The Interleukin-1β Gene Is Transcribed from a Poised Promoter Architecture in Monocytes*

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Michael D. Liang†, Yue Zhang‡, Daniel McDevit*, Sylvia Marecki†, and Barbara S. Nikolajzycky* §¶

From the Department of Pathology, Boston University School of Medicine, the Department of Medicine, Immunobiology Unit, Evans Memorial Department of Clinical Research, Boston Medical Center, and the Department of Microbiology, Boston University School of Medicine, Boston, Massachusetts 02118

Cytokine transcription is usually regulated by transcription factor binding and chromatin remodeling following an inducing signal. By contrast, these data showed the interleukin (IL)-1β promoter assembles into a “poised” structure, as evidenced by nucleosome accessibility and loss of core histones immediately surrounding the transcription start site. Strikingly, these properties do not change upon transcriptional activation by lipopolysaccharide. Furthermore, association of two key transcriptional activators, PU.1 and C/EBPB, is robust pre- and post-stimulation indicating the IL-1β promoter is packaged into a nontranscribed but poised promoter architecture in cells capable of rapidly inducing IL-1β. Monocyte stimulation causes recruitment of a third factor, IRF-4, to the IL-1β enhancer. PU.1 phosphorylation at a CK2 kinase consensus element is required for this recruitment. We showed that CK2 phosphorylates PU.1, CK2 inhibitors abrogate IL-1β induction, and CK2 inductively associates with the IL-1β enhancer. Taken together, these data indicate a novel two-step mechanism for IL-1β transcription: 1) formation of a poised chromatin architecture, and 2) phosphorylation of an enhancer-bound factor that recruits other activators. We propose that this poised structure may generally characterize rapidly activated genes.

Members of the monocyte/macrophage lineage are the major IL-1β source in vivo. Processes regulating the first step of IL-1β production, transcription, have been studied in detail by transient transfection and DNA binding assays. These analyses have putatively identified transcription factors and regulatory elements playing nonredundant roles in IL-1β transcription in response to endotoxin stimulation. IL-1β transcription is regulated by a proximal promoter and an enhancer located at about 3 kb upstream of transcription start. Inducible transcriptional activation has been localized to both promoter and enhancer elements and appears to be regulated similarly in mouse and human (1–4).

Classical reporter and in vitro DNA binding assays have demonstrated that PU.1, C/EBPB, and NF-κB can activate the IL-1β promoter (2, 5, 6). The IL-1β enhancer is activated by PU.1, C/EBPB, AP-1 (Fos/Jun), and a novel STAT transcription factor dubbled LIL-STAT (7). Members of the interferon regulatory family (IRF) family also activate IL-1β transcription (8). However, activation by any of the enhancer-binding proteins is minimal in the absence of intact PU.1 promoter site(s) (8). Importantly, ectopic PU.1 expression in nonhematopoietic cells results in IL-1β transcription from the endogenous (i.e. chromatinized) locus, further emphasizing the role PU.1 plays in this process (8). Work herein therefore focuses on the critical role PU.1 plays in inducible IL-1β transcription.

Monocytic cells activate IL-1β transcription rapidly (i.e. within minutes) following stimulation with the endotoxin lipopolysaccharide (LPS). The mechanisms driving inducible IL-1β transcription appear to hinge on PU.1 phosphorylation at serine residue 148. Mutation of serine 148, located within a protein kinase CK2 consensus element, substantially blunts PU.1-mediated transcriptional activation of an IL-1β-regulated reporter gene (9). Because CK2 has been shown to be activated by LPS, and CK2 can target PU.1 serine 148 in cells (10), it is likely that CK2-mediated PU.1 phosphorylation leads to IL-1β transcription in monocyte lineage cells. Furthermore, PU.1 phosphorylation is required for transcriptional activation via recruitment of IRF-4 to an ETS/IRF composite element, found in both the κ3 and IL-1β enhancers (9, 10). This model of PU.1-activated transcription via IRF-4 recruitment has not been tested in the context of a chromatinized gene. Most analyses aimed at understanding the role PU.1 or other transcription factors play in inducible IL-1β transcription were completed before the importance of the potential regulatory context of cellular chromatin was fully realized. The lone exception is the demonstration that PU.1 overexpression can activate IL-1β transcription from the endogenous locus. Whether PU.1 functions through direct (i.e. IL-1β enhancer or promoter association) or indirect mechanisms was not discerned (8).

Changes in promoter chromatin structure regulate inducible transcription of most cytokine genes tested to date. The typical scenario is that a histone octamer sequestering the transcription start site is remodeled in response to stimulation, resulting in more or less rapid gene transcription. This mechanism was described with variable rigor for the IL-2, granulocyte-macrophage colony-stimulating factor, IL-4, interferon-γ, IL-12 p35, IL-12 p40, IL-13/IL-5, TNF-α, IL-10, MCP-1, and interferon-β promoters (11–24). Whether this mechanism results in the very rapid induction of IL-1β transcription has not been reported, despite the recent demonstration that modifications of histones packing the locus change following monocyte stimulation (25).

We have analyzed chromatin structure and protein association at the IL-1β locus in resting and stimulated monocyte lineage cells. Results herein demonstrate that, in resting cells capable of rapid transcriptional activation, the IL-1β promoter is packaged into an accessible, poised chromatin architecture not yet shown at any cytokine locus. Further analyses suggest that phosphorylation of constitutively associated PU.1 by DNA-associated CK2 upon cellular stimulation results in recruitment of IRF-4 to the IL-1β enhancer. RNA polymerase II is concomitantly recruited to the promoter.
to result in the robust IL-1β transcription characteristic of stimulated monocytes. Overall, the data demonstrate that the IL-1β gene is inducibly transcribed from a poised promoter architecture likely due to post-translational modification of DNA-associated protein.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Mono-Mac-6 (MM6) cells were maintained in RPMI containing 15% heat-inactivated fetal calf serum, 2 mM L-glutamine, and OPI media supplement to a final concentration of 1 mM oxaloacetate, 0.45 mM pyruvate, and 0.2 units/ml insulin. 293 human embryonic kidney cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% heat-inactivated fetal calf serum and 50 μM β-mercaptoethanol. THP-1 and THP-1/CD14 cells were maintained in RPMI containing 10% heat-inactivated fetal calf serum and 1 mM sodium pyruvate. HL-60 cells were maintained in RPMI containing 10% heat-inactivated fetal calf serum and 2 mM L-glutamine. U937 cells were maintained in RPMI containing 10% heat-inactivated fetal calf serum. RAW 264.7 cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% heat-inactivated fetal calf serum. All culture media contained 100 units of penicillin and 100 μg of streptomycin per ml.

Bone marrow-derived macrophages (BMDM) were derived from 6- to 8-week-old BALB/cByJ femurs according to Vicente et al. (26). Specifically, marrow from femurs and tibias were flushed out with 1–2 ml of RPMI 1640 containing 15% fetal bovine serum using a 30-gauge needle. Viable cells were purified through Lympholyte M. Cells from each specific, marrow from femurs and tibias was flushed out with 1–2 ml of RPMI 1640 containing 15% fetal bovine serum using a 30-gauge needle. Viable cells were purified through Lympholyte M. Cells from each

**RNA Isolation and Quantitative PCR Analysis**—Total RNA was isolated from 5 × 10⁶ cells using RNeasy (Qiagen). cDNA was prepared by standard methods. PCRs amplified 10 ng of cDNA, using SYBR Green (Bio-Rad) incorporation for quantitation and the Stratagene Mx3000p Real Time PCR System (Stratagene, La Jolla, CA). Each amplification was performed in triplicate under the following conditions: hot start, 3 min at 95 °C, melting, 15 s at 95 °C and then annealing and extension at 60 °C for 1 min, for 40 cycles. The primers used for IL-1β transcribe were as follows: sense, 5’-ACGAATCTGGGACACCATC-3’, and antisense, 5’-CCAATGCAGGTTGTTGCG-3’. PCRs were performed in parallel using β2 microglobulin (β2m) primers (sense, 5’-CTCCGTTGCTTTCCGAGC-3’, and antisense, 5’-TTTGGAGTACGCTG-3’) were used to normalize for differences in cDNA synthesis and RNA input. mRNA copy number was calculated according to the equation: copies = 10(Cq – Ct) / (Cq – Ct) - 3.32).

**Nuclease Probing of Chromatin Accessibility Assayed on Southern Blots**—Nuclease probing of chromatin accessibility was performed as described (28). 2 × 10⁶ cells were used for each condition. For the accessibility cut, 100 units of HindIII were added, and the nuclei were incubated at 37 °C for 1 h with agitation. Ten μg of purified genomic DNA was digested to completion with Xmal. Digestion products were separated on agarose gels. The accessibility fragment was detected on Southern blots using a radiolabeled probe specific for the IL-1β promoter (~860 to ~352 bp relative to transcription start) and standard methods.

**Chromatin Accessibility by Real Time (CHART) PCR**—Accessibility of DNA to digestion with MNase was analyzed using CHART-PCR as published (29) using 10⁵–10⁶ cells. 5–10 ng of DNA was analyzed by SYBR Green incorporation during quantitative PCR. All products were run on acrylamide gels to verify product size and displayed a single transcript characteristic of stimulated monocytes. Overall, the data demonstrate that the IL-1β gene is inducibly transcribed from a poised promoter architecture likely due to post-translational modification of DNA-associated protein.

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FIGURE 1. The IL-1β promoter is packaged into a poised chromatin structure in monocytes. A, schematic of amplicons amplified by primers sets in Table 1. +1 is position of transcription start. B, CHART-PCR analysis of human MNase-resistant IL-1β promoter DNA in 293 cells (open bars), resting Mono-Mac-6 monocytes (MM6 U, black bars), or MM6 monocytes stimulated with LPS (MM6 S, gray bars). Numbers at top represent center nucleotide of the quantitated amplicon. High levels of accessibility denote a relatively accessible chromatin structure as indicated. S.E. was <5% over at least three independent experiments. C, CHART-PCR of resting human monocyte lines THP-1 (black bars), U937 (gray bars), HL-60 (stippled bars), and 293 cells (open bars). Each cell line was analyzed at least twice with less than 5% deviation between experiments. * indicates value was not determined at this position in THP-1 cells. D, chromatin accessibility at the IL-1β promoter in resting and stimulated control (293, lanes 1 and 2, respectively) and monocytes (THP-1, lanes 3 and 4, respectively) as measured by restriction endonuclease cutting. Southern blots of indirect end labeling results are shown. Arrow denotes accessibility fragment. E, chromatin accessibility at the IL-1β enhancer in resting control (293, lane 1) and monocytes (THP-1, lane 2) and in stimulated THP-1 cells (lane 3), as in D. Data represent at least three independent analyses.
appropriate amount of antibody was determined empirically, and the same microgram amount of α-histidine tag antibody was added in the control samples. ChIP-competent antibodies were as follows: α-histidine tag antibody (SC-803; Santa Cruz Biotechnology) as a nonspecific isotype/species-matched control, α-acetylated histone H3 (06-599; Upstate), rabbit α-PU.1 (SC-352; Santa Cruz Biotechnology), α-C/EBPβ (C-19; Santa Cruz Biotechnology), α-histone H3 (antibody 1791-100, Abcam), α-C-K2α (19278-100; Abcam) and α-RNA polymerase II (N-20; Santa Cruz Biotechnology). Oligonucleotides used for amplifying the precipitated IL-1β promoter region were from promoter primer set −1 (Tables 1 and 2). IL-1β enhancer-specific primers were 5′-AGCGGTCCTCTTGGAAAAGA-3′ and 5′-ACCATCCCATCCATCTCAGG-3′. β₀m promoter primers were 5′-CCCCAGTCTAGTGCATGCCTTC-3′ and 5′-ACGCGTGCGCCAGGTAGAGAG-3′.

Recombinant Protein Production and Kinase Assays—Recombinant His-tagged PU.1 protein production has been described (32). Multiple protein preparations were analyzed in replicate experiments. Recombinant PU.1 was incubated in 20 μl of incubation buffer (100 mM Tris, pH 8, 100 mM NaCl, 50 mM KCl, 20 mM MgCl₂, and 100 μM sodium orthovanadate) with 1 μl of CK2 (New England Biolabs) and either 1 μl of [γ-32P]ATP or 1 μl of 100 mM ATP for 10 min at 30 °C. The reaction was stopped with 4 μl of SDS sample buffer. Western blots were probed with 1:300 rabbit anti-PU.1 (sc-352; Santa Cruz Biotechnology).

Kinase Inhibitor Analyses—Human Mono Mac-6 monocytes were pretreated with pharmacological inhibitors of various intracellular signaling pathways for 3 h as detailed in Table 3. Alternatively, SP600125 and SB203580 were added 10 min prior to further treatment. LPS was then added to stimulate IL-1β transcription. Transcripts were measured by quantitative reverse-transcriptase PCR and SYBR green incorporation as detailed above. Primers approximated >97% efficiency; hence this slope is within experimental error. Values graphed are the ratio of normalized IL-1β copy number in stimulated cells: normalized IL-1β copy number in resting cells for a fold induction. Error of triplicate PCRs was in no case >10%.

RESULTS

The IL-1β Promoter Is Accessible in Resting Monocyte Lineage Cells—Toward characterizing putative inducible changes in IL-1β promoter chromatin structure in monocytes, we completed quantitative chromatin accessibility analyses. Specifically, we measured MNase sensitivity of the IL-1β promoter chromatin in multiple cell lines by CHART-PCR. Fig. 1A shows the approximate location of amplicons used to measure MNase-resistant DNA and therefore promoter chromatin packaging. The center position of each amplicon is listed (relative to transcription start) in Fig. 1, B and C. By using this method, we showed that less than 50% of the IL-1β promoters in the cellular population were packaged into an MNase-accessible structure in 293 cells, which cannot produce IL-1β, at all positions tested (Fig. 1B, open circle). Similarly, monocyte IL-1β DNA located 700 to 300 bp upstream of the transcription start site was relatively MNase-resistant (Fig. 1B, black and gray bars). In contrast, DNA positioned from −200 to +100 relative to transcription start was highly accessible to MNase digestion in human Mono-Mac-6 monocytes (up to 80%). Surprisingly, accessibility was indistinguishable in resting versus LPS-stimulated cells (black versus gray bars),
Despite the demonstration that only stimulated cells produced measurable levels of transcript (Fig. 3A, MM6.5). This CHART-PCR result suggests that the IL-1β promoter is packaged into a relatively accessible chromatin structure in resting monocytes that do not transcribe this gene. Further analyses focused on confirming this “poised” chromatin structure.

To dismiss the trivial possibility that this poised chromatin structure is characteristic of a single cell line, we analyzed the structure of the IL-1β promoter in additional resting human cell lines (Fig. 1C). THP-1 (Fig. 1C, black bars), U937 (gray bars) and HL-60 (stippled bars) monocyte lineage cells package the IL-1β promoter into a highly MNase-accessible structure proximal to the transcription start site as compared with 293 cells (open bars). Taken together, the data strongly indicate the IL-1β promoter is packaged into MNase-accessible chromatin in monocyte lineage cells independent of transcriptional status.

To confirm further the constitutively accessible promoter structure and to determine its extent, we performed more traditional restriction endonuclease accessibility assays on THP-1 monocytes measuring the appearance of an accessibility fragment on Southern blots (33). The IL-1β promoter was relatively accessible to restriction endonuclease in THP-1 cells (Fig. 1D, compare lanes 1 and 2 to lanes 3 and 4, arrow shows accessibility fragment), and the amount of accessibility fragment did not change upon LPS stimulation (lane 3 versus 4), consistent with the interpretation that the promoter is packaged into a poised chromatin structure. In contrast, Fig. 1E demonstrates that accessibility of the IL-1β enhancer, as indicated by the presence of an accessibility fragment (arrow), is approximately equivalent in IL-1β-negative 293 cells (lane 1), and resting and stimulated THP-1 cells (lanes 2 and 3, respectively). These data suggest that the chromatin packaging the IL-1β enhancer, in contrast to the promoter, is similarly accessible in expressing and nonexpressing cell types; hence changes in promoter but not enhancer chromatin structure may regulate the IL-1β gene in monocytes.

The Poised Promoter Chromatin Structure of IL-1β Is Monocyte Lineage-specific—Toward understanding how specific the identified poised chromatin structure is among hematopoietic cell types, we measured IL-1β promoter MNase accessibility in primary B and T cells by CHART-PCR. Although both types of cells have been shown to produce IL-1β upon appropriate stimulation (34, 35), the promoter is MNase-insensitive from approximately −500 to −100 relative to the transcription start site in resting lymphocytes (Fig. 2A, dotted bars). This difference is not because of species differences, because murine RAW 264.7 macrophages package the promoter into an MNase-accessible structure (Fig. 2A, white bars). In contrast, murine 3T3 fibroblasts package the IL-1β promoter into an inaccessible chromatin structure (Fig. 2A, black bars). Overall, the data in Figs. 1 and 2 are consistent with the interpretation that the poised IL-1β promoter chromatin structure uniquely prepares the monocyte for rapid IL-1β production post-stimulation.

To test further the novelty of the poised IL-1β promoter in monocytes/macrophages, we analyzed chromatin accessibility at several positions along the TNF-α promoter (TNF1,4,5) in resting and stimulated Mono-Mac-6 monocytes. As shown previously (36), MNase accessibility of DNA proximal to the TNF-α promoter increased by ∼5-fold upon LPS stimulation (Fig. 2B, TNF1). Less dramatic increases in MNase accessibility were detected upstream from transcription start (TNF4 and TNF5). Taken together, data in Fig. 2 show the poised IL-1β promoter structure is both cytokine- and lineage-specific.

The IL-1β Promoter Is Packaged into a Unique Poised Chromatin Structure in the Absence of Transcription—Toward ensuring that the various monocyte lines tested for IL-1β promoter structure were capable of inducible IL-1β transcription, we stimulated each line with LPS (100 ng/ml) and quantitated IL-1β transcript normalized to βm transcript by real time PCR. Mono-Mac-6 monocytes responded rapidly to LPS (Fig. 3A, filled squares). As expected, HeLa cells do not transcribe IL-1β (Fig. 3B, open and filled circles), consistent with results from 293 cells (data not shown). Most surprisingly, THP-1 monocytes did not transcribe IL-1β in response to LPS (Fig. 3B, open circles), demonstrating that promoter accessibility is insufficient for inducible IL-1β transcription. This finding is consistent with our interpretation of a poised promoter. This interpretation is bolstered by identical findings for U937 monocytes (Fig. 3B, filled squares). Stable expression of the LPS co-receptor CD14 in THP-1 enabled the cells to respond to LPS by transcribing IL-1β (open squares), indicating THP-1 cells express many of the additional signaling molecules required for IL-1β transcription in response to LPS. Overall, these results demonstrate that monocytes, even those incapable of transcribing IL-1β in response to LPS, package the IL-1β promoter into an accessible chromatin structure.
The Poised IL-1β Promoter Is Not Packaged by Hyperacetylated Histones—Toward further characterizing the poised chromatin structure of the IL-1β promoter, we measured association of hyperacetylated histone H3 (AcH3) with the human promoter in Mono-Mac-6 cells. DNA relatively distal to the IL-1β transcription start site (primer set VII) associated with AcH3 (Fig. 4). Both downstream regions tested (primer sets I and II) are also packaged by hyperacetylated histones. In contrast, DNA amplified by primer sets V to I lacked hyperacetylated H3 packaging, as indicated by amplification of less than 2-fold over input. AcH3/IL-1β association was not significantly different in stimulated versus resting monocytes. Overall, the pattern of AcH3/IL-1β promoter association is consistent with the proposed poised chromatin structure. α-His control antibody did not precipitate the promoter in any cell tested (data not shown).

Decreased AcH3 Packaging Proximal to the IL-1β Transcription Start Site Correlates with Decreased Histone H3 Packaging in the Poised Promoter Structure—The decreased apparent AcH3 association proximal to transcription start could be explained two ways. Either H3 acetylation is low or overall H3 packaging is absent. Loss of histone packaging upon transcriptional activation has been measured on only a
few promoters at present, namely Pho5, IL-2, and granulocyte-macrophage colony-stimulating factor (37, 38). Toward differentiating between these possibilities for the IL-1β promoter, we completed ChIPs on resting and stimulated Mono-Mac-6 cells with antibodies recognizing total histone H3. H3 is insignificantly associated with the IL-1β promoter proximal to the transcription start site in both resting and stimulated monocytes (DNA amplified by primer sets -III, -I, and +I; Fig. 4B). DNA located more distal to the transcription start site is packaged by H3 (primer sets -VII and +II). Furthermore, H3/IL-1β association does not change significantly upon monotype stimulation. H3/IL-1β association patterns overall mirror those found for AcH3/IL-1β association demonstrating that the lack of AcH3/promoter packaging is because of lack of chromatin packaging which additionally characterizes the poised promoter structure.

The IL-1β Promoter Is Poised for Activation in Primary Murine Macrophages—To further test whether the poised IL-1β structure is evolutionarily conserved, we measured histone packaging at the murine promoter in RAW 264.7 cells. AcH3-specific and histone H3-specific ChIP analyzing IL-1β chromatin packaging from about 500 bp upstream to 100 bp downstream of transcription start demonstrated low association at all sites in resting and stimulated macrophages (Fig. 5, A and B, left or right panels, respectively). Like the human promoter, the lowest association was detected proximal to the murine transcription start site (+1) and was unchanged upon cellular stimulation (Fig. 5, A and B, right panels). The modest increase in AcH3 association detected at murine amplicon -V upon stimulation mirrored findings in human cells. Nonspecific antibody/DNA associations were detected at background levels, as measured by α-His-specific ChIP (data not shown). Overall, the murine association pattern recapitulated results from human monocytes, suggesting (along with Fig. 2A) the poised chromatin structure is evolutionarily conserved.

Finally, to determine whether human and murine cell line analyses accurately reflect IL-1β chromatin packaging in primary cells, we analyzed the IL-1β promoter structure in primary murine BMDMs. To confirm homogeneity of BMDMs harvested after 7 days in culture, we performed flow cytometry with cells stained for α-CD11c and α-Mac-1. Greater than 90% of the mature BMDM population stained appropriately for these markers (CD11c+ Mac-1+, Fig. 5C, middle and right panels, respectively), ensuring that the proper primary cell population was indeed being analyzed. ChIP analyses specific for H3 showed again that the IL-1β promoter is histone-free proximal to the transcription start site in BMDMs (Fig. 5D). Finally, IL-1β promoter chromatin accessibility to MNase in BMDMs demonstrated a pattern highly similar to the accessibility pattern demonstrated by human monocyte lines (Fig. 5E versus Fig. 1, B and C). We therefore conclude that cell line analyses reflect a naturally poised IL-1β promoter structure in primary monocyte lineage cells.

Transcription Factors Constitutively Associate to Define a Poised Promoter Architecture—Because PU.1 and C/EBPβ are two important IL-1β activators, we focused additional ChIP analyses on association of these proteins with IL-1β regulatory elements in monocytes. Fig. 6 shows C/EBPβ or PU.1 association with the IL-1β promoter (A) or enhancer (C) in Mono-Mac-6 human monocytes. Both PU.1 and C/EBPβ associated with the IL-1β promoter approximately equivalently in resting and stimulated monocytes (Fig. 6A). PU.1 or C/EBPβ/IL-1β promoter association is not detected in control 293 cells (Fig. 6A, dark bars), consistent with the inaccessible, transcriptionally inactive status of the gene in these cells. Western blot analyses demonstrated C/EBPβ is expressed at approximately equivalent levels in Mono-Mac-6 and 293 cells (data not shown), indicating specificity of this interaction in monocytes.

In a final series of transcription factor-specific ChIP assays on the IL-1β promoter, we assessed association of RNA polymerase II (pol II). pol II did not associate substantially with the IL-1β promoter in resting monocytes (Fig. 6A), explaining the lack of transcript production shown in Fig. 3A. Upon monocyte stimulation pol II was recruited to the promoter, as indicated by an approximate 8-fold enrichment of promoter sequence in ChIP products (Fig. 6A, stippled bar, far right). pol II was not recruited to the IL-1β promoter in 293 cells as expected. A series of control ChIP assays further demonstrated specificity of PU.1 and C/EBPβ association with the IL-1β promoter. The transcriptionally
active β₂m promoter constitutively associated with AcH3 and pol II (Fig. 6B). Neither PU.1 nor C/EBPβ associated with the monocyte β₂m promoter, indicating IL-1β promoter specificity. Overall, the data show that association of two sequence-specific DNA binding proteins, C/EBPβ and PU.1, does not change upon IL-1β transcriptional activation. The data therefore show the promoter is poised by two criteria, an accessible chromatin structure and constitutive transcription factor association, and hence is termed a “poised promoter architecture.”

It is possible that changes in protein association at the IL-1β enhancer may play a role in recruiting pol II to the promoter. We therefore used ChIP to measure protein association with the IL-1β enhancer in resting and stimulated Mono-Mac-6 monocytes. As for the promoter, association of C/EBPβ and PU.1 with the IL-1β enhancer was similar in resting and stimulated monocytes (Fig. 6C). Additional analyses detected no pol II/enhancer association in resting or stimulated monocytes (Fig. 6C, rightmost bars). This finding strongly suggests that pol II is not recruited to the promoter by the enhancer but instead by the promoter complex. Furthermore, the IL-1β enhancer is not packaged by hyperacetylated histones (data not shown) and is not selectively accessible (Fig. 6E), suggesting large regions of the gene may not be similarly structured, in contrast to findings for Igα and globin loci (31, 39).

Inducible PU.1 Phosphorylation at the IL-1β Enhancer Plays an Important Role in IL-1β Transcription from a Pooled Promoter Architecture—Based on the demonstration of constitutive PU.1 association at both promoter and enhancer, we hypothesized that IL-1β-bound PU.1 is modified to somehow trigger pol II recruitment only in stimulated monocytes. Toward testing this possibility, we focused analysis on the LPS-inducible protein kinase CK2, proposed to activate IL-1β transcription by modifying PU.1 serine 148 (9, 40). Direct kinase assays testing the ability of CK2 to modify PU.1 in vitro demonstrate that CK2 transfers radioactive phosphate to PU.1 (Fig. 7A, lanes 2 and 3). Notably, phospho-PU.1 had decreased mobility on SDS-PAGE as compared with unmodified PU.1 (40 versus 35 kDa, respectively). Ets-1, a factor related to PU.1, was not radiolabeled in parallel reactions (Fig. 7A, lanes 4 and 5, arrow indicates position of the 54-kDa Ets-1 protein; signal is absent because Ets-1 is not phosphorylated), demonstrating specificity. CK2 autophosphorylation (Fig. 7A, lane 1) is as expected. In contrast, PU.1 mutated serine-to-alanine at position 148 was not phosphorylated by recombinant CK2 (Fig. 7B, compare lanes 2 and 3). To confirm the labeled species was PU.1, we repeated the analysis using a nonradioactive phosphate donor, detecting CK2- or mock-treated PU.1 on Western blots. Anti-PU.1 reactivity confirmed the 40-kDa band was PU.1 (Fig. 7C, lane 2). In contrast, PU.1 S148A was not extensively phosphorylated by CK2 (Fig. 7C, lane 6), as indicated by a lack of super-shifting on SDS-PAGE. Because CK2 is detectable throughout the cell (41–43), these biochemical data demonstrate that CK2 can theoretically phosphorylate DNA-bound PU.1 at serine 148 in response to LPS stimulation.

Toward determining whether CK2 activity is required for IL-1β transcription, we treated Mono-Mac-6 monocytes with a cadre of kinase inhibitors prior to LPS stimulation and then measured IL-1β mRNA (normalized to β₂m mRNA). Treating cells with Me₂SO, bisindolylmaleimide (protein kinase C inhibitor), PD98059 (MAP kinase inhibitor), or LY294002 (phosphatidylinositol 3-kinase inhibitor) did not ablate inducible IL-1β transcript levels (Fig. 7D and data not shown). The lack of MAP kinase involvement is interesting given C/EBPβ is phosphorylated by MAP kinases (44). Pretreating cell with the c-Jun NH₂-terminal kinase inhibitor SP600125 decreased IL-1β transcript levels modestly (~29%), indicating c-Jun NH₂-terminal kinase may play a minor role in inducible IL-1β transcription. Treating monocytes with the p38 inhibitor SB203580 resulted in a 58% decrease in IL-1β mRNA, indicating p38, shown to phosphorylate PU.1 serine 142 (45), may act in inducible IL-1β transcription. This result agrees with a previous demonstration that p38 plays a role in activating IL-1β transcription in murine RAW 264.7 cells (46). The CK2 inhibitors apigenin and emodin decreased IL-1β transcript most dramatically (by 97 or 68%, respectively). These inhibitor data suggest that CK2 activity is essential for inducible IL-1β transcription.
Previous analyses demonstrated that CK2-mediated PU.1 phosphorylation at serine residue 148 is critical for recruiting a co-activator, IRF-4, to the PU.1/IRF composite enhancer element implicated in both IL-1β and Igκ transcription (10, 47). If IL-1β-associated PU.1 is phosphorylated in response to LPS, IRF-4 would associate with the IL-1β enhancer in stimulated (but not resting) monocytes. ChIP using α-IRF-4 antibody demonstrated that IRF-4 fails to associate with the IL-1β enhancer in resting Mono-Mac-6 cells (Fig. 8A, leftmost stippled bar). Upon stimulation, IRF-4 associates with the enhancer (~6.9-fold enrichment compared with input chromatin; Fig. 8A, leftmost open bar), but not with the promoter, which lacks an IRF-4-binding site (data not shown).

Because CK2 undergoes a relatively strong interaction with substrate proteins, we reasoned that if CK2 is responsible for IRF-4 recruitment via PU.1 phosphorylation, CK2 should associate with the IL-1β enhancer in stimulated, but not resting, Mono-Mac-6 cells. To test this prediction, we performed ChIP with an antibody specific for CK2 to measure CK2/enhancer association. This association was undetectable in resting Mono-Mac-6 monocytes (Fig. 8B, leftmost stippled bar). In contrast, CK2 clearly associates with the IL-1β enhancer in stimulated Mono-Mac-6 cells (~4.1-fold enrichment compared with input chromatin; Fig. 8B, leftmost open bar). CK2 failed to associate with the promoter in resting or stimulated monocytes (data not shown). Taken together, these data support the model that cellular stimulation triggers CK2 association and subsequent PU.1 phosphorylation for recruitment of additional activators to the IL-1β gene.

**DISCUSSION**

Data herein demonstrate by multiple independent measures that the IL-1β promoter chromatin structure is equivalently accessible and associated with transcription factors in resting and stimulated monocytes/macrophages. Additional studies support the model that CK2-mediated phosphorylation occurs on enhancer-bound PU.1 upon LPS stimulation, leading to recruitment of additional transcription factors concomitant with pII/promoter association. This model provides a mechanistic explanation of how IL-1β is transcriptionally activated within minutes of monocyte stimulation.

These findings markedly extend the first characterization of the monocyte IL-1β promoter in the context of chromatin (25). These authors demonstrated that some histone tail modifications change upon LPS stimulation in THP-1 monocytes, but did not compare DNA accessibility in stimulated versus resting cells. Furthermore, single sites of chromatin were analyzed in the published work, rendering the more comprehensive structure we have presented impossible to detect. Interestingly, p50 association (which we did not test) was constitutive, in agreement with our poised promoter architecture model. One caveat to the demonstration that the poised promoter is largely devoid of histone H3 (or acetylated H3) packaging is that the resolution of the ChIP assay is limited by the length of the sheared DNA fragments, or ~500 bp. However, because shearing randomly breaks the DNA and results in amplification of randomly overlapping fragments, our results most likely underestimate differences in histone association at the transcription start site versus regions upstream or downstream. It is possible that the chromatin preferentially sheared in the accessible nucleosome-free regions to result in unexpectedly high ChIP resolution.

The data herein are consistent with a two-step model of IL-1β transcriptional activation (Fig. 9). First (Fig. 9, a and b), the monocyte-specific poised chromatin architecture is established. Based on the general paradigm that chromatin of tissue-specific genes becomes selectively accessible during cell development, and the demonstration that the IL-1β promoter is inaccessible to MNase in B and T cells, it is likely that the combination of transcription factors associated with either the promoter or enhancer established this architecture during monocyte development. Because hematopoietic stem cells, a multipotential blood cell precursor, express IL-1β (48), we presume HSCs pack-

**FIGURE 8. Inducible protein association with the IL-1β enhancer.** IRF-4 (A) or CK2 (B) associates with the IL-1β enhancer only upon monocyte stimulation. ChIP using antibodies to IRF-4/CK2 (left set of bars in A or B, respectively) or the His6 tag (right set of bars in both A and B) in Mono-Mac-6 cells is shown. Bars show average plus S.D. of three independent experiments.

**FIGURE 9. Model for IL-1β transcription in the context of chromatin.** a, the IL-1β promoter is likely packaged into inaccessible chromatin in multipotent precursor cells. b, in resting monocytes, the promoter is accessible, based on lack of nucleosome association. Sequence-specific binding proteins such as PU.1 and C/EBPβ may promote formation of accessible chromatin and the poised promoter architecture. c, LPS stimulation results in CK2 activation, CK2/enhancer association, and phosphorylation of PU.1 at serine 148. Phospho-PU.1 recruits IRF-4 to the IL-1β enhancer. d, RNA polymerase II is recruited to the poised promoter by unknown mechanisms resulting in IL-1β transcription.
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age the promoter into an accessible chromatin structure. If so, the poised promoter architecture must be established in a very early multipotent precursor, unless HSCs activate the locus using alternative mechanisms. Furthermore, because PU.1 can act as a chromatin accessibility factor in other contexts (28, 49), and ectopic PU.1 expression can induce IL-1β from the endogenous locus in nonhematopoietic cells (8), PU.1 is a likely candidate for establishing the poised chromatin structure. HSCs express PU.1 (50, 51). Although lymphocytes are capable of producing IL-1β (34, 35), our data may explain why monocytes appear uniquely sensitive in activating the IL-1β gene in response to physiological stimuli.

The second step required for IL-1β transcription occurs only upon monocyte stimulation (Fig. 9, c and d). Because 1) CK2 is inducibly recruited to the IL-1β enhancer; 2) CK2 can directly phosphorylate PU.1; 3) this reaction requires serine 148 of PU.1; and 4) IRF-4 associates with the IL-1β enhancer only upon monocyte stimulation, the data (along with published findings that CK2 is activated by LPS, and only phosphorylated PU.1 recruits IRF-4 to DNA (8–10)) strongly suggest that enhancer-associated PU.1 is phosphorylated by CK2 in situ and that this post-translational modification catalyzes transcription through recruitment of at least one additional factor. The demonstrations that PU.1 can interact directly with the basal transcription complex TFIIID (53) and that PU.1 may induce assembly of a TBP-dependent complex on the IL-1β promoter (54) raise the possibility that PU.1 may be responsible for the demonstrated pol II recruitment to the IL-1β promoter post-stimulation.

Although we have characterized a poised promoter architecture at a cytokine promoter, partial evidence suggests similar structures at other cytokine promoters. For example, RUNX1 constitutively associates with the MIP-1α promoter in resting and stimulated Jurkat T cells (55). In a second example, analysis of the IL-8 promoter suggests that the transcription start site is nucleosome-free prior to transcriptional activation (56) and hence perhaps packaged in a poised chromatin architecture. Recent work by Smale and co-workers has demonstrated that immediate early genes are generally characterized by a poised promoter chromatin structure; whether activating proteins are constitutively bound and post-translationally modified upon macrophage stimulation remains to be determined (57). This study therefore highlights the general importance of IL-1β analyses toward establishing paradigms for regulating rapidly inducible pro-inflammatory genes.

The most extensive, convincing example of a poised promoter architecture to our knowledge is the receptor locus c-fms. The promoter for this cytokine receptor is fully occupied by DNA binding factors prior to protein production, and as for IL-1β, additional proteins associate at the enhancer upon transcription initiation (58). This promoter is packaged into accessible chromatin in resting macrophages (59) and monocyte precursors (58). However, in contrast to our findings for IL-1β, recruitment of additional proteins because of post-translational modification of bound proteins has not been demonstrated for c-fms. Finally, constitutive association of both sequence-specific binding proteins and the basal transcriptional machinery to the A20 promoter suggests that pre-assembly of even more extensive complexes than that shown for the IL-1β promoter may be required for rapid activation of this NF-kB target (60). Overall, identification of a poised promoter architecture specifically in a quick-response cytokine promoter suggests a more thorough analysis of inducible genes is important to determine the extent cells utilize this mechanism toward rapid transcriptional activation.

There are few examples in which phosphorylation of a DNA-associating sequence-specific protein, the second step in IL-1β activation, is used to activate transcription. The yeast Sko1 transcription factor is phosphorylated while bound to DNA to activate transcription (61). In a second example, phosphorylation of promoter-associated Elk-1 occurs upon cellular stimulation, resulting in transcription of the Egr gene (62). However, cAMP-response element-binding protein, the classical example of a DNA-bound factor phosphorylated following cellular stimulation (63), has been discrepantly recently (64). Hence, the number of examples of transcriptional activation by phosphorylation of DNA-associated protein is extremely limited.

Overall, our data introduce a unique combination of two recently identified mechanisms enabling monocytes to inducibly achieve rapid, robust IL-1β gene transcription. Understanding the development of the poised promoter architecture and precisely how RNA polymerase II becomes associated with the promoter are the next logical steps toward establishing additional mechanistic explanations for this model of rapid cytokine gene induction.

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REFERENCES

1. Shirakawa, F., Saito, K., Bonagura, C. A., Galson, D. L., Fenton, M. J., Webb, A. C., and Auron, P. E. (1993) Mol. Cell. Biol. 13, 1332–1344
2. Zhang, Y., and Rom, W. N. (1993) Mol. Cell. Biol. 13, 3831–3837
3. Buras, J. A., Monks, B. G., and Fenton, M. J. (1994) J. Immunol. 152, 4444–4454
4. Godambe, S. A., Chaplin, D. D., Takova, T., Read, L. M., and Bellowe, C. J. (1995) Mol. Cell. Biol. 15, 112–119
5. Kominato, Y., Galson, D., Waterman, W. R., Webb, A. C., and Auron, P. E. (1995) Mol. Cell. Biol. 15, 59–68
6. Monks, B. G., Martell, B. A., Buras, J. A., and Fenton, M. J. (1994) Mol. Immunol. 31, 139–151
7. Tsukada, J., Waterman, W. R., Koyama, Y., Webb, A. C., and Auron, P. E. (1996) Mol. Cell. Biol. 16, 2183–2194
8. Marecki, S., Riendeau, C. J., Liang, M. D., and Fenton, M. J. (2001) J. Immunol. 166, 6829–6838
9. Lodie, T. A., Savedra, R. J., Golenbock, D. T., Van Beveren, C. P., Imai, R. A., and Fenton, M. J. (1997) J. Immunol. 158, 1848–1856
10. Pongubala, J. M. R., Beveren, C. V., Nagalapalli, S., Klemmz, M. J., McKercher, S. R., Imai, R. A., and Atchison, M. L. (1993) Science 259, 1622–1626
11. Rao, S., Gerendaklis, S., Wolfring, D., and Shannon, M. F. (2003) J. Immunol. 170, 3724–3731
12. Holloway, A. F., Rao, S., Chen, X., and Shannon, M. F. (2003) J. Exp. Med. 197, 413–423
13. Fields, P. E., Kim, S. T., and Flavell, R. A. (2002) J. Immunol. 169, 647–650
14. Avni, O., Lee, D., Macian, F., Szabo, S. J., Glinscher, L. H., and Rao, A. (2002) Nat. Immun. 3, 643–651
15. Brema, J. H., Hodge, D. L., Gonsky, R., Leonard, W. J., Krebs, S., Targan, S., Morinobu, O., O’Shea, J. J., and Young, H. (2004) J. Biol. Chem. 279, 41249–41257
16. Kwan, M., Powell, D. R., Nachman, T. Y., and Brown, M. A. (2005) Eur. J. Immunol. 35, 1267–1274
17. Goriely, S., Demonte, D., Nizet, S., De Wit, D., Willems, F., Goldmann, M., and Van Lint, C. (2003) Blood 101, 4894–4902
18. Weinmann, A. S., Pley, S. E., and Smale, S. T. (1999) Immunity 11, 665–675
19. Takemoto, N., Kamogawa, Y., Jun Lee, H., Kunita, H., Ariai, K. I., O’Garra, A., Ariai, N., and Miyatake, S. (2000) J. Immunol. 165, 6687–6691
20. Barthel, R., and Goldfeld, A. E. (2003) J. Immunol. 171, 3612–3619
21. Lucas, M., Zhang, X., Prasanna, V., and Mosser, D. M. (2005) J. Immunol. 175, 469–477
22. Im, S. H., Huerbe, A., Monticelli, S., Kang, K. H., and Rao, A. (2004) J. Biol. Chem. 279, 46818–46825
23. Boekhoudt, G. H., Guo, Z., Beresford, G. W., and Boss, J. M. (2003) J. Immunol. 170, 4139–4147
24. Agliotti, T., Lomvardas, S., Parekh, B., Yie, J., Maniatis, T., and Thanos, D. (2000) Cell 103, 667–678
25. Chan, C., Li, L., McCall, C. E., and Yozu, B. K. (2005) J. Immunol. 175, 461–468
26. Vicente, R., Escalada, A., Soler, C., Grande, M., Celada, A., Tamkun, M. M., Solsona, C., and Felipe, A. (2005) J. Immunol. 174, 4736–4744
27. McDevit, D. C., Perkins, L., Atchison, M. L., and Nikolajczyk, B. S. (2005) J. Immunol. 174, 2834–2842
28. Nikolajczyk, B. S., Sanchez, J. A., and Sen, R. (1999) Immunity 11, 11–20
29. McDevit, D. C., Perkins, L., Atchison, M. L., and Nikolajczyk, B. S. (2005) J. Immunol. 174, 2834–2842
30. McDevit, D. C., Perkins, L., Atchison, M. L., and Nikolajczyk, B. S. (2003) J. Biol. Chem. 278, 42106–42114
31. Litt, M. D., Simpson, M., Recillas-Targa, F., Prioleau, M. N., and Felsenfeld, G. (2001) EMBO J. 20, 2224–2235
32. Nikolajczyk, B., Nelsen, B., and Sen, R. (1996) Mol. Cell. Biol. 16, 4544–4554
33. Boyes, J., and Felsenfeld, G. (1996) EMBO J. 15, 2496–2507
34. Wrenshall, L. E., Stevens, R. B., Cerra, F. B., and Platt, J. L. (1999) J. Leukocyte Biol. 66, 391–400
35. Kim, H. Y., and Rikihisa, Y. (2000) Infect. Immun. 68, 3394–3402
36. Lee, J. Y., Kim, N. A., Sanford, A., and Sullivan, K. E. (2003) J. Leukocyte Biol. 73, 862–871
37. Boeger, H., Strattan, J. S., and Kornberg, R. D. (2004) Mol. Cell 14, 667–673
38. Chen, X., Wang, J., Woltring, D., Gerondakis, S., and Shannon, M. F. (2005) Mol. Cell. Biol. 25, 3209–3219
39. Chowdhury, D., and Sen, R. (2001) EMBO J. 20, 6394–6403
40. Lodier, T. A., Reiner, M., Coniglio, S., Vigliani, G., and Fenton, M. J. (1998) J. Immunol. 161, 268–276
41. Krek, W., Maridor, G., and Nigg, E. A. (1992) J. Cell Biol. 116, 43–55
42. Penrose, C. G., Wang, Z., and Litchfield, D. W. (1997) J. Cell. Biochem. 64, 525–537
43. Filhol, O., Nueda, A., Martel, V., Gerber-Scokaert, D., Benitez, M. J., Souchier, C., Saoudi, Y., and Cochet, C. (2003) Mol. Cell. Biol. 23, 975–987
44. Bradley, M. N., Zhou, L., and Smale, S. T. (2003) Mol. Cell. Biol. 23, 4841–4858
45. Wang, J. M., Lai, M. Z., and Yang-Yen, H. F. (2003) Mol. Cell. Biol. 23, 1896–1909
46. Baldassare, J. J., Bi, Y., and Bellone, C. J. (1999) J. Immunol. 162, 5367–5373
47. Marecki, S., Atchison, M. L., and Fenton, M. J. (1999) J. Immunol. 163, 2713–2722
48. Watari, K., Mayani, H., Lee, F., Dragowska, W., Lansdorp, P. M., and Schrader, J. W. (1996) J. Clin. Investig. 97, 1666–1674
49. Marecki, S., McCarthy, K., and Nikolajczyk, B. S. (2004) Mol. Immunol. 40, 723–731
50. Iwasaki, H., Somoza, C., Shigematsu, H., Duprez, E. A., Iwasaki-Arai, J., Mizuno, S., Aritobu, Y., Geary, K., Zhang, P., Dayaram, T., Fengus, M. L., Elf, S., Chan, S., Kastner, P., Huettner, C. S., Murray, R., Tenen, D. G., and Akashi, K. (2005) Blood 106, 1590–1600
51. Kim, H. G., de Guzman, C. G., Swindle, C. S., Cotta, C. V., Gartland, L., Scott, E. W., and Klug, C. A. (2004) Blood 104, 3894–3900
52. Deleted in proof
53. Hagemeier, C., Bannister, A. J., Cook, A., and Kouzarides, T. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 1580–1584
54. Buras, J. A., Reenstra, W. R., and Fenton, M. J. (1995) Mol. Immunol. 32, 541–554
55. Bristow, C. A., and Shore, P. (2003) Nucleic Acids Res. 31, 2735–2744
56. Lomvardas, S., and Thanos, D. (2002) Cell 110, 261–271
57. Ramirez-Carrozzo, V. R., Nazarian, A. A., Li, C. C., Goire, S. L., Sridharan, R., Imbalzano, A. N., and Smale, S. T. (2006) Genes Dev. 20, 282–296
58. Tagoh, H., Himes, R., Clarke, D., Leenen, P. J., Biggs, A. D., Hume, D., and Bonifer, C. (2002) Genes Dev. 16, 1721–1737
59. Himes, S. R., Tagoh, H., Goonetilleke, N., Sasmono, T., Oceandy, D., Clark, R., Bonifer, C., and Hume, D. A. (2001) J. Leukocyte Biol. 70, 812–820
60. Ainbinder, E., Revach, M., Wolstein, O., Moshonov, S., Diamant, N., and Dikstein, R. (2002) Mol. Cell. Biol. 22, 6354–6362
61. Proft, M., and Struhl, K. (2003) Mol. Cell 9, 1307–1317
62. Wang, G., Balamutis, M. A., Stevens, J., Yamaguchi, Y., Handa, H., and Berk, A. J. (2005) Mol. Cell 17, 683–694
63. Mayr, B., and Mottmorny, M. (2001) Nat. Rev. Mol. Cell Biol. 2, 599–609
64. Cha-Molstad, H., Keller, D. M., Yochum, G. S., Imphey, S., and Goodman, R. H. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 13572–13577