BRG1 Increases Transcription of Proinflammatory Genes in Renal Ischemia

Masayo Naito,*† Richard A. Zager,*† and Karol Bomsztyk*

*Department of Medicine, University of Washington, and †Fred Hutchinson Cancer Research Center, Seattle, Washington

ABSTRACT

Acute kidney injury stimulates renal production of inflammatory mediators, including TNF-α and monocyte chemoattractant protein 1 (MCP-1). These responses reflect, in part, injury-induced transcription of proinflammatory genes by proximal tubule cells. Because of the compact structure of chromatin, a series of events at specified loci remodel chromatin to provide access for transcription factors and RNA polymerase II (Pol II). Here, we examined the role of Brahma-related gene-1 (BRG1), a chromatin remodeling enzyme, in the transcription of TNF-α and MCP-1 in response to renal ischemia. Two hours after renal ischemic injury in mice, renal TNF-α and MCP-1 mRNA increased and remained elevated for at least 1 wk. Matrix chromatin immunoprecipitation assays revealed sustained increases in Pol II at these genes, suggesting that the elevated mRNA levels were, at least in part, transcriptionally mediated. The profile of BGR1 binding to the genes encoding TNF-α and MCP-1 resembled Pol II recruitment. Knockdown of BRG1 by small interfering RNA blocked an ATP depletion–induced increase in TNF-α and MCP-1 transcription in a human proximal tubule cell line; this effect was associated with decreased recruitment of BRG1 and Pol II to these genes. In conclusion, BRG1 promotes increased transcription of TNF-α and MCP-1 by the proximal tubule in response to renal ischemia.

J Am Soc Nephrol 20: 1787–1796, 2009. doi: 10.1681/ASN.2009010118

It has been well documented that diverse forms of acute renal failure (ARF) evoke cytokine (*e.g.*, TNF-α) and chemokine (*e.g.*, monocyte chemoattractant protein 1 [MCP-1]) production by the kidney.[1–4] Together with the potential involvement of resident lymphocytes, neutrophils, and monocytes/macrophages, these and other kidney cell–derived mediators cause intrarenal inflammation.[5–7] These inflammatory changes have a number of potentially important clinical consequences: (*1*) Renal inflammation can exacerbate the severity of renal failure[7,8]; (*2*) renal cytokines and chemokines can be released into the systemic circulation, where they may evoke extrarenal tissue injury and contribute to multiorgan failure[6,9]; and (*3*) secondary inflammatory responses may postpone renal functional recovery and potentially promote ESRD. Given the importance of TNF-α, MCP-1, and other proinflammatory mediators in kidney disease, it is surprising how little is known about their regulation in response to kidney injury.

ARF-mediated intrarenal synthesis of inflammatory mediators cells may reflect increased transcription and changes in chromatin dynamics along target loci.[10] The compact structure of chromatin, composed of proteins, DNA, and RNA, represents a physical obstacle to molecular interactions that mediate transcription.[11,12] Multiple processes exist to open up, or relax, chromatin structure, thereby exposing docking sites for the binding of transcription factors and providing polymerase II (Pol II) access to the DNA template. The dynamics of chro-

Received January 30, 2009. Accepted April 15, 2009.

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Karol Bomsztyk, UW Medicine Lake Union, Box 358050, University of Washington, Seattle, WA 98109. Phone: 206-616-7949; Fax: 206-616-8591; E-mail: karolb@u.washington.edu

matin structure are regulated by diverse processes, such as DNA methylation, covalent histone modifications, chromatin remodeling, histone eviction, and deposition of histone variants.[11,12] The term "chromatin remodeling" refers to processes by which the energy derived from ATP hydrolysis is used to loosen histone–DNA contacts, thereby allowing the sliding of nucleosomes along DNA. This renders the promoter, enhancer, and transcribed regions more accessible to transcription factor and Pol II binding.

There are several highly conserved multiprotein chromatin remodeling complexes, which include SWItch/Sucrose NonFermentable (SWI/SNF), Nucleosome remodeling factor (NURF), Nucleosome remodeling and histone deacetylation (NuRD) (classified into SWItch2 (Swi2)-, Imitation switch (ISWI)-, Chromodomain helicase DNA-binding (Chd)-, or INO80-containing complexes).[13–17] These chromatin remodelers have different subunit compositions; however, all depend on helicase-like ATPase activity that regulates chromatin structure in a similar manner. The general view is that the ATPases of these complexes act as molecular motors that facilitate dynamic changes in chromatin structure at both active and inactive genes. The human SWI/SNF complex contains either the Brahma-related gene 1 (BRG1) or Brahma (BRM) ATPase catalytic chromatin remodeler subunit. In the mouse, BRG1 but not BRM is essential *in vivo*,[18] suggesting that BRG1-containing SWI/SNF nucleosomal remodeling complexes are critical in mammalian organisms. The functional importance of BRG1 is further underscored by the observation that this protein alone is capable of inducing chromatin remodeling *in vitro*.[19] Because BRG1 regulates chromatin structure in response to stress,[20,21] we explored its role in transcription of *TNF-α* and *MCP-1* genes in an *in vivo* and *in vitro* model of ischemic renal injury.

RESULTS

Ischemia-Reperfusion Induces Increasingly Sustained Expression of TNF-α and MCP-1 mRNA in Renal Cortex

Acute kidney injury increases expression of TNF-α and MCP-1 mRNA in the kidney.[4,10,22,23] We used reverse transcriptase–PCR (RT-PCR) to follow renal cortical TNF-α and MCP-1 mRNA levels soon after that injury and during the subsequent week. By 2 h of reperfusion, there was an increase in the MCP-1 mRNA levels, whereas TNF-α was not yet significantly increased, but at both 3 and 7 d after ischemia, marked elevations of both mRNAs were observed (Figure 1).

Ischemia-Reperfusion Induces Sustained Chromatin Changes and Co-recruitment of BRG1 and Pol II along the TNF-α and MCP-1 Genes in Renal Cortex

The aforementioned ischemia-reperfusion (I/R)-induced increases in renal cortical TNF-α and MCP-1 mRNA levels could reflect enhanced Pol II recruitment to these loci, leading to increased transcription rates.[10] We used the matrix chromatin immunoprecipitation (ChIP) platform[24,25] to profile Pol II levels along these genes (Figures 2 and 3). At 2 h after acute injury, the levels of Pol II at the start ($P < 0.003$) and end exon ($P < 0.05$) of TNF-α (Figure 2A) were higher in postischemic kidneys, compared with contralateral controls. Like the mRNA levels (Figure 1), the I/R-induced Pol II changes persisted for at least 1 wk after the injury. The levels of Pol II at a 3′ flanking region 5 kb from the end of the *TNF-α* gene were low and not different between injured and contralateral kidneys (Figure 2B), indicating the specificity of the ChIP assay. Similar results were obtained along the *MCP-1* gene locus (Figure 3). The ChIP analysis of Pol II density along these genes suggests that the I/R-induced TNF-α and MCP-1 mRNA increases reflected, at least in part, increased transcription.

Activation of gene expression is often associated with specific changes in chro-

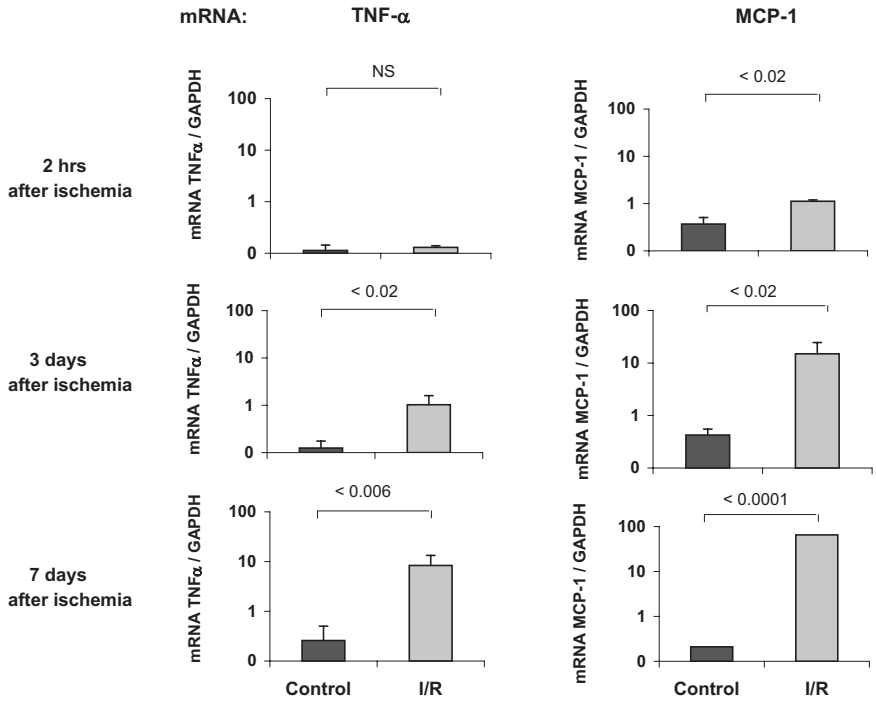

Figure 1. Expression of *TNF-α* and *MCP-1* genes after I/R. RNA was extracted from mouse cortical samples at 2 h, 3 d, or 7 d after 30 min of I/R (▨). The contralateral kidneys, not subjected to I/R, served as time-matched controls (■). The mRNA levels of TNF-α and MCP-1 were assessed by competitive PCR and are expressed as a ratio to the simultaneously obtained glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcript. Data are means ± 1 SD ($n = 4$ mice).

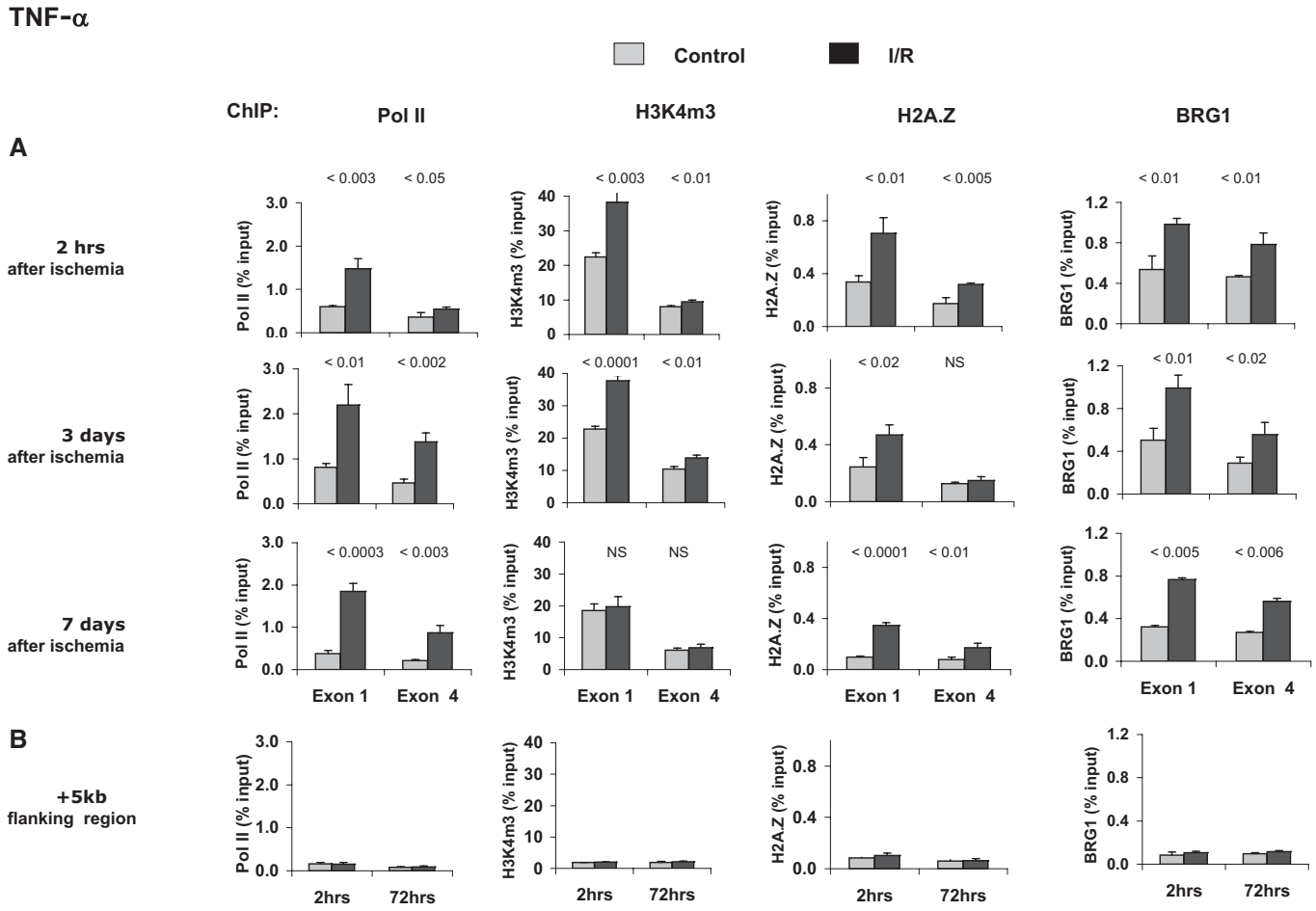

Figure 2. Profiles of Pol II, H3K4m3, H2AZ, and BRG1 after I/R along the *TNF-α* gene. Renal cortical chromatin was prepared from mice subjected to unilateral I/R (■). Renal cortical chromatin from the contralateral kidney of the same mouse was used as control (▨). (A) Levels at the TNF-α first (exon 1) and last (exon 4) exons were assessed using matrix ChIP assays.[24,25] (B) Levels measured in an intergenic region 5 kb downstream of the end of the *TNF-α* gene served as a control. Data are percentage of input DNA, mean ± 1 SD ($n = 3$ mice).

matin structure that are important for induction of transcription.[12,26–29] Trimethylation of lysine 4 of histone H3, H3K4m3, is one of the best studied covalent modifications that mark an open chromatin.[30] Like Pol II, H3K4m3 levels are highest near the transcription start sites.[12,28,31] We previously described increased H3K4m3 levels along several genes in renal cortex from different mouse models of ARF.[10,25] Recently, an increase in H3K4m3 was also reported in response to hypoxia in hepatocytes.[32] In this study, H3K4m3 levels at the first and last exons of the *TNF-α* gene were higher in postischemic kidneys, compared with contralateral controls ($P < 0.003$ and $P < 0.01$; respectively; Figure 2A). Furthermore, H3K4m3 in the intergenic 3′ 5-kb flanking region was very low and not different between the clamped and contralateral kidneys (Figure 2B), serving as a negative internal control. Interestingly, the differences between the injured and control kidneys remained at 3 but not 7 d after I/R. Similar results were obtained along the *MCP-1* gene locus (Figure 3). These results suggest that whereas transcription of *TNF-α* and *MCP-1* genes remains enhanced in the injured kidney, the classical H3K4m3 mark of open chromatin does not remain higher. This observation suggests that there is evolution of epigenetic processes to maintain genomic access after injury.

The H2A.Z histone variant is highly enriched at sites of active transcription,[33] and the substitution of H2A.Z for the canonical H2A histone is increased with stimulation of transcription.[29] At transcribed loci, the H2A.Z levels are highest at the transcription start site.[33] Two hours after I/R, H2A.Z levels at the *TNF-α* gene were higher in the I/R kidneys *versus* their contralateral controls (Figure 2A). Unlike H3K4m3, these differences were sustained for 7 d. The H2A.Z density in the intergenic 3′ 5-kb flanking region were low and not different between the clamped and control contralateral kidneys (Figure 2B; negative internal control). Similar results were obtained along the *MCP-1* gene locus (Figure 3). These results provide further evidence that (*1*) in response to I/R, the chromatin environments encompassing the *TNF-α* and *MCP-1* genes are altered and (*2*) these differences persist for some (H2A.Z) but

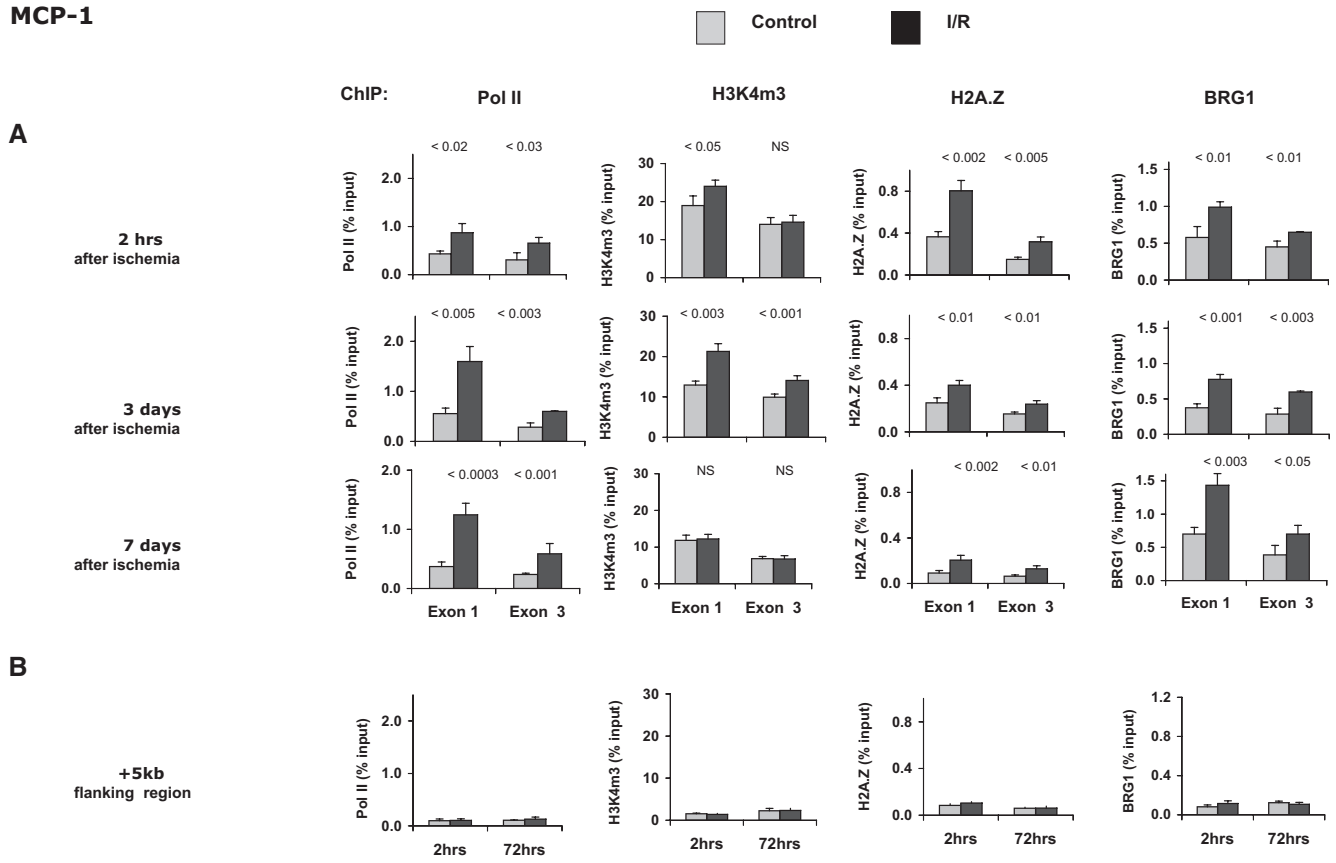

Figure 3. Profiles of Pol II, H3K4m3, H2A.Z, and BRG1 after I/R along the *MCP-1* gene. Renal cortical chromatin was prepared from mice subjected to unilateral I/R (■). Renal cortical chromatin from contralateral kidney of the same mouse was used as control (▨). (A) Levels at the MCP-1 first (exon 1) and last (exon 3) exons were assessed using matrix ChIP assays. (B) Levels measured in an intergenic region 5 kb downstream of the end of the *MCP-1* gene served as a control. Data are percentage of input DNA, mean ± 1 SD ($n = 3$ mice).

not all (H3K4m4) epigenetic marks for at least 1 wk after injury induction.

The ATP-dependent SWI/SNF multisubunit chromatin remodeling complexes are important in the regulation of transcription.[13,29] With respect to our results (Figures 2 and 3) and those obtained from yeast[14,34] to mammals,[29,35] SWI/SNF complexes play a role in the replacement of H2A with H2A.Z. SWI/SNF complexes contain either the BRG1- or the BRM-ATPase catalytic subunit and several BRG-associated factors.[13] The induction of the erythropoietin gene in response to hypoxia depends on BRG1 recruitment to this locus.[21] Recruitment of BRG1 is also required for the induction of heme oxygenase 1 gene transcription in response to oxidative stress.[20] BRG1 recruitment also plays a role in the transcriptional regulation of inflammatory mediators, including TNF-α[36,37]; therefore, we next assessed BRG1 levels along the TNF-α locus in response to I/R. These studies revealed higher levels of BRG1 at the first and last TNF-α exons in injured kidneys, compared with contralateral controls. These effects were observed as early as 2 h after I/R and were sustained for 1 wk. The BRG1 levels in the intergenic 3′ 5-kb flanking region were low and not different between the clamped and contralateral kidneys (Figure 2B). Similar results were obtained along the *MCP-1* gene locus (Figure 3). Thus, the time course of increased levels of BRG1 along the TNF-α and MCP-1 loci is similar to that observed for Pol II and H2A.Z. Matrix ChIP observations suggest that similar epigenetic mechanisms underlie the increased transcription of *TNF-α* and *MCP-1* genes in response to renal ischemia (Figure 1). These observations also suggest that the inducible recruitment of BRG1 plays a role in the induction of these genes in response to renal ischemia.

Simulated I/R in Cultured Proximal Tubule Cells Alters Chromatin and Enhances Recruitment of Pol II and BRG1 along TNF-α and MCP-1 Genes

The combination of antimycin A (AA) and 2-deoxyglucose (DOG) depletes intracellular ATP, and this has been used to simulate oxygen deprivation in cultured cells.[23,38] For example, AA treatment stimulates TNF-α mRNA and protein synthesis in HK-2 cells,[23] recapitulating the *in vivo* proinflammatory mediator expression in response to ischemia. Thus, this system is a potentially useful model to define epigenetic and transcriptional mechanisms that promote cytokine/chemokine expression in response to hypoxic stress. We used matrix ChIP assay[24,25] to

compare Pol II, BRG1, and chromatin profiles along TNF-α and MCP-1 genes in untreated and in reversibly ATP-depleted HK-2 cells (AA + DOG for 4 h, followed by 2 h of ATP recovery [AA + DOG washout]; Figure 4). As in the case of renal ischemia (Figures 2 and 3), ATP depletion in HK-2 cells increased Pol II density along both the *TNF-α* (Figure 4A) and *MCP-1* (Figure 4B) genes. This observation suggests that the reversible ATP depletion–induced cytokine/chemokine mRNA increases in HK-2 cells (Figure 5) is, at least in part, transcription dependent. As in the case of *in vivo* I/R (Figures 2 and 3), the ATP-mediated increase in Pol II density along *TNF-α* and *MCP-1* genes was associated with higher levels of H3K4m3 and H2A.Z at these loci. In addition, there was an injury-mediated recruitment of BRG1 to these genes, which may play a role in the increased expression of TNF-α and MCP-1 in response to ATP depletion. This possibility was tested in the next series of experiments.

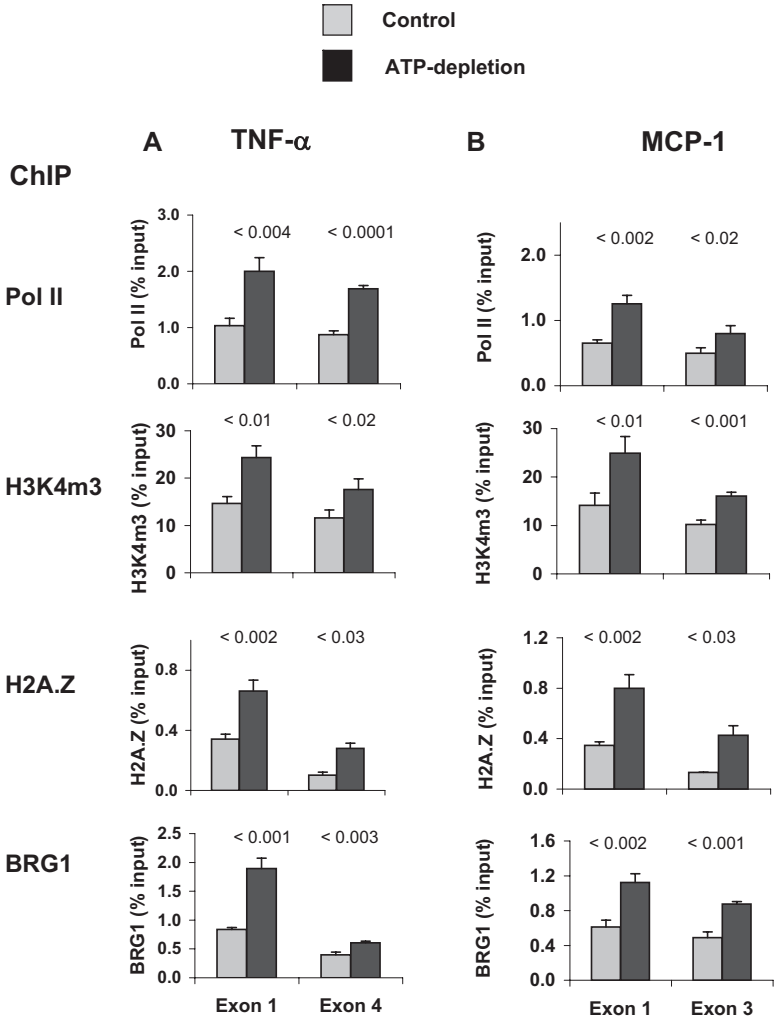

Figure 4. (A and B) Effects of ATP depletion on density profiles of BRG1, Pol II, H3K4m3, and H2A.Z at the *TNF-α* (A) and *MCP-1* (B) genes in proximal tubule HK-2 culture. Cells were subjected to 4 h of ATP depletion and 2 h of recovery (ATP depletion; see the Concise Methods section) (). Simultaneously treated cells, subjected to the same experimental protocol but without a previous ATP depletion (AA + DOG exposure), served as controls (Control). Chromatin was isolated and sheared. Density of given factors and marks at the first and the last exons of *TNF-α* and *MCP-1* genes were assessed using matrix ChIP assays. Data are percentage of input DNA, mean ± 1 SD ($n = 3$ experiments).

Knockdown of BRG1 Attenuates Injury-Induced TNF-α and MCP-1 mRNA Expression in Proximal Tubule HK-2 Cells

We used small interfering RNA (siRNA) knockdown to examine the role of BRG1 in the aforementioned changes (Figure 5). ATP depletion increased BRG-1 mRNA expression. Transfection of cells with BRG1 siRNA decreased BRG1 mRNA levels by approximately 75% in both control ($P < 0.004$) and ATP-depleted ($P < 0.002$) cells (Figure 5). In response to ATP depletion, there was a >2× increase in TNF-α ($P < 0.02$) and MCP-1 ($P < 0.02$) mRNAs in cells transfected with noncomplementary (NC) siRNA. In cells transfected with siRNA BRG1 for 24 h, the constitutive and inducible levels of both TNF-α and MCP-1 mRNAs were greatly decreased. In contrast, there was no change in β-actin mRNA expression in response to injury, and the levels of β-actin transcripts were not different between NC and BRG1 siRNA-transfected cells. Consistent with previous studies,[20,29,36,37,39] these results suggest that the BRG1 chromatin remodeler regulates expression of inducible subsets of genes. The increased expression of *TNF-α* and *MCP-1* genes in response to renal ischemia (Figure 1) that is associated with increased co-recruitment of Pol II and BRG1 to these loci (Figures 2 and 3) suggests that BRG1 plays a role in injury-induced transcription. This point was addressed next.

Knockdown of BRG1 Attenuates Injury-Induced Recruitment of Pol II to TNF-α and MCP-1 Genes in Proximal Tubule HK-2 Cells

Reversible ATP depletion caused a more than threefold increase in the density of BRG1 at the first and last exons of the *TNF-α* gene ($P < 0.001$ and $P < 0.001$, respectively; Figure 6). There was 40 to 80% decrease in BRG1 density at the *TNF-α* locus in cells transfected with BRG siRNA under baseline conditions. Furthermore, the ATP depletion–mediated BRG1 increase was prevented. Similar effects of BRG1 siRNA were seen at the *MCP-1* gene (Figure 7). Together with the renal I/R data (Figures 2 and 3), this experiment indicates that ischemia increases recruitment of the BRG1 chromatin remodeler to the *TFN-α* and *MCP-1* genes in proximal tubule cells.

In agreement with the ChIP results from post-ischemic kidneys (Figure 2), reversible ATP depletion increased Pol II density at the first ($P < 0.08$) and last ($P < 0.001$) exons of the *TNF-α* gene (Figure 6). BRG1 knockdown reduced both the constitutive and the inducible recruitment of Pol II to the *TNF-α*

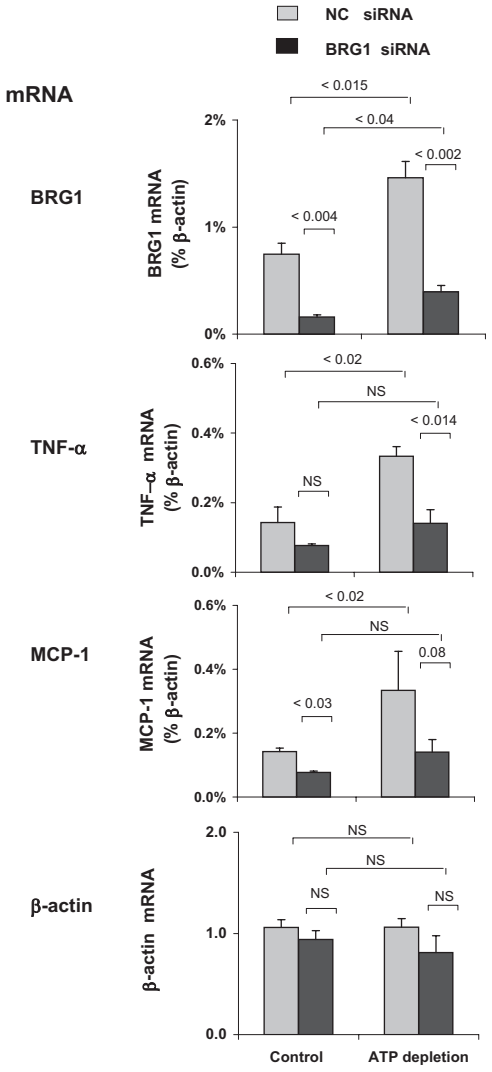

Figure 5. Effects of siRNA BGR1 knockdown on the ATP depletion–induced expression of *TNF-α* and *MCP-1* genes in proximal tubule HK-2 cell culture. After transfection with either BRG1 siRNA (■) or noncomplementary (NC) siRNA (▨), HK-2 cells were treated either without (Control) or with (ATP depletion AA + DOG). Total RNA was extracted and reverse-transcribed, and transcript levels were assessed by real-time PCR done in triplicate using specific primers. mRNA levels are expressed as percentage of β-actin transcript. Data are means ± 1 SD (n = 3 experiments).

gene. Similar results were obtained along the *MCP-1* gene (Figure 7). In sum, these results suggest that the increased expression of *TNF-α* and *MCP-1* transcripts in response to reversible ATP depletion in HK-2 cells (Figure 5) is, at least in part, transcriptionally mediated and that this effect depends on the recruitment of BRG1 to these genes. That BRG1 is also recruited to *TNF-α* and *MCP-1* genes *in vivo* (Figures 2 and 3) suggests that BRG1 plays a role in expression of *TNF-α* and *MCP-1* genes after *in vivo* renal I/R (Figure 1). Unlike the effects on Pol II, changes in the levels of BRG1 recruitment to the *TNF-α* (Figure 6) and *MCP-1* (Figure 7) genes had no effects on H3K4m3. This suggests a hierarchically ordered series of epigenetic events that enhance chromatin access to transcribing Pol II, as suggested in other studies.[12,14,17,40] The lack of BRG1 effect on the level of H3K4m3 suggests that in this chain of events, H3K4m3 changes may operate upstream and are independent of BRG1.[17]

DISCUSSION

Here, we show that 30 min of renal ischemia increases the expression of TNF-α and MCP-1 mRNAs and that these changes persist for up to 1 wk (Figure 1). The prolonged duration of these mRNA changes was associated with comparably long-lasting increases in Pol II and BRG1 recruitment to these proinflammatory genes (Figures 2 and 3). In our *in vitro* model of reversible proximal tubule ATP depletion (Figure 4), siRNA knockdown of BRG1 blocked injury-induced expression of TNF-α and MCP-1 mRNAs (Figure 5) and decreased Pol II and BRG1 recruitment to these genes (Figures 6 and 7). These results provide evidence for the role of BRG1 in the sustained

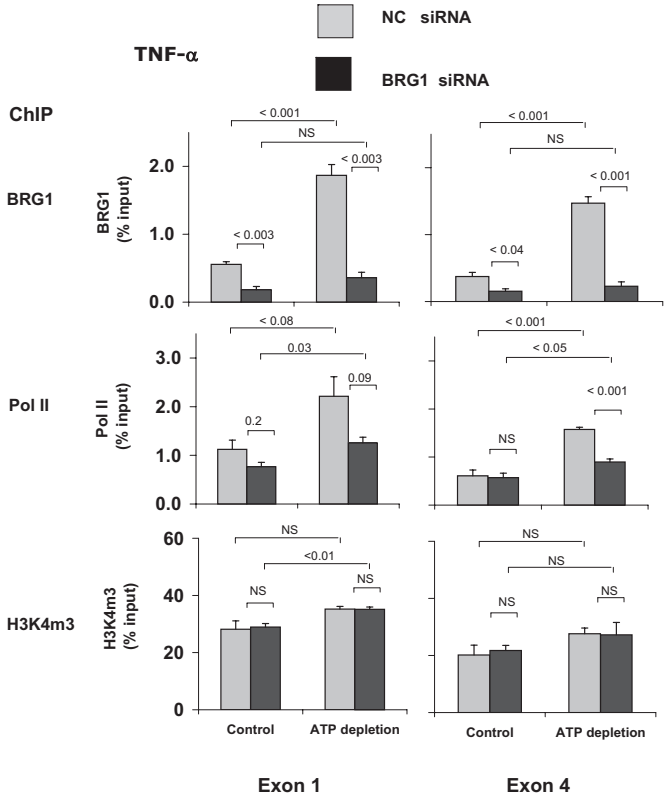

Figure 6. Effects of siRNA-induced BGR1 knockdown on the ATP depletion–induced co-recruitment of Pol II and BRG1 to the *TNF-α* gene in HK-2 cells. Cells were transfected with either BRG1 siRNA (■) specific or NC siRNA (▨). After transfection, HK-2 cells were treated either without (Control) or with (ATP depletion) AA + DOG; chromatin was extracted and sheared. Density at the *TNF-α* first (exon 1) and last (exon 4) exons were assessed using matrix ChIP assay. Data are percentage of input DNA, mean ± 1 SD (n = 3 experiments).

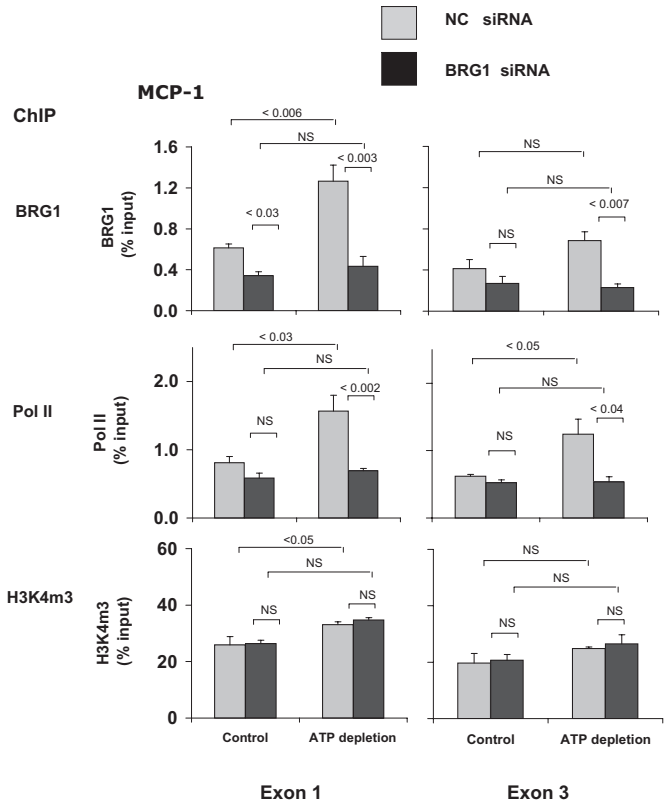

Figure 7. Effects of siRNA BGR1 knockdown on the ATP depletion–induced co-recruitment of Pol II and BRG1 to the *MCP-1* gene in HK-2 cells. Cells were transfected with either BRG1 siRNA (■) specific or NC siRNA (▨). After transfection, HK-2 cells were treated either without (Control) or with (ATP depletion) AA + DOG; chromatin was extracted and sheared. Density at the *MCP-1* first (exon 1) and last (exon 3) exons were assessed using matrix ChIP assay. Data are percentage of input DNA, mean ± 1 SD ($n = 3$ experiments).

transcription of *TNF-α* and *MCP-1* genes in the kidney in the aftermath of renal ischemia.

Given the complexity of epigenetic mechanisms,[27,41] it seems plausible that multiple chromatin alterations could account for sustained increases in *TNF-α* and *MCP-1* gene transcription that follows renal ischemia (Figures 1 through 3). In this (Figures 2 and 3) and in previous studies,[10,25] we found changes in histone marks and the deposition of H2A.Z histone variant along ARF-induced genes, thereby supporting this hypothesis. Others have reported that renal I/R demethylates 5-methylcytosine in the cytosine phosphoguanine (^{5m}CpG) dinucleotides within the NF-κB–binding sites contained in the complement C3 promoter, a process that coincides with a rapid increase in renal C3 synthesis.[42] Presumably, this epigenetic modification exposes docking sites for NF-κB binding. Induction of ^{5m}CpG demethylation of a subset of genes could be one of the epigenetic changes that follow hypoxia.[43] Although not examined here, DNA methylation/demethylation could also be involved in the regulation of *TNF-α* and *MCP-1* gene expression after ischemia. We found that, unlike the sustained increase in the H2A.Z levels, the H3K4m3 mark returned to control levels by 7 d after I/R (Figures 2 and 3). This observation may indicate that whereas a subset of epigenetic processes initiates chromatin changes, different sets of events maintain the open chromatin state. If so, then the persistently higher BRG1 levels along the *TNF-α* and *MCP-1* genes suggest that BRG1 could be involved in both the initiation and the maintenance of Pol II access and elongation. BRG1 is a component of numerous chromatin-modifying complexes.[13,44] These observations are consistent with the suggestion that BRG1 is involved in multiple epigenetic events.

BRG1 is a large protein (predicted molecular weight 185 kD). In addition to its evolutionarily conserved ATPase domain, BRG1 contains domains that mediate multiple interactions with components of SWI/SNF complexes as well as a host of other proteins.[13,44] BRG1 does not directly bind to DNA. Instead, BRG1 recruitment to target loci is indirect and mediated through the association of BRG1 and/or other SWI/SNF components with DNA-binding proteins, such as transcription factors, co-activators, and histone tails. Transcription factors, such as nuclear receptors, are thought to play a role in the recruitment of BRG1 to promoters of genes.[20,29,37,45] BRG1 is also found at enhancer sites.[46] At the promoter and enhancer regions, BRG1 likely serves to open access to DNA-binding transcription factors. These results expand on our understanding of this issue, because we found that BRG1 also binds along the entire transcribed regions of *TNF-α* and *MCP-1* genes. Indeed, this achieved a pattern that was similar to that observed for Pol II (Figures 2 through 4, 6, and 7). This new insight could potentially have mechanistic relevance for Pol II binding and, hence, gene transcription rates. Elongating Pol II carries a large molecular cargo, but, thus far, there is no evidence that BRG1-SWI/SNF travels with the polymerase. Instead, upon induction and before Pol II binding, SWI/SNF complexes are recruited to histone tails along transcribed regions[17] to remove the nucleosomal barrier, allowing efficient polymerase elongation.[47,48] Future studies will address such a possibility and search for BRG1 docking sites along the proinflammatory genes in response to acute kidney injury and how BRG1 recruitment to these genes may affect cell phenotype in response to injury.

In conclusion, this study has presented new evidence that I/R-mediated *TNF-α* and *MCP-1* gene induction is associated with changes in the histone profiles along these genes (most notably, increases in H2A.Z and H3K4m3). Furthermore, there is a parallel recruitment of both BRG1 and Pol II to these sites. Because BRG1 knockdown in HK-2 cells reduced Pol II binding, as well as TNF-α and MCP-1 mRNAs, a mechanistic link between BRG1, Pol II binding, and, ultimately, gene transcription seems to exist. This suggests a novel pathway by which I/R elicits an inflammatory response. It also allows new potential therapeutic approaches (*e.g.*, interruption of BRG1 enzymatic activity) to mitigate postischemic renal injury, potentially circulating cytokine levels, and multiorgan failure. The technological and computational advances that are currently being made in this area provide exciting new opportunities to explore fully such issues.

Table 1. List and sequences of RT-PCR primers

Primer	Sequence
Human	
TNF-α exon 4 (ID 7124)	
left	5′-TAGATGGGCTCATACCAGGG-3′
right	5′-CCGTCTCCTACCAGACCAAG-3′
MCP-1 exon 3 (ID 6347)	
left	5′-AGCTGCAGATTCTTGGGTTG-3′
right	5′-AAGGAGATCTGTGCTGACCC-3′
BRG1 exon 2/3 (ID 6597)	
left	5′-CCCATTCCTTTCATCTGGTTG-3′
right	5′-ACCCTCAGGACAACATGCAC-3′
β-actin exon 4/5 (ID 60)	
left	5′-AGAGCTACGAGCTGCCTGAC-3′
right	5′-AAGGTAGTTTCGTGGATGCC-3′
Mouse	
TNF-α exon 1 (ID 21926)	
left	5′-ACCGTCAGCCGATTTGCTATCTCA-3′
right	5′-TGTAGGGCAATTACAGTCACGGCT-3′
MCP-1 exon 1/2 (ID 20296)	
left	5′-TCACCTGCTGCTACTCATTCACCA-3′
right	5′-AAAGGTGCTGAAGACCCTAGGGCA-3′
GAPDH (ID 14433)	
left	5′-CTGCCATTTGCAGTGGCAAAGTGG-3′
right	5′-TTGTCATGGATGACCTTGGCCAGG-3′

CONCISE METHODS

In Vivo I/R Protocol

Male CD 1 mice (Charles River Laboratories, Wilmington, MA; 30 to 35 g), maintained under routine vivarium conditions and subjected to institutional animal care and use committee–approved protocols, were used for all experiments. In brief, mice were anesthetized and subjected to a midline abdominal incision under sterile conditions. Left renal ischemia was induced with an atraumatic microvascular clamp applied to the renal pedicle. After 30 min of vascular occlusion, the clamp was released and reperfusion of the entire kidney was assessed visually (by loss of global cyanosis). A 30-min ischemic insult was selected for study because it induces moderately severe but reversible ischemic kidney damage. At variable times after ischemia, the mice were re-anesthetized and the kidneys were resected and processed for analyses. With the unilateral ischemia protocol, azotemia is not predicted. As previously documented,[23] the right nonischemic, contralateral (control) kidney recapitulates what is seen in sham-operated kidneys and, hence, served as an internal control.

In Vitro "Chemical Ischemia" (ATP depletion) Experimental Protocol

The HK-2 immortalized proximal tubule cell culture maintained in keratinocyte serum-free medium was seeded into T75 flasks, as described previously.[38] Culture conditions included 100 U/ml penicillin plus 25 μg/ml streptomycin. For experimentation, the cells were seeded into six-well Costar plates and then studied approximately 18 h later.

To assess the effects of reversible ATP depletion in HK-2 cells, we used AA-induced mitochondrial inhibition (7.5 μM) + DOG-induced (20 mM) inhibition of glycolysis, as described previously.[23,38] After 4 h of AA + DOG exposure, ATP depletion was reversed by removal of the cell culture medium, washing with fresh medium, and then allowing a 2-h recovery period.

siRNA Knockdown Experiments

siRNA knockdowns were done using siRNA Gene Silencing Santa Cruz Protocol (http://www.scbt.com/protocols.html). Briefly, cells were grown in six-well plates to 30 to 50% confluence. Lipofectamine 2000 (Invitrogen, Carlsbad, CA) and either BRG1 siRNA (sc-29827; Santa Cruz Biotechnology, Santa Cruz, CA) or NC siRNA (sc-37007; Santa Cruz Biotechnology) Transfection Reagents was diluted separately with siRNA Transfection Medium (Opti-MEM I; Invitrogen). After 5 min of incubation, the diluted siRNA and Lipofectamine 2000 were combined and the mixture was incubated for another 30 min at

Table 2. List and sequences of matrix ChIP PCR primers

Primer	Sequence
Human	
TNF-α (ID 7124)	
exon 1	
right	5′-CCACGATCAGGAAGGAGAAG-3′
left	5′-CCTGGAAAGGACACCATGAG-3′
exon 4	
right	5′-TAGATGGGCTCATACCAGGG-3′
left	5′-CCGTCTCCTACCAGACCAAG-3′
MCP-1 (ID 6347)	
exon 1	
right	5′-GAATGAAGGTGGCTGCTATG-3′
left	5′-AACCCAGAAACATCCAATTCTC-3′
exon 3	
right	5′-AGCTGCAGATTCTTGGGTTG-3′
left	5′-AAGGAGATCTGTGCTGACCC-3′
Mouse	
TNF-α (ID 21926)	
exon 1	
right	5′-GCAGGTTCTGTCCCTTTCAC-3′
left	5′-AGTGCCTCTTCTGCCAGTTC-3′
exon 4	
right	5′-TATGGCTCAGGGTCCAACTC-3′
left	5′-GCTCCAGTGAATTCGGAAAG-3′
intergenic 3′ 5 kb	
right	5′-CCAGACTCAGAACTAGGACCG-3′
left	5′-GGTAAACAGGAAGCTGGGTG-3′
MCP-1 (ID 20296)	
exon 1	
right	5′-GCCAACACGTGGATGCTC-3′
left	5′-AGCCAACTCTCACTGAAGCC-3′
exon 3	
right	5′-TTAAGGCATCACAGTCCGAG-3′
left	5′-TTGAATGTGAAGTTGACCCG-3′
intergenic 3′ 5 kb	
right	5′-TTTCATCATGGCAGGGAAAC-3′
left	5′-GCTGCTATCTTGGACTATGCG-3′

Table 3. List of antibodies used in matrix ChIP

Antibody	Type	Source	Catalog	Amount/ChIP (μg)
Pol II CTD (4H8)	Monoclonal	Gene Tex	GTX25408	0.25
H2A.Z	Rabbit polyclonal	Abcam	ab4174	0.50
H3K4m3	Rabbit polyclonal	Abcam	ab8580	0.50
BRG1	Rabbit polyclonal	Upstate	07-478	0.50

room temperature. The mixture was then overlain onto the medium bathing cells in the wells. After 12 to 24 h, the overlay was aspirated and replaced with fresh culture medium for additional 24 to 48 h before mRNA and ChIP experiments.

RNA Extraction

TRIzol was used to purify RNA according to the manufacturer's protocol (Invitrogen). Excess TRIzol was used to separate clearly the RNA and DNA phases. Total extracted RNA was dissolved in 20 μl of sterile water and stored at −80°C. RNA concentrations were measured with a spectrophotometer (Bio Mate3; Thermo Fisher Scientific, Waltham, MA), and the 260/280 ratio of RNA was >1.7.

RT-PCR

First-strand cDNA was synthesized by priming 1 μg of total RNA with 10 μM random hexamers (Promega, Madison, WI) by heating at 65°C for 10 min and snap-cooling on ice. Reverse transcription (37°C for 1 h) was performed in the presence of 10 mM each of dATP, dCTP, dTTP, and dGTP (Invitrogen), 4 μl of 5× first-strand buffer (Invitrogen), 0.1 M dithiothreitol (Invitrogen), 200 U of Maloney-murine leukemia virus reverse transcriptase (Invitrogen), and 20 U of RNase inhibitor (Invitrogen). After the reaction, the sample was heated at 94°C for 5 min and cooled on ice; after addition of 180 μl of water, samples were stored at −80°C. RT cDNA samples were measured either with competitive (Figure 1) or real-time PCR (Figure 5). List of RT-PCR primers is shown in Table 1.

Sonication of Renal Cortex Chromatin

Approximately 25 mg of minced renal cortex was fixed with formaldehyde (final concentration 1.42% in PBS for 15 min; 22°C) and then quenched with 125 mmol/L glycine (5 min, 22°C). The cross-linked tissues were then extensively washed with PBS (4°C). For shearing the chromatin, the washed cross-linked tissue pellets were resuspended in IP buffer.[49] The shearing was done using either Masonic 3000 microprobe (1 ml of IP buffer, six rounds of sonication power 5, 15 s, on ice)[49,50] or Diagenode Bioruptor (100 μl of IP buffer, 30 rounds for 30 s on/30 s off, high power, 4°C). The suspension was cleared by centrifugation at 12,000 × *g* (10 min at 4°C), and the supernatant, representing sheared chromatin, was aliquotted and stored at −80°C.

Microplate-Based Matrix ChIP Platform

ChIP assays were done using the Matrix ChIP platform in 96-well polystyrene high-binding capacity microplates as described previously.[24,25,49] ChIP DNA samples were assayed by quantitative PCR. All PCR reactions were run in triplicate. PCR primers were designed using the Primer3 software (http://frodo.wi.mit.edu/). At least four samples were run for each determination. PCR calibration curves were generated for each primer pair from a dilution series of total mouse or human sheared genomic DNA. The PCR primer efficiency curve was fit to cycle threshold *versus* log (genomic DNA dilutions) using an r^2 best fit. DNA concentration values for each ChIP and input DNA samples were calculated from their respective average cycle threshold values. Final results are expressed as percentage input DNA.[24] Matrix ChIP PCR primers are shown in Table 2 and the list of antibodies in Table 3.

Statistical Analysis

All results are presented as means ± 1 SD. Statistical comparisons were made by unpaired *t* test (significance judged by $P < 0.05$). When multiple statistical comparisons were made, the Bonferroni correction was applied.

ACKNOWLEDGMENTS

This work was supported by research grants from the National Institutes of Health (DK-R37-45978 and GM45134 [K.B.] and DK-R37-38431 and DK-68520 [R.Z.]).

We thank Ali Johnson for expert technical support on this project.

DISCLOSURES

None.

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