodimer or as a heterodimer with STAT2 [31]. LPS-induced TFs, including NF-κB and STAT family members, have been shown to bind preferentially at loci that are pre-bound by PU.1, C/EBPβ, IRF4, JUNB, and ATF3 [25]. Accordingly, histone modifications were also more frequently observed at regions that were pre-bound by these five TFs (Fig. S14).

Weaker associations were found for LPS-induced binding by other NF-κB subunits (NFKB1, REL, and RELB), TFs that are widely active even before stimulation (C/EBPβ, ATF3, JUNB, and IRF4), and E2F1, which has been shown to be recruited by NF-κB through interaction with Rela [32].

Together, these results suggests a strong correlation between increases in activation marker histone modifications and LPS-induced binding by RelA, IRF1, STAT1 and STAT2.

STAT1 and STAT2 Binding Coincides with Accumulation of H3K9K14ac, and Precedes Accumulation of H3K4me3

The relative timing of LPS-induced TF binding events and increases in histone modifications can reflect potential causal relationships. As described above, H3K9K14ac levels increase mainly during the first 3 hours after LPS stimulation (Fig. 2D). Particularly, many LPS-induced promoters show increases in H3K9K14ac between 2 and 3 hours after stimulation, and we found a strong overlap between increases in H3K9K14ac and binding by STAT1 (Fig. 4). STAT1 is not active before stimulation, and its activity is only induced about 2 hours after LPS stimulation [33], resulting in a strong increase in STAT1-bound loci (from 56 STAT1-bound loci at 0h to 1,740 loci at 2h; Fig. S12B).

We observed a particularly strong coincidence in timing between STAT1 binding and increases in H3K9K14ac (Fig. 5A): genomic regions that become bound by STAT1 at 2h show a coinciding sharp increase in H3K9K14ac around the STAT1 binding sites. At promoters and enhancers that became bound by STAT1 at 2h the induction of H3K9K14ac was particularly frequent (Fig. 5B,C). At the 2 hour time point, STAT1 binds 378 enhancers and 409 promoters, and at the same time there is a widespread induction of H3K9K14ac at these target regions (Fig. 5B,C). Accumulation of H3K9K14ac was very rare at these regions before STAT1 binding. At the end of our time series, 222 (54.2%) of these promoters, and 214 (56.6%) of these enhancers, had significantly increased H3K9K14ac levels (versus only 3.0% of promoters and 3.3% of enhancers lacking STAT1 binding).

Similar to H3K9K14ac, we observed a general increase in H3K4me3 around STAT1 binding sites (Fig. 5D). However, in contrast with H3K9K14ac, STAT1 binding immediately precedes the induction of H3K4me3 (between 2-4 hours). Accordingly, only 21 STAT1-bound promoters (out of 409; 5.6%) had significant increases at 2 h, but an additional 111 promoters (27.1%) experienced increases at the following time points (3-4 hours; Fig. 5E). As noted above, H3K4me3 was in general absent at enhancers.

Similar patterns were observed for enhancers and promoters bound by STAT2 2 hours after stimulation (Fig. S15). In contrast, regions bound by RelA and IRF1 showed increased levels of H3K27ac and to a lesser degree H3K9K14ac at earlier time points (Fig. S16 and S17). Associations with H3K9K14ac induction after 2 hours were weak compared to STAT1/2. Average increases in H3K4me3 at RelA- and IRF1-bound regions were only modest (Fig. S16G-I and S17G-I), suggesting that the association between RelA- and IRF1-binding and H3K4me3 as seen in Fig. 4 is mostly through co-binding at STAT1/2-bound regions. Associations between histone modifications and binding by other TFs were in general weak (not shown; see also Fig. 4). No changes were observed in H3K4me1 at STAT1/2-bound regions (Fig. S18A). Although there was a tendency for STAT1/2-bound loci to have increases in H3K27ac, binding seemed to slightly lag behind H3K27ac induction (Fig. S18B). Finally, although STAT1/2-bound regions tended to experience increases in H3K36me3, the time lag between binding and induction was large (Fig. S18C). This is also true for other TFs, such as RelA and IRF1, and even PU.1 and C/EBPβ, regardless of the timing of TF binding (Fig. S13C-F).

These results suggest possible causal relationships between STAT1/2 binding and the accumulation of H3K9K14ac and H3K4me3. The specific timing of increases in these modifications might reflect the timing of activation of these TFs, resulting in the recruitment of acetyl transferases and methyl transferases to specific promoter and enhancer regions.

LPS-induced Accumulation of H3K9K14ac and H3K4me3 is especially frequent at STAT1/2-bound Promoters of TRIF-dependent Genes

In *Trif*$^{-/-}$ cells, LPS-induced type I IFN production, activation of the JAK-STAT pathway, and induction of STAT1 and STAT2 target genes are severely impaired. Under the hypothesis that there is a causal relation between STAT1/2 binding and induction of H3K9K14ac and/or H3K4me3, we would therefore expect an especially high overlap between genes with decreased expression in *Trif*$^{-/-}$ cells, and promoters with induction of H3K9K14ac or H3K4me3 following stimulation in the WT. On the other hand, MyD88-dependent but TRIF-independent genes should mostly lack STAT1 and STAT2 binding, and are therefore expected to lack induction of these modifications.

Using RNA-seq data from LPS-stimulated *Myd88*$^{-/-}$ and *Trif*$^{-/-}$ cells, we defined a set of 141 TRIF-dependent genes (Fig. 6A). These genes typically have induction around 3-6 hours after stimulation in WT and *Myd88*$^{-/-}$, but lack induction of transcription in the *Trif*$^{-/-}$ cells. We found that a high fraction of the TRIF-dependent genes were bound by STAT1 and STAT2 in the WT ChIP-seq samples 2 hours after stimulation (STAT1: 64.5%, STAT2: 57.4%; compare: MyD88-dependent genes: 10.6% and 9.1%; Fig. 6B). This suggests that these genes are indeed under the control of STAT1 and/or STAT2 in WT.

Within these 141 TRIF-dependent genes, 97 (68.8%) had increases in H3K9K14ac at time points 2-3 hours (Fig. 6C). After dividing these TRIF-dependent genes by presence or absence of STAT1/2 binding, a clear difference in H3K9K14ac induction was observed: 81 out of 91 (89.0%) TRIF-dependent STAT1-bound genes, and 75 out of 81 (92.6%) of TRIF-dependent STAT2-bound genes had induction of H3K9K14ac between 2-3 hours. In contrast, only 13 out of 47 (27.7%) of TRIF-dependent genes lacking binding by STAT1 and STAT2 had induction of H3K9K14ac. As a reference, only 11 out of 66 (16.7%) of MyD88-dependent genes had H3K9K14ac induction at these time points.

A similar tendency was observed for H3K4me3 induction at time points 2-4h (Fig. 6D). 78 (55.3%) of TRIF-dependent genes had increases in H3K4me3 at time points 2-4 hours. Only 10 out of 47 (21.3%)

TRIF-dependent genes lacking STAT1 and STAT2 binding, and only 10 out of 66 (15.2%) MyD88-dependent genes had induction of H3K4me3 at these time points. In contrast, 66 (72.5%) of STAT1-bound, and 62 (76.5%) of STAT2-bound promoters had increases.

A Subset of STAT1/2 Target Genes lack Induction of H3K9K14ac and H3K4me3 in *Trif*$^{-/-}$, *Irf3*$^{-/-}$, and *Ifnar1*$^{-/-}$ cells

We further analyzed the roles of the TRIF-dependent signaling pathway and STAT1/2 in regulating LPS-induced genes, using RT-qPCR and ChIP-qPCR in WT, *Trif*$^{-/-}$, *Irf3*$^{-/-}$, and *Ifnar1*$^{-/-}$ cells. Experiments were performed on a selection of known TRIF-dependent and TRIF-independent genes, which showed increases in H3K9K14ac and H3K4me3 in WT (Fig. 7).

A set of known TRIF-independent genes (*Tnf*, *Il1b*, *Cxcl1*, and *Nfkbiz*) had no change between WT and knock outs (KOs) in the induction of gene expression, H3K9K14ac and H3K4me3 (Fig. 7A). These genes are not bound by STAT1/2 (except for *Cxcl1* which is bound only by STAT2), and the induction of histone modifications at their promoter regions is likely to be STAT1/2-independent.

A second subset of LPS-induced genes (*Ifit1* and *Rsad2*) has TRIF-dependent expression. These genes become bound by STAT1/2 2 hours after LPS stimulation. Their expression and the induction of H3K9K14ac and H3K4me3 were completely abrogated in *Trif*$^{-/-}$ cells, in *Irf3*$^{-/-}$ cells, and in *Ifnar1*$^{-/-}$ cells (Fig. 7B). These results further support a role of STAT1 and/or STAT2 in the control of chromatin modifications at the promoters of these genes.

A third subset of genes (*Cxcl10*, *Ccl5*, and *Il6*) was partially dependent on TRIF, IRF3, and IFNR in their induction of gene expression and histone modification changes (Fig. 7C). Induction of H3K4me3 was abrogated for *Cxcl10* in all three KOs, although H3K9K14ac was only affected in *Trif*$^{-/-}$cells. Modifications at *Ccl5* and *Il6* too, showed a dependency of TRIF, but not on IFNR. Although these three genes were bound by STAT1 or STAT2 2 hours after LPS stimulation, it is likely that their gene expression and histone modifications are regulated by additional, partly redundant signaling

pathways downstream of TLR4 [34]. Indeed, RelA and IRF1 bind to the promoters of these genes, supporting the notion of combinatorial control of gene expression and histone modifications.

Furthermore, stimulation of WT cells using IFN-β induced expression of *Ifit1* and *Rsad2*, and accumulation of H3K9K14ac and H3K4me3 at their promoters (Fig. S19B). In this system, the activation of the IFNR signaling pathway, and of STAT1/2, is independent of TRIF. Accordingly, this accumulation of H3K9K14ac and H3K4me3 was not affected in *Trif*$^{-/-}$ cells, further supporting a role for STAT1/2 in the control of these modifications at these genes. Similar accumulations were observed for *Cxcl10*, *Ccl5*, and *Il6* (Fig. S19C). Although the resolution of the data is not enough to make a definite statement, accumulation of H3K9K14ac following IFN-β stimulation had a tendency to start earlier at several of these five promoters in both WT and *Trif*$^{-/-}$ cells, compared to LPS-stimulated cells, possibly reflecting a faster activation of STAT1/2. In contrast, no or only limited accumulation was not observed for *Tnf*, *Il1b*, *Cxcl1*, and *Nfkbiz* (Fig. S19A).

Discussion

The concept of active genes being in an open chromatin conformation was introduced several decades ago [35], yet the contribution of histone modifications to the control of gene activity remains controversial [15]. On the other hand, the contribution of TFs to regulating gene expression is widely recognized [36], and a few studies have identified crosstalk between TFs and histone modifiers as important in the regulation of the response to immune stimuli. Induction of enhancer histone modifications was found to be associated with LPS stimulation-induced gene expression [7]. IκBζ was shown to control gene expression of *Lcn2* and *Il12b* as well as H3K4me3 in their promoters [37]. This process further induces recruitment of the SWI/SNF chromatin remodeling complex, which is regulated by Akirin2 [38]. Histone modifications such as deacetylation by histone deacetylases [39] and H3K9me3 demethylation by Jmjd2d [40], have been linked to the induction of a small set of genes upon LPS stimulation. Nevertheless, our understanding about causal

372 relationships between TF binding, changes in histone modifications, and changes in transcriptional
373 activity of genes in response to stimuli is still lacking.

374 Analysis of the ordering of events over time can reveal insights into possible causal relationships or
375 independence between them. Here, we presented an integrative study of the timing and ordering of
376 changes in histone modifications, in function of transcriptional induction in response to an immune
377 stimulus. Our results suggest that, rather than a clear temporal order between stimulus-induced
378 chromatin remodeling followed by transcriptional activation, specific histone modifications appear
379 to be induced at specific time frames after stimulation. These time frames appear to be relatively
380 independent of the timing of induction of transcription.

381 In our dataset, we roughly observed three “waves” of modifications. The first was early induction of
382 H3K9K14ac, which occurs mainly in the first three hours after stimulation. Here, we did observe that
383 some immediate-early promoters tended to have immediate induction of acetylation. However,
384 changes were concentrated at time points 2h and 3h. A second wave consisted of H3K4me3,
385 occurring mainly at 2-4 hours following stimulation. For genes with early transcriptional induction,
386 this modification therefore only occurs *after* induction of transcription, while it *precedes* induction of
387 transcription for genes with later induction times. Although H3K4me3 is widely used as a marker for
388 active genes, the functional role of this modification is still unclear. For example, the deletion of Set1,
389 the only H3K4 methyltrasferase in yeast, resulted in slower growth than in wild type, but otherwise
390 appears to have only limited effects on transcription [17]. Other studies too have reported a lack of a
391 direct effect of H3K4me3 on transcription [18,19]. Another study showed that H3K4
392 methyltransferase Wbp7/MLL4 controls expression of only a small fraction of genes directly [41].
393 Together, these and our results hint at a lack of a causal relationship between transcription and
394 H3K4me3 on a genome-wide scale. Finally, a third and last wave consisted of changes in H3K36me3
395 and H3K9me3, occurring only around 16-24 hours after stimulation. On the other hand, induction of
396 H3K27ac was not limited to a specific time frame, but was more correlated with transcription

induction times. Interestingly, fluorescence microscopy experiments have shown that H3K27ac levels, and not H3K4me3, can alter Pol2 kinetics by up to 50% [19]. The induction time patterns of histone modifications could therefore indeed present hints about active or passive roles of different histone modifications.

Since the induction of remodeling appears to occur specifically at LPS-induced genes, it is likely that histone modifiers are recruited by one or more LPS-activated TFs to specific target regions in the genome defined by the binding specificity of the TFs. In this scenario, the timing of activation of the TFs could explain the "waves" of histone modification changes. This fits well with our observations for STAT1/2 and the induction of H3K9K14ac and H3K4me3, within specific time frames and mostly restricted to LPS-induced promoters. Other studies have reported associations between STAT1 binding and changes in epigenetic markers following environmental stimulation, including the activation of latent enhancers [7] and histone acetylation [39,42]. Moreover, epigenetic priming by histone acetylation through STAT1 binding to promoters and enhancers of *Tnf*, *Il6*, and *Il12b* has been reported [43]. Interestingly, these genes lack canonical STAT1 binding sites, and the acetylation at these genes did not directly result in transcriptional induction, but enhanced TF and Pol2 recruitment after subsequent TLR4 activation. Since TFs such as STAT1 are also known to induce gene expression, one might expect induction of histone modifications to co-occur with induction of expression. However, as we described here, and as supported by the above studies, this is not necessarily the case. Gene expression is known to be regulated by combinations of TFs, and in this study too we noticed that LPS-activated TFs such as NF-κB, IRF1 and STATs often bound to the same loci (Fig. S12), which were moreover often pre-bound by several other TFs, including PU.1 and C/EBPβ. Discrepancies between timing of expression induction and accumulation of histone modifications could be caused by different requirements for combinatorial binding. This could also explain widely-reported "non-functional" TF binding, where TF binding does not seem to affect the activity of nearby genes [44]. Such "non-functional" TF binding might instead trigger changes in histone modifications that remain unnoticed and affect gene activity in more subtle ways.

Although many studies have compared histone modifications before and after stimulation, most lack sufficient time points and resolution to allow analysis of temporal ordering of changes. One recent study in yeast reported results that are partly similar to ours [45]: specific modifications (especially, but not only, acetylation) occur at earlier time frames during the response of yeast to diamide stress, and others at later time points. Interestingly, even late changes in histone modifications in yeast (including H3K36me3) were reported to occur within just one hour after stimulation. In contrast, changes in H3K36me3 in our data were concentrated between 16-24 hours after stimulation. Thus, the time scales of stimulus-induced epigenetic changes in multicellular, higher mammalian systems might be much longer. Interestingly, increases in H3K36me3 around 16-24 h often coincide with a decrease in histone acetylation towards pre-stimulation levels at LPS-induced promoters. A study in yeast suggested that H3K36me3 plays a role in the activation of a histone deacetylase [46], and might therefore play a role in the return to a basal state of histone modifications and terminating the response to stimulus.

Conclusions

Our time series ChIP-seq data and analysis present a first genome-wide view of the timing and order of accumulation of histone modifications during a stress response in mammalian immune cells. The stimulus-induced accumulation of H3K9K14ac and H3K4me3 occurred within specific time frames, and appears to be independent of transcriptional induction times. Integrative analysis suggests a role for STAT1/2 in the induction of these markers at stimulus-dependent promoters and enhancers. Together these findings hint at the absence of direct causal relationship between these histone modifications and transcription. Stimulus-induced changes in modifications more likely reflect the activation of stimulus-dependent TFs and their interactions with chromatin modifiers.

Material and Methods

Reagents, cells, and mice

Bone marrow cells were prepared from C57BL/6 female mice, and were cultured in RPMI 1640 supplemented with 10 % of fetal bovine serum under the presence of murine granulocyte/monocyte colony stimulating factor (GM-CSF, purchased from Peprotech) at the concentration of 10 ng/mL. Floating cells were harvested as bone marrow-derived dendritic cells (BM-DCs) after 6 days of culture with changing medium every 2 days. The cells were stimulated with LPS (Salmonella minnessota Re595, purchased from Sigma) at the concentration of 100 ng/mL for 0, 0.5, 1, 2, 3, 4, 6, 8, 16, and 24 hours, and were subjected to RNA extraction or fixation. Murine IFN-β was purchased from Pestka Biomedical Laboratories, and was used to stimulate the cells at the concentration of 1x10^2 unit/mL. All animal experiments were approved by the Animal Care and Use Committee of the Research Institute for Microbial Diseases, Osaka University, Japan (IFReC-AP-H26-0-1-0). TRIF-, IRF3-, or IFNR-deficient mice have been described previously [47–49].

ChIP-seq experiments

For each time point, thirty million BM-DCs were stimulated with LPS and subjected to fixation with addition of 1/10 volume of fixation buffer (11% formaldehyde, 50 mM HEPES pH 7.3, 100 mM NaCl, 1 mM EDTA pH 8.0, 0.5 mM EGTA pH8.0). The cells were fixed for 10 minutes at room temperature, and immediately washed with PBS three times. ChIP and sequencing were performed as described (Kanai et al, DNA Res, 2011). Fifty microliter of lysate after sonication was aliquoted as “whole cell extract” (WCE) control for each IP sample. Antibodies used were Pol2 (05-623, Millipore), H3K4me3 (ab1012, Abcam), H3K9K14ac (06-599, Millipore), H3K36me3 (ab9050, Abcam), H3K9me3 (ab8898, Abcam), H3K27me3 (07-449, Milllipore), H3K4me1 (ab8895, Abcam), and H3K27ac (ab4729, Abcam).

RNA extraction and RT-qPCR.

One million BM-DCs were stimulated with LPS for indicated times and subjected to RNA extraction by using TRIzol (Invitrogen) according to manufacturer's instruction. RNAs were reverse transcribed by using RevaTra Ace (Toyobo). The resulting cDNAs were used for qPCR by using Thunderbird SYBR master mix (Toyobo) and custom primer sets (Table S1). QPCR was performed by using LightCycler Nano (Roche).

ChIP-qPCR

ChIP was done as above, except 4x10^6 cells were used. The resulting ChIP-DNAs were subjected to qPCR as same as RT-qPCR, using custom primer sets (Table S2).

Peak calling and processing of ChIP-seq data

For each histone modification and for Pol2 binding data, we aligned reads to the genome, conducted peak calling and further processing as follows.

We mapped sequenced reads of ChIP-seq IP and control (WCE) samples using Bowtie2 (version 2.0.2), using the parameter "very-sensitive", against the mm10 version of the mouse genome [50]. Processing of alignment results, including filtering out low MAPQ alignments (MAPQ score < 30) was performed using samtools [51].

We predicted peaks for each time point using MACS (version 1.4.2) [52], using each IP sample as input and its corresponding WCE sample as control. To improve the detection of both narrow and broad peaks, peak calling was performed using default settings and also using the "nomodel" parameter with "shiftsize" set to 73. Negative control peaks were also predicted in the control sample using the IP sample as reference. Using the predicted peaks and negative control peaks, we set a threshold score corresponding to a false discovery rate (FDR) of 0.01 (number of negative control peaks vs true peaks), for each time point separately. All genomic regions with predicted peaks were collected over all 10 time points, and overlapping peak regions between time points

were merged together. Moreover, we merged together peak regions separated by less than 500 bps. This gave us a collection of all genomic regions associated with a peak region in at least one sample of the time series.

In a next step, we counted the number of reads mapped to each region at each time point for both the IP samples and WCE control samples. Using these counts, we performed a read count correction, as described by Lee et al. [53]. Briefly, this method subtracts from the number of IP sample reads aligned to each peak region the expected number of non-specific reads given the number of reads aligned to the region in the corresponding WCE sample. The resulting corrected read count is an estimate of the number of IP reads in a region that would remain if no WCE reads are present [53]. This correction is necessary for the quantitative comparison of ChIP signals over time in the downstream analysis.

Finally, the corrected read counts were converted to reads per kilobase per million reads (RPKM) values (using read counts and the lengths of each region), and normalized using quantile normalization, under the assumption that their genome-wide distribution does not change substantially during each time series. The normalized RPKM values were converted to reads per million read (ppm) values.

TSS-seq data processing and promoter definition

TSS-seq data for BM-DCs before and after stimulation with LPS was obtained from the study by Liang *et al*. [22] (DDBJ accession number DRA001234). TSS-seq data reflects transcriptional activity, but also allows for the detection of TSSs on a genome-wide scale at a 1 base resolution [54]. Mapping of TSS-seq samples was done using Bowtie2, as for ChIP-seq data. The location (5’ base) of the alignment of TSS-seq reads to the genome indicates the nucleotide at which transcription was started. In many promoters, transcription is initiated preferably at one or a few bases. Because of this particular distribution of TSS-seq reads mapped to the genome, default peak calling approaches

cannot be applied. Instead, we used the following scanning window approach for defining regions with significantly high number of aligned TSS-seq reads.

The number of TSS-seq reads mapped to the genome in windows of size 1, 10, 50, 100, 500, and 1000 bases were counted in a strand-specific way, in steps of 1, 1, 5, 10, 50, and 100 bases. As a control, a large number of sequences was randomly selected from the mouse genome, and mapped using the same strategy, until an identical number of alignments as in the true data was obtained. For these random regions too, the number of reads was counted using the same scanning window approach. The distribution of actual read counts and control read counts were used to define a FDR-based threshold (FDR: 0.001) for each window size. For overlapping regions with significantly high read counts, the region with the lowest associated FDR was retained.

In order to remove potentially noisy TSSs, we removed TSSs that were located within 3' UTRs, and TSSs located >50 kb upstream of any known gene. For remaining TSSs, we used a simple model (see Supplementary material) 1) to decide the representative TSS location in case a promoter region contained several candidate main TSSs, 2) to remove TSS-seq hits lacking typical features of promoters (e.g. presence of only TSS-seq reads in absence of histone modifications and Pol2 binding), and 3) to decide the main promoter of a gene in case there were multiple candidates. Finally, we obtained 9,964 remaining high-confidence TSSs, each assigned to 1 single Refseq gene.

These TSS-seq-based TSSs were supplemented with 14,453 non-overlapping Refseq-based TSSs for all Refseq genes which did not have an assigned high-confidence TSS-seq-based TSS. Most of the genes associated with these TSSs had lower expression in our RNA-seq data (mostly RPKM is 0 or < 1; not shown). Together, TSS-seq-based TSSs and Refseq-based TSSs resulted in a total of 24,416 promoter regions.

CpG-associated promoters were defined as those having a predicted CpG island (from the UCSC Genome Browser Database) in the region -1kb to +1kb surrounding the TSS [55]. Other promoters were considered to be non-CpG promoters.

Definition of enhancers

Enhancers were defined based on the signals of H3K4me1 and H3K4me3. First, we collected all genomic regions with significantly high levels of H3K4me1 (see section "Peak calling and processing of ChIP-seq data") in at least one of the ten time points. Regions located proximally (<2kb distance) to promoter regions and exons were removed, because they are likely to be weak H3K4me1 peaks observed around promoters, as were H3K4me1-positive regions of excessively large size (>10kb). Finally, we removed regions with H3K4me1 < H3K4me3 * 5, resulting in 34,072 remaining enhancers.

Enhancers were naively assigned to the nearest promoter (TSS-seq based or Refseq-based) that was < 150kb separated from it (center-to-center). For 30,448 enhancers (89%) a promoter could be assigned.

Public ChIP-seq data for TFs

Genome-wide binding data (ChIP-seq) is available for mouse DCs before and after stimulation with LPS, for a set of 24 TFs with a known role of importance and/or high expression in DCs [25] (GEO accession number GSE36104). TFs (or TF subunits) included in this dataset are Ahr, ATF3, C/EBPβ, CTCF, E2F1, E2F4, EGR1, EGR2, ETS2, HIF1a, IRF1, IRF2, IRF4, JUNB, MafF, NFKB1, PU.1, Rel, RelA, RelB, RUNX1, STAT1, STAT2, and STAT3. Typically time points in this data are 0h, 0.5h, 1h, and 2h following LPS stimulation (some TFs lack one or more time points). We used the ChIP-seq-based peak scores and score threshold as provided by the original study as an indicator of significant TF binding.

Promoters (region -1kb to +1kb around TSS) and enhancers (entire enhancer region or region -1kb to +1kb around the enhancer center for enhancers < 2 kb in size) were considered to be bound by a TF if they overlapped a ChIP-seq peak with a significantly high peak score. New binding events by a TF at a region were defined as time points with a significantly high score where all previous time points lacked significant binding.

Definition of induction of histone modifications and Pol2 binding

In order to analyze induction times of increases in histone modifications and Pol2 binding, we defined the induction time of a feature as the first time point at which a significant increase was observed compared to its original basal levels (at 0h). Significant increases were defined using an approach similar to methods such as by DESeq and voom [56,57], which evaluate changes between samples taking into account the expected variance or dispersion in read counts in function of mean read counts. This approach is necessary because regions with low read counts typically experience high fold-changes because of statistical noise in the data. Here we slightly modified this approach to be applicable to our data (10 time points without replicates; ppm values per promoter/enhancer region).

The values of all histone modifications, Pol2, RNA-seq, TSS-seq reads (ppms, for each time point) were collected for all promoters (region -1kb to +1kb) and enhancers (entire enhancer region or region -1kb to +1kb around the enhancer center for enhancers < 2 kb in size). For each feature (all histone modifications and Pol2 binding), we calculated the median and standard deviation in ppm values for each region, over the 10 time points. Dispersion was defined as follow:

$$d_{x,f} = \left(\frac{s_{x,f}}{m_{x,f}}\right)^2 \quad (1)$$

where $d_{x,f}$, $s_{x,f}$, and $m_{x,f}$ represent the dispersion, standard deviation, and median of feature f in region x over the 10 time points of the time series. Fitting a second order polynomial function on the $\log(d_{x,f})$ as a function of $\log(m_{x,f})$ for all promoter and enhancer regions, we obtained expected dispersion values in function of median ppm value (see for example Fig. S20 for H3K9K14ac). From fitted dispersion values, fitted standard deviation values $s_{x,f,fitted}$ were calculated (see Eq. 1), and 0h-based Z-scores were calculated as follows:

$$Z_{x,f,t} = \frac{(ppm_{x,f,t} - ppm_{x,f,0h})}{s_{x,f,fitted}} \quad (2)$$

where $Z_{x,f,t}$ is the Z-score of feature f in region x at time point t, and $ppm_{x,f,t}$ is the ppm value of feature f in region x at time point t. The induction time of a feature f at region x was defined as the first time point where $Z_{x,f,t}$ ≥4. To further exclude low-signal regions we added this additional threshold: the region should have a ppm value ≥ the 25 percentile of non-0 values in at least 1 time point. If Z-scores did not exceed 4 at any time point, the feature was regarded as not induced at a region. We used a similar approach to define LPS-induced promoters using TSS-seq data (see below).

For the analysis of induction times of H3K9K14ac, H3K4me3, and H3K36me3 at enhancers in function of their pre-stimulation basal levels (Fig. S7), we divided promoters into three classes according to their basal levels of each modifications as follows: Promoters lacking a modifications altogether (0 tag reads after correction described above) were considered as one class ("absent"). The remaining promoters were sorted according to their basal level of the modification, and were divided into two classes ("low basal level", and "high basal level") containing the same number of promoters.

Definition of LPS-induced promoters, unchanged promoters

LPS-induced promoters were defined using TSS-seq ppm values. LPS-induced promoters should have $Z_{x,TSS-seq,t}$ ≥4 for at least 1 time point and have TSS-seq ppm ≥1 at at least 1 time point. Only TSS-seq reads aligned in the sense orientation were considered for this (e.g. they should fit the orientation of the associated gene). For each of the thus obtained 1,413 LPS-induced promoters, the transcription induction time was defined as the first time point for which $Z_{x,TSS-seq,t}$ ≥4 was observed. Unchanged promoters were defined as those promoters having absolute values of $Z_{x,TSS-seq,t}$ < 1 for all time points, leading to 772 promoters.

RNA-seq data processing for wild type, *Trif*$^{-/-}$ and *Myd88*$^{-/-}$ cells

RNA-seq data for mouse BM-DCs treated with LPS were obtained from the study by Patil *et al.* [58] (SRA accession number DRA001131). This data includes time series data for WT, as well as *Trif*$^{-/-}$ mice and *Myd88*$^{-/-}$ mice.

Mapping of RNA-seq data was performed using TopHat (version 2.0.6) and Bowtie2 (version 2.0.2) [50,59]. Mapped reads were converted to RPKM values [60] using gene annotation data provided by TopHat. RNA-seq data obtained from the *Myd88*$^{-/-}$ and *Trif*$^{-/-}$ mice was processed in the same way. RPKM values were subjected to quantile normalization over all 10 time points.

For genes corresponding to the LPS-induced promoters, the maximum fold-induction was calculated in the WT RNA-seq data. The same was done in the *Trif*$^{-/-}$ RNA-seq data, and in the *Myd88*$^{-/-}$ RNA-seq data. TRIF-dependent genes were defined as genes for which the fold-induction was more than 5 times lower in the *Trif*$^{-/-}$ data than in WT, leading to 141 TRIF-dependent genes (see Fig. 6A). Similarly, 66 MyD88-dependent genes (not shown) were defined as having more than 5 times lower induction in the *Myd88*$^{-/-}$ than in WT.

Fisher's exact test

We used Fisher's exact test to evaluate the significance of differences between induced and non-induced promoters and enhancers (Fig. 1A,B), the significance of associations between changes of pairs of features (Fig. 1C,D), and the association between TF binding and increases in histone modifications, Pol2 binding and transcription (Fig. 4 and Fig. S14).

List of abbreviations

BM-DC	bone marrow-derived dendritic cells
DC	dendritic cell
FDR	false discovery rate
GM-CSF	granulocyte/monocyte colony stimulating factor
IFN	interferon
IFNR	interferon receptor
KO	knock out
LPS	lipopolysaccharide
Pol2	RNA polymerase II
ppm	reads per million reads
RPKM	reads per kilobase per million reads
TF	transcription factor
TLR	Toll-like receptor
TSS	transcription start site

WCE	whole cell extract
WT	wild type

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The ChIP-seq datasets generated and analysed during the current study are available in the DDBJ repository, accession number DRA004881.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AV, YK, YS, and KN designed the project. YK, ML and YS conducted ChIP-seq experiments, and YK additional experiments. AV and YK performed data analysis. All authors contributed to the interpretation of the data. AV and YK wrote the manuscript. All authors read and approved the final manuscript.

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813 Figure Legends

814 **Figure 1:** Frequencies of induction of features at LPS-induced promoters. (A) Heatmap showing the
815 changes (white: no change; red: induction; blue: repression) in transcriptional activity of 1,413 LPS-
816 induced promoters, relative to time point 0h. At the right, induction times and the number of
817 promoters induced at each time point are indicated. (B) The fraction of promoters (y-axis) with
818 increases in features (x-axis) are shown for the genome-wide set of promoters (green), and for the
819 LPS-induced promoters (orange). Increases in H3K4me3, H3K9K14ac, H3K27ac, H3K36me3, RNA, and
820 Pol2 binding are observed relatively frequently at LPS-induced promoters. Significance of differences
821 was estimated using Fisher's exact test; *: $p < 1e-4$; **: $p < 1e-6$; ***: $p < 1e-10$. (C) Same as (B), for
822 enhancers. (D-E) Heatmaps indicating the overlap in induction of pairs of features. Colors represent
823 p values ($-\log_{10}$) of Fisher's exact test. White: low overlap; Red: high overlap. Plots are shown for
824 promoters (D), and enhancers (E).

825

826 **Figure 2:** Induction times of transcription, Pol2 binding and histone modifications at promoters in
827 function of induction of transcriptional activation times. (A-F) The fraction (top) and cumulative
828 fraction (bottom) of promoters with an induction in RNA-seq reads (A), Pol2 binding (B), H3K9K14ac
829 (C), H3K4me3 (D), H3K36me3 (E), and H3K27ac (F) are shown. Line colors represent promoters with
830 different transcriptional activation times. Black boxes indicate time frames with frequent induction
831 of a feature.

832

833 **Figure 3:** Induction times of RNA-seq reads (A), Pol2 binding (B), H3K9K14ac (C), and H3K27ac (D) at
834 enhancers of LPS-induced promoters. Axes and color codes are the same as in Fig. 2.

835

836 **Figure 4:** Associations between LPS-induced TF binding at promoters (left) and enhancers (right) and
837 increases in histone modifications, Pol2 binding and transcription at the newly bound regions. Colors
838 in the heatmap represent the degree of co-incidence (Fisher's exact test, $-\log_{10}$ p values) between
839 new TF binding events (rows) and increases (columns). TFs (rows) have been grouped through
840 hierarchical clustering by similarity of their association pattern.

841

842 **Figure 5:** Interaction between STAT1 binding and accumulation of H3K9K14ac and H3K4me3. (A) For
843 all genomic regions bound by STAT1 at 2h after LPS stimulation, mean H3K9K14ac signals are shown
844 over time. Left: profile of mean values (y axis) over time in bins of 100 bps in function of distance (x
845 axis) to the TF binding site. Right: mean values (y axis) summed over the region -2kb to +2kb over all
846 bound regions, over time (x axis). The red arrow indicates the time at which these regions become
847 bound by STAT1. (B) The fraction of promoters with induction of H3K9K14ac over time after
848 stimulation (x axis). Blue: regions bound by STAT1 at time 2h. Red: regions not bound by STAT1 at
849 any time point. Numbers in parentheses show the number of regions bound and not bound by
850 STAT1 (C) As in (B) for enhancer regions bound (and not bound) by STAT1. (D) As in (A), for H3K4me3
851 at the genomic regions bound by STAT1 2 hours after LPS stimulation. (E) As in (B), for promoter
852 regions with induction in H3K4me3.

853

854 **Figure 6:** Increase of H3K9K14ac and H3K4me3 at STAT1/2-bound promoters of TRIF-dependent
855 genes. (A) Heatmap showing gene expression changes in WT, *Myd88*$^{-/-}$, and *Trif*$^{-/-}$ mice after LPS

856 stimulation of mouse DCs for TRIF-dependent genes. (B) TF binding ratios of promoter regions with
857 stable expression ("unchanged", blue), all LPS-induced promoters in WT (red), TRIF-dependent
858 promoters (green), and MyD88-dependent promoters (purple). TRIF-dependent promoters are often
859 bound by STAT1 and/or STAT2. (C) Fraction of regions with induction of H3K9K14 for TRIF- and
860 MyD88-dependent promoters. For TRIF-dependent genes, plots are also shown specifically for STAT1
861 and/or STAT2 bound and unbound regions. Colors of bars indicate the timing of H3K9K14ac
862 induction. (D) Same as (C) for H3K4me3 induction. Induction of H3K9K14ac and H3K4me3 is highly
863 specific for STAT1/2 bound TRIF-dependent genes, and concentrated at time points 2-3 h and 2-4 h,
864 respectively. Percentages indicate the fraction of regions with induction between 2-3 hours for
865 H3K9K14ac, and between 2-4 hours for H3K4me3.

866

867 **Figure 7:** Gene expression (mRNA), H3K9K14ac and H3K4me3 dynamics in WT, *Trif*$^{-/-}$, *Irf3*$^{-/-}$, and *Ifnar*$^{-}$
868 $^{/-}$ cells following LPS stimulation. We distinguished genes of which expression and histone
869 modifications are independent (A), dependent (B), and partially dependent (C) on TRIF, IRF3, and
870 IFNR. Error bars represent the standard deviation based on duplicate experiments. The red dotted
871 line in each graph represents the mean value at 0h. Y axes represent fold induction (for mRNA)
872 and % input (for H3K9K14ac and H3K4me3). Binding of promoters by RelA, IRF1, STAT1 and STAT2 is
873 indicated at the right hand side (white: no binding, purple: binding by RelA, green: binding by IRF1,
874 blue: binding by STAT1, red: binding by STAT2).