In Vivo Chromatin Remodeling Events Leading to Inflammatory Gene Transcription under Diabetic Conditions*

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The transcription factor NF-κB (NF-κB) plays a pivotal role in regulating inflammatory gene expression. Its effects are optimized by various coactivators including histone acetyltransferases (HATs) such as CBP/p300 and p/CAF. Evidence shows that high glucose (HG) conditions mimicking diabetes can activate the transcription of NF-κB-regulated inflammatory genes. However, the underlying in vitro transcription and nuclear chromatin remodeling events are unknown. We therefore carried out chromatin immunoprecipitation (ChIP) assays in monocytes to identify 1) chromatin factors bound to the promoters of tumor necrosis factor-α (TNF-α) and related NF-κB-regulated genes under HG or diabetic conditions, 2) specific lysine (Lys (K)) residues on histone H3 (H3K) and H4 acetylated in this process. HG treatment of THP-1 monocytes increased the transcriptional activity of NF-κB p65, which was augmented by CBP/ p300 and p/CAF. ChIP assays showed that HG increased the recruitment of NF-κB p65, CBP, and p/CAF to the TNF-α and COX-2 promoters. Interestingly, ChIP assays also demonstrated concomitant acetylation of H3K at Lys6 and Lys14, and H4K at Lys5, Lys8, and Lys12 at the TNF-α and COX-2 promoters. Overexpression of histone deacetylase (HDAC) isosforms inhibited p65-mediated TNF-α transcription. In contrast, a HDAC inhibitor stimulated gene transcription and histone acetylation. Finally, we demonstrated increased H3K acetylation at TNF-α and COX-2 promoters in human blood monocytes from type 1 and type 2 diabetic subjects relative to nondiabetic. These results show for the first time that diabetic conditions can increase in vivo recruitment of NF-κB and HATs, as well as histone acetylation at the promoters of inflammatory genes, leading to chromatin remodeling and transcription.

Certain key factors have been implicated in diabetes-induced accelerated atherosclerotic and inflammatory disease including hyperglycemia (1, 2), oxidant stress (3, 4), advanced glycation end products (AGEs) (5, 6) and protein kinase C (7). Normalizing mitochondrial superoxide production could block three pathways induced by hyperglycemic damage (8). However, the specific molecular mechanisms and nuclear events involved in the transcription of pathological genes under diabetic conditions are not fully resolved. Circulating monocytes in diabetic individuals would be continuously exposed to hyperglycemic conditions. We recently showed that in vitro culture of monocytes under high glucose (HG) versus normal glucose (NG) conditions led to the activation of the transcription factor nuclear factor κB (NF-κB) and significantly increased the expression of several inflammatory chemokines and cytokines (9, 10). The potent inflammatory cytokine, tumor necrosis factor-α (TNF-α), chemokine, and monocyte chemoattractant protein-1 (MCP-1) were among the factors induced (9, 10). Furthermore, these factors were transcriptionally regulated by HG with NF-κB being a major regulatory factor. In addition, the involvement of multiple upstream signaling pathways such as oxidant stress, protein kinase C, and mitogen-activated protein kinases were demonstrated (9, 10). Recent studies, including our own, indicate that HG, AGEs, and the receptor of AGE ligand S100B can activate NF-κB in vitro in monocytes and lead to the expression of inflammatory genes such as TNF-α, MCP-1, interleukins, and cyclooxygenase-2 (COX-2) (9–14). NF-κB activity was also increased in monocytes from diabetic individuals (13). However, very little is known about the specific molecular in vivo transcription mechanisms and nuclear chromatin remodeling events underlying the regulation under diabetic conditions of these NF-κB-regulated inflammatory genes implicated in monocyte activation and dysfunction.

NF-κB is an inducible transcription factor that plays a pivotal role in regulating the expression of more than 100 genes including TNF-α, MCP-1, and COX-2 (15, 16). NF-κB is a heterodimer that usually consists of 65- and 50-kDa subunits (p65 and p50) complexed to its inhibitor, IκB-α, in the cytoplasm (15, 16). Upon cell stimulation by stimuli such as TNF-α, the IκB unit is phosphorylated, ubiquitinated, and degraded, thereby allowing free NF-κB heterodimer to be transported to the nucleus and participate in gene regulation (16). p65 is a key transcriptionally active component of NF-κB. Our recent data shows that HG can increase nuclear p65 and decrease cytosolic IκB-α levels in monocytes (9).

The nuclear transactivation potential of NF-κB has been shown to require a number of different coactivators including CBP/p300, p/CAF, and SRC-1 (17). These cofactors have histone acetyltransferase (HAT) activity and play key roles in the transcription machinery (18, 19). Histone acetylation has been shown to be associated with increased gene transcription (18–20). Histone acetylation is an important early event in the transcriptional activation by NF-κB, and HATs play a pivotal role in regulating inflammatory gene expression.

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The abbreviations used are: AGE, advanced glycation end product; HG, high glucose; NG, normal glucose; TNF-α, tumor necrosis factor-α; HAT, histone acetyltransferase; HDAC, histone deacetylase; ChIP, chromatin immunoprecipitation; H3K, histone H3; H4K, histone H4; TSA, trichostatin; IL, interleukin; RT, reverse transcription; PBMC, peripheral blood monocytes; Ab, antibody; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CBP, CREB-binding protein (where CREB is CAMP-responsive element-binding protein).
22). Acetylated histone proteins confer accessibility of the DNA template to the transcriptional machinery for gene expression. Histone deacetylases (HDACs), on the other hand, catalyze removal of acetyl groups on amino-terminal lysine residues and act as transcriptional repressors or silencers of genes (23, 24). The exchange of HATs for HDACs can mediate regulated gene expression. It is now clear that, apart from binding of transcription factors to their promoter DNA binding sites, transcriptional activation or repression is also linked to the recruitment of protein complexes that alter chromatin structure and architecture via enzymatic modifications of histone tails and/or nucleosome remodeling. Thus, although NF-κB and p65 seem to be involved in gene transcription under diabetic conditions, the specific in vivo regulation mechanisms at the level of chromatin are not known.

In the present study, we hypothesized that diabetic conditions lead to chromatin remodeling and key alterations in the nuclear transcriptional mechanisms that induce inflammatory gene transcription and enhanced monocyte activation. We have effectively used chromatin immunoprecipitation (ChIP) assays to demonstrate for the first time the occurrence of chromatin rearrangements at the promoters of inflammatory genes in vivo in monocytes under diabetic conditions. We noted that H3 culture of monocytes that mimics diabetic conditions could specifically increase the recruitment of coactivator HATs such as CBP and p/CAF to the promoters of inflammatory genes and leading to the acetylation of nucleosomal factors histone H3 (H3H) and H3H4 while also decreasing the association of HDACs. There was concomitant recruitment of NF-κB p65 to the promoters of key HG-induced NF-κB-dependent genes such as TNF-α and COX-2. Additionally, we demonstrated in vivo relevance by examining histone acetylation patterns in monocytes from diabetic patients. Our results reveal for the first time that in vivo chromatin remodeling occurs under diabetic conditions in cell culture and in patients.

EXPERIMENTAL PROCEDURES

Materials—Anti-p65, anti-p50, and anti-CBP antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). All other antibodies were from Upstate Biotechnology (Lake Placid, NY). Trichostatin (TSA) was from Sigma. 293TNF-αLuc plasmid was a gift from Dr. J. S. Economou (University of California, Los Angeles). p65 expression vector (hemagglutinin-tagged) was a gift from Dr. E. Zandi (University of Maloney murine leukemia virus RT using a GeneAmp RNA PCR kit. 1/20th fraction was used in multiplex RT-PCRs with cytokine-specific primers paired with Quantum 18 S RNA internal standards (10, 12). The primers used to amplify TNF-α cDNA were 5′-ATGGCCACACT-GACCTCCTC-3′ and 5′-TAGATGGGCTCATACCAAGG-3′ (product: 346 bp) from the coding region; and for COX-2, 5′-CAGCACCTTCCAAGC-GAACTG-3′ and 5′-TCTGGTCACTAGGTTTTCGTT-3′ (product: 756 bp). PCRs were performed for 25–30 cycles at 94°C for 30 s, 60°C for 1 min, and 72°C for 1 min. PCR products were fractionated on 2% agarose gels and quantitated as described (10, 12). Results are expressed as relative changes in intensity after normalization for 18 S RNA levels.

RESULTS

p65-dependent Transcription from the TNF-α Promoter Is Augmented by CBP and p/CAB and Further Enhanced by HG—Evidence shows that NF-κB p65 can interact with CBP and its structural homolog p300, and also p/CAB (17, 20, 26), to stimulate NF-κB-dependent transactivation and gene expression. There is also evidence that the HAT activity of p/CAB, but not that of CBP, is required to coactivate p65-dependent transcription (17). Since HG can augment the expression of NF-κB-dependent genes such as TNF-α, we first examined whether HG can affect CBP/p300, p/CAB-, and p65-mediated transcriptional activation of the TNF-α promoter. We transfected THP-1 cells with a minimal hTNF-α promoter (295TNF-αLuc) with or without expression vectors for p65, CBP, p300 or p/CAB for 16 h and then cultured the cells under NG (5.5 mM) or HG (25 mM) conditions for 24 h. Luciferase activities in Fig. 1A show that TNF-α transcription is dependent on p65. Furthermore, Fig. 1B shows that this is increased under HG conditions (second bar set). Furthermore, CBP, p300, and p/CAB could each clearly enhance p65-mediated increase in TNF promoter activity under NG conditions and these are slightly, but not significantly, stimulated by HG versus NG conditions (third, fourth, and fifth bar sets). Interestingly, the transcriptional activities of p65 (second bar set) as well as coactivator effects of CBP, p300, or p/CAB (sixth, seventh, and eighth bar sets) are...
ChIP assays were then performed with anti-p65 Ab. Enrichment of specific DNA sequences in chromatin immunoprecipitations indicating association of the antigen (p65) with specific NF-κB DNA binding sites within intact chromatin was visualized by PCR. The target sequences for PCR were located in the gene promoter region around specific NF-κB sites, except with GAPDH and TNF-α exon controls. A, ChIP assay of p65 binding at the TNF-α promoter. PCR analysis was performed on immunoprecipitation samples without (w/o) Ab, p65 Ab, and purified total input DNA (Input DNA). The first four lanes of the upper panel represent PCR products of TNF-α promoter amplification: w/o Ab, NG (5.5 mM), HG (25 mM), MANN (5.5 mM glucose + 20 mM mannitol); the last two lanes are GAPDH (HG cells and GAPDH primers in PCR) and EXON (HG cells with TNF-α exon primers in PCR). The lower panel is amplification of input DNA prior to immunoprecipitation. B, ChIP assay to determine p65 binding at the IL6, COX-2, and MCP-1 promoters under NG versus HG conditions in THP-1 cells. PCRs were performed to amplify regions of each of these promoters around NF-κB sites.

ChIP Assays to Evaluate p65 Binding to the Promoters of Inflammatory Genes—The ChIP assay is a powerful technique to determine true in vivo binding of transcription factors and other nucleosomal proteins to chromatin in response to an agonist (25, 27). We used this assay to determine the status of the NF-κB transcription complex at the promoters of key NF-κB-regulated genes in THP-1 cells in response to HG. Specifically we evaluated the recruitment of NF-κB p65 and p50 subunits and the HAT coactivators, CBP and p/CAF, using antibodies specific for each of these factors. After the ChIP protocol, gene promoter regions were amplified and analyzed by semiquantitative PCRs using specific primer pairs around NF-κB binding regions on the promoters of TNFα, MCP-1, COX-2, and IL-6. All of these genes are known to be regulated at least in part by NF-κB.

THP-1 cells cultured for 3 days in NG or HG were subjected to ChIP first with an antibody (Ab) to p65. Enrichment of specific DNA sequences in the chromatin immunoprecipitates, which indicates association of these ligand factors to DNA strands within intact chromatin, were visualized by PCR amplification. PCR was designed to amplify a 486-bp region of the hTNF-α promoter (−453 to +33) (24 bp from either end used as primers), which contains key κB binding elements. An increase in the relative amount of amplified TNF-α promoter-specific PCR product indicates binding. Fig. 2A clearly shows selective increased occupancy by p65 on the TNFα promoter by 48-h HG treatment (over 5-fold). Very little or no binding is seen in NG. Important controls demonstrate the specificity of HG effects, since there is no amplified band in the absence of Ab (lane 1), with mannitol (MANN, band similar to NG), with primers that amplify GAPDH promoter, or to an open reading frame exon-coding region on TNF-α gene (EXON). Control amplification is with total input DNA (Input DNA) (lower panel of Fig. 2A). There is no change in the amplification of input DNA in all the cases.

In addition, we also examined the promoters of other NF-κB target genes that are regulated by HG, namely MCP-1, COX-2, and IL-6. p65 is again similarly recruited in vivo to these gene promoters in response to HG (Fig. 2B).
We also conducted ChIP assays to evaluate changes at the human COX-2 promoter, which is also regulated by NF-xB (12, 28). Fig. 3B shows the time course (16–24 h) of HG effects and indicates that CBP, p/CAF, and p65 are recruited in a temporal sequence to the COX-2 promoter similar to TNF-α promoter. p50 is also recruited with similar kinetics. The intriguing new result is that, while the transcriptional repressor HDAC1 is associated to the COX-2 promoter under basal NG conditions (lane 1), however, with increasing time of HG treatment, the levels of bound HDAC1 steadily decreased while bound CBP levels increased. Interestingly, HDAC1 is reported to appear in complexes containing various corepressors like Sin3 and Mi-2-NuRD complex (29) and hence can mediate gene repression. These new results suggest that HG conditions can elicit dynamic alterations in HDAC to HAT activities, in vivo recruitment of transcription factors and coactivators, and histone acetylation at inflammatory gene loci in vivo, culminating in increased gene expression.

Evidence shows that TNF-α can auto regulate its own gene expression. Fig. 3C shows that TNF-α increases p65 and CBP recruitment and also the acetylation of H3 at the TNF-α promoter similar to the actions of HG. Furthermore, TNF-α effects are augmented under HG conditions.

Identification of Specific Lysines Acetylated on HH3 and HH4—Emerging data indicates the importance of specific patterns of histone acetylation at lysine residues for the final outcome of gene expression versus silencing. We therefore wanted to determine which specific lysines of HH3 and HH4 are acetylated at the TNF-α promoter in response to HG. For these, we performed ChIP assays with specific Abs to HH3 acetylated at lysine (Lys (K)) 9 or Lys 14 or Abs to HH4 acetylated at Lys8, Lys12, or Lys16 (key histone modifications involved in gene transcription). Fig. 4A shows that HG leads to a time-dependent increase in the acetylation of Lys9 and Lys14 on HH3 at the TNF promoter region, with Lys9 effects being more intense. Furthermore, it appears that, temporally, these events occur parallel to p65, CBP, and p/CAF recruitment. This is supported by data showing that CBP can in fact acetylate H3K9 and H3K14 (20) and that the HAT activity of p/CAF is important for NF-xB-mediated transactivation (17). Acetylation at H4K5 and H4K12 appear to be low and also only marginally responsive to HG, suggesting that acetylation at these sites may not be critical to the transcriptional response of p65 by HG. Interestingly, HH4 associated with DNA appears to be constitutively acetylated at Lys8 even in the basal state (fourth panel from the top). It is tempting to conjecture that this site would be associated with constitutively expressed genes.

We similarly evaluated the nature of lysines acetylated on the COX-2 promoter in response to HG. Fig. 4B shows that here also H3K9 acetylation is increased in response to HG in a time-dependent manner similar to CBP and p65, but there were distinct differences in acetylation status and acetylation kinetics between the two promoters. HH3 and HH4 on the TNF-α promoter appeared to be more easily acetylated compared with COX-2 promoter. Furthermore, while H4K8 around the TNF promoter was constitutively acetylated, it appeared to be only mildly acetylated at the COX-2 promoter in the basal NG state and then further acetylated by HG. Unlike the TNF promoter, H3K14 and H4K12 were not acetylated at the COX-2 promoter. These new results indicate a novel critical level of regulation at the level of chromatin. Thus, these results demonstrate that, although several genes have NF-xB binding sites on their promoters, specific agonist-dependent differential gene expression can be dictated by the status of histone acetylation as well as occupancy of other chromatin factors in vivo.

HDACs Inhibit p65-mediated Transcriptional Activation; Reversal by TSA—Histone acetylation is regulated by HATs, which promote acetylation, and HDACs, which promote deacetylation. HDACs remove the acetyl moieties from the lysine residues of histones causing rewinding/condensation of DNA, displacement of transcription factors from their cognitive DNA binding sites, thereby leading to silencing of gene transcription. To prove that histone acetylation is vital to NF-kB mediated transcription in our system, we cotransfected 295TNF-αLuc with either class I (hHDAC1–3) or class II (hHDAC4–6) expression vectors into THP-1 cells. Results show that this clearly decreased p65-mediated TNF-α promoter activity (Fig. 5). On the other hand, the HDAC inhibitor TSA, or HG, could enhance luciferase activity in each condition. However, HDAC2, HDAC3, and HDAC5 were much more potent inhibitors in the presence or absence of TSA or HG.

TSA TreatmentEnhances p65-mediated Transcriptional Activation and Histone Acetylation—TSA is a specific inhibitor of multiple HDACs (20, 29) and broadly inhibits the action of HDACs. Hence it is believed to enhance transcription through inhibiting HDACs and increasing histone acetylation around the promoter of a gene. To demonstrate the in vivo relationship between p65-mediated gene transcription and specific histone acetylation, THP-1 cells were first treated with 300 nM TSA for...
24 h, then ChIP assays were performed to analyze the status of acetylation of histones at H3K9, H3K14, H4K5, H4K8, H4K12, and H4K16 at the TNF-α and COX-2 promoters. The results in Fig. 6A show that TSA treatment increases acetylation at all these sites. Interestingly, histones at the TNF promoter appear to be relatively more sensitive to TSA than on COX-2 promoter. This could be a reflection of their different acetylation status or the difference in action of the specific HDACs and proteins associated with these promoters.

We also performed RT-PCRs to evaluate TNF-α and COX-2 gene expression under these conditions. Fig. 6B shows that TSA, similar to HG, could enhance TNF-α and COX-2 mRNA expression. 18S RNA as internal control in the relative RT-PCR is shown in the upper bands in each of the two panels. These results further prove that histone acetylation at these specific lysine sites directly increases gene expression.

**Increased Histone H3K9 and H3K14 Acetylation in Monocytes from Patients with Diabetes**—Evidence shows that hyperglycemia can lead to the transcription of inflammatory genes with NF-κB being one of the key transcription factors involved. Our new data now shows that in cultured monocytes, HG can increase histone acetylation. Circulating monocytes in vivo in diabetic individuals would be continuously exposed to hyperglycemic conditions. To evaluate the in vivo functional relevance of our observations in THP-1 cells, we compared histone acetylation status in blood monocytes from patients with diabetes relative to non-diabetic healthy volunteers. PBMC isolated from these human subjects were subjected to ChIPs with Abs to acetylated histones H3K9 or H3K14 as indicated. Fig. 7 shows the results of these ChIP assays. Data shown is from two normal control volunteers and four diabetic patients (two with type 1 diabetes (patients 1 and 3) and two with type 2 diabetes (patients 2 and 4)). Interestingly, it is seen that all four diabetic patients showed marked H3K9 acetylation at the TNF-α promoter and that, except for patient 2, they also showed H3K9 acetylation even at the COX-2 promoter. On the other hand, H3K14 acetylation was less evident overall and could be clearly seen in only one patient (patient 1). However, there was no significant H3K9 or H3K14 acetylation in the normal controls. Quantitation of the band densities after normalization with corresponding input DNA shows that diabetic patients have significant increase in H3K9 acetylation relative to controls at both the TNF and COX-2 promoters (Fig. 7B). These new results demonstrate evidence of higher H3K9 and possibly Lys14 acetylation (modifications associated with positive gene expression) at the promoters of inflammatory genes in diabetic patients, implicating a more open state of chromatin primed for transcription. These data with diabetic monocytes provide in vivo functional relevance and molecular mechanisms by which HG, AGEs, and diabetes itself can augment the expression of key inflammatory genes.

**DISCUSSION**

Histone acetylation has been shown to be associated with increased gene transcription (18–22) and hence with transcriptionally active chromatin domains. Acetylated histones confer accessibility of the DNA template to the transcriptional machinery for gene expression. HDACs (23, 24), on the other hand, mediate the recruitment of chromatin modifiers such as HATs, histone kinases, and methyltransferases. These re- sultant epigenetic changes and posttranslational modifications occur on histone NH2-terminals in the form of specific patterns of acetylation, phosphorylation, methylation, or ubiquitination and thus extend the information content of the DNA code (35–37). Furthermore, a more sophisticated additional regulatory role in gene transcription is the idea that distinct acetylation sites in histone tails perform different roles.

An important implication of the histone code hypothesis is that promoter DNA alone and binding of transcription factors are not sufficient for the deployment of complex patterns of gene expression. Instead, inputs received at promoter DNA in the form of multiple transcription factor binding events can mediate the recruitment of chromatin modifiers such as HATs, HDACs, histone kinases, and methyltransferases. These re- sultant epigenetic changes and posttranslational modifications occur on histone NH2-terminals in the form of specific patterns of acetylation, phosphorylation, methylation, or ubiquitination and thus extend the information content of the DNA code (35–37). Furthermore, a more sophisticated additional regulatory role in gene transcription is the idea that distinct acetylation sites in histone tails perform different roles.
nuclear transcriptome in a temporal fashion, thereby showing that diabetic conditions can induce novel in vivo chromatin remodeling events to induce inflammatory genes. Additionally, we obtained key in vivo relevance, since our ChIP assays revealed for the first time that monocytes obtained from diabetic patients have increased acetylation of H3K9 at the TNF-α and COX-2 promoters relative to non-diabetic volunteers. This provides evidence that, under diabetic conditions, chromatin region around the locus of inflammatory genes is in an open state primed for gene transcription.

Identification of histone modification patterns in a cell under normal conditions and during transcription form the basis of functional significance of the histone code (30, 31). The results reported here add to the understanding of the connection between histone acetylation and transcription in the following ways. First, there exists a set of basal histone marks such as acetylated H4K8, which ensure that the chromatin around specific NF-κB-regulated genes is in an activation mode (transcription ready mode). Second, H3K9, H3K14, H4K5, and H4K12 are acetylated during NF-κB activation. Third, various NF-κB-regulated genes appear to respond to the stimulus differently. The TNF-α promoter appeared to be relatively more sensitive than COX-2 as reflected by the extent of histone acetylation. More precisely, H4K8 was highly acetylated at the TNF-α promoter in the basal state while only slightly acetylated at the COX-2 promoter. Thus subtle differences in the nature and extent of acetylation of specific H3 and H4 lysines may dictate the nature of the specific gene regulated by NF-κB, thereby implicating additional epigenetic nuclear regulatory events at the level of chromatin.

The mechanism by which HDACs regulates NF-κB activity is presumably through deacetylation, since TSA could inhibit this activity. However, the mechanisms regulating the interactions between NF-κB and HDACs are still not clear. In mammalian cells, HDAC1 and HDAC2 are typically found in a large complex with either the Sin3 protein or as part of the Mi-2-NuRD complex (32). HDAC3, HDAC4, and HDAC5 can be found in the complex containing SMRT/N-CoR (33, 34). The Sin3 complex is brought to promoters through its interaction, either directly or indirectly, with sequence-specific transcription factors. For example, the Sin3 protein complex contributes to Mad-Max repression by interacting directly with the Mad family of proteins (35). Alternatively, the Sin3 complex represses nuclear receptor-mediated activation by interacting indirectly with liganded nuclear receptors through either N-CoR or SMRT corepressor proteins (36). Although HDAC1 can interact with p65, interactions between p65 and either mSin3a or N-CoR were not detected by co-immunoprecipitation (37). However, the fact that various HDACs could inhibit NF-κB-mediated transcription in our study opens up the possibility that several corepressor complexes could be involved in the inhibition of transcription. We noticed that HDAC1, HDAC2, and HDAC6 inhibited p65-mediated transcription, while TSA as well as HG reversed this. On the other hand, HDAC3, HDAC4, and HDAC5 could inhibit p65-mediated transcription to a greater extent in our luciferase assay, and TSA did not relieve this. These results suggest that Sin3, SMRT/N-CoR corepressors may modulate NF-κB-regulated gene transcription in our system as shown elsewhere (37, 38). Evidence shows that HDAC3 could coprecipitate with HDAC4 and HDAC5 (39). Using ChIP assays, we observed the presence of HDAC4 and HDAC5 around the TNF-α promoter at the basal level, while HDAC3 was recruited to the TNF-α promoter after TNF-α treatment. Taken together, our results provide new information on how HDACs repress NF-κB-mediated transcription.

The role of histone acetylation under diabetic conditions is not yet known. These results represent to our knowledge the first demonstration that HG conditions and diabetes can induce in vivo chromatin remodeling and histone modifications to allow increased binding of key transcription factors that regulate inflammatory and diabetes-related genes. Our new findings of specific gene stimulatory histone H3K9 acetylation in diabetic monocytes represent a novel molecular mark indicative of increased inflammatory state in these individuals. There is tremendous interest in these posttranslational histone modifications dictating promoter access to specific transcription factors. However, although immense amount of work has re-

### Fig. 7. The acetylation status of H3K9 and H3K14 in monocytes obtained from diabetic versus non-diabetic volunteers. PBMC were prepared from blood obtained from four subjects with documented diabetes (patients 1–4) or from two normal healthy controls (1 and 2) and ChIP assays performed with antibodies to histone H3K9 and H3K14. PCR analyses were performed using immunoprecipitated ChIPed DNA samples with primers amplifying the TNF-α and COX-2 promoters individually. A shows PCR products from the ChIP assays along with input DNA control. B shows densitometric quantitation. *, p < 0.001 versus control; **, p < 0.05 versus control.

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\* F. Miao and R. Natarajan, unpublished data.
ciently gone into the identification of various histone modifications that lead to gene activation or repression, the biological role and function in higher eukaryotes and, in particular, their role in disease states such as diabetes has not been studied. Given that inflammatory gene regulation is an integral feature of these diseases, our results could lead to significant new information, particularly in light of the urgency to uncover diabetes susceptibility factors at the post-genome level.

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REFERENCES

1. Ruderman, N., Williamson, J. R., and Brownlee, M. (1992) FASEB J. 6, 2905–2914
2. Pugliese, G., Tilton, R. G., and Williamson, J. R. (1991) Diabetes. Metab. Rev. 7, 35–59
3. Baynes, J. W. (1991) Diabetes 40, 405–412
4. Ido, Y., Kilo, C., and Williamson, J. R. (1997) Diabetologia 40, S115–S117
5. Brownlee, M., Cerami, A., and Vlassara, H. (1988) N. Engl. J. Med. 318, 1315–1321
6. Schmidt, A. M., Hori, O., Brett, J., Yan, S. D., Wautier, J. L., and Stern, D. (1999) J. Mol. Med. 77, 76–88
7. Ishii, H., Daisuke, K., and King, G. L. (1998) Mol. Cell. Biol. 18, 275–286
8. Nishikawa, T., Edelstein, D., Du, X. L., Yamagishi, S., Matsumura, T., Kameda, Y., Yorek, M. A., Beebe, D., Oates, P. J., Hammes, H. P., Giardino, L., and Brownlee, M. (2000) Nature 404, 787–796
9. Guha, M., Bai, W., Nadler, J., and Natarajan, R. (2000) J. Biol. Chem. 275, 17728–17739
10. Shanmugam, N., Reddy, M. A., Guha, M., and Natarajan, R. (2003) Diabetes 52, 1256–1264
11. Morohoshi, M., Fujisawa, K., Uchimura, I., and Numano, F. (1995) Ann. N. Y. Acad. Sci. 748, 562–570
12. Shanmugam, N., Kim, Y. S., Lanting, L., and Natarajan, R. (2003) J. Biol. Chem. 278, 34834–34844
13. Hoffman, M. A., Schiekofler, S., Kanitz, M., Klevesath, M. S., Josig, M., Lee, V., Marcos, M., Tritschler, H., Ziegler, B., Wahl, P., Bierhaus, A., and Nawroth, P. P. (1998) Diabetes Care 21, 1310–1316
14. Hoffmann, M. A., Drury, S., Fu, C., Wu, Q., Taguch, A., Lu, Y., Avial, C., Kambham, N., Bierhaus, A., Nawroth, P., Neurath, M. F., Slattery, T., Beach, D., McClary, J., Nagashima, N., Morser, J., Stern, D., and Schmidt, A. M. (1999) Cell 97, 889–901
15. Leonard, M. J., and Baltimore, D. (1998) Cell 98, 227–229
16. Baasaru, R. A., and Baltimore, D. (1988) Science 242, 540–546
17. Sheppard, R. A., Rose, D. W., Haque, Z. A., Kurokawa, R., M-clinerney, E., Westin, S., Thonas, D., Rosenfeld, M. G., Glass, C. K., and Collins, T. (1999) Mol. Cell. Biol. 19, 6367–6378
18. Cheung, W. L., Briggs, S. D., and Allis, C. D. (2000) Curr. Opin. Cell Biol. 12, 326–333
19. Kurdistani, S. K., and Grunein, M. (2003) Nat. Rev. Mol. Cell Biol. 4, 276–284
20. Jouveniec, T., and Allis, C. D. (2001) Science 293, 1074–1079
21. Sterner, D. E., and Berger, S. L. (2000) Microbiol. Mol. Biol. Rev. 64, 435–439
22. Roth, S. Y., Jenu, J. M., and Allis, C. D. (2001) Annu. Rev. Biochem. 70, 81–120
23. de Ruijter, A. J., van Gennip, A. H., Caron, H. N., Kemp, S., and van Kuijlenburg, A. B. (2003) Biochem. J. 370, 737–749
24. Wade, P. A. (2001) Hum. Mol. Genet. 10, 695–698
25. Boyd, K. E., and Farnham, P. J. (1999) Mol. Cell. Biol. 19, 8393–8399
26. Zong, H., May, M. J., Jimi, E., and Ghosh, S. (2002) Mol. Cell 9, 625–636
27. Orlando, V. (2000) Trends Biochem. Sci. 25, 99–104
28. Appley, S. B., Ristimaki, A., Nielsen, K., Narko, K., and Hla, T. (1994) Biochem. J. 302, 723–727
29. Finnin, M. S., Donigian, J. R., Cohen, A., Richon, V. M., Rifkind, R. A., Marks, P. A., Breslow, R., and Pavletich, N. P. (1998) Nature 401, 188–193
30. Berger, S. L. (2002) Curr. Opin. Genet. Dev. 12, 142–148
31. Nakayama, J. I., Rice, J. C., Strahl, B. D., Allis, C. D., and Grewal, S. I. S. (2001) Science 292, 110–113
32. Knoepfler, P. S., and Eisenman, R. N. (1999) Cell 99, 447–456
33. Fuchiche, W., Dequadt, F., Hendzel, M. J., Guenther, M. G., Lazar, M. A., Voelter, W., and Verdin, E. (2002) Mol. Cell 9, 45–57
34. Guenther, M. G., Barak, O., and Lazar, M. A. (2001) Mol. Cell. Biol. 21, 6091–6101
35. Barrera-Hernandez, G., Cultraro, C. M., Panetti, S., and Segal, S. (2001) Mol. Cell. Biol. 20, 4255–4264
36. Chen, C. K., Rose, D. W., and Rosenfeld, M. G. (1997) Curr. Opin. Cell Biol. 9, 222–232
37. Ashburner, B. P., Westerheider, S. D., and Baldwin, A. S., Jr. (2001) Mol. Cell. Biol. 21, 7065–7077
38. Chen, L. F., Fuchiche, W., Verdin, E., and Greene, W. C. (2001) Science 293, 1653–1657
39. Yang, W. M., Tsai, S. C., Wen, Y. D., Fejer, G., and Seto, E. (2002) J. Biol. Chem. 277, 9447–9454
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