During the onset of diabetes, pancreatic β cells become unable to produce sufficient insulin to maintain blood glucose within the normal range. Proinflammatory cytokines have been implicated in impaired β cell function. To understand more about the molecular events that reduce insulin gene transcription, we examined the effects of hyperglycemia alone and together with the proinflammatory cytokine interleukin 1 (IL-1)β on signal transduction pathways that regulate insulin gene transcription. Exposure to IL-1β in fasting glucose activated multiple protein kinases that associate with the insulin gene promoter and transiently increased insulin gene transcription in β cells. In contrast, cells exposed to hyperglycemic conditions were sensitized to the inhibitory actions of IL-1β. Under these conditions, IL-1β caused the association of the same protein kinases, but a different combination of transcription factors with the insulin gene promoter and began to reduce transcription within 2 h; stimulatory factors were lost, RNA polymerase II was lost, and inhibitory factors were bound to the promoter in a kinase-dependent manner.

**ERK1/2 | histone acetylation | interleukin 1-β**

During the development of type II diabetes, pancreatic β cells become progressively unable to produce sufficient insulin to prevent hyperglycemia (1–3). This is in part due to decreased insulin gene transcription (4–8) and has been associated with impaired β cell function. To understand more about the molecular events that reduce insulin gene transcription, we examined the effects of hyperglycemia alone and together with the proinflammatory cytokine interleukin 1 (IL-1)β on signal transduction pathways that regulate insulin gene transcription. Exposure to IL-1β in fasting glucose activated multiple protein kinases that associate with the insulin gene promoter and transiently increased insulin gene transcription in β cells. In contrast, cells exposed to hyperglycemic conditions were sensitized to the inhibitory actions of IL-1β. Under these conditions, IL-1β caused the association of the same protein kinases, but a different combination of transcription factors with the insulin gene promoter and began to reduce transcription within 2 h; stimulatory factors were lost, RNA polymerase II was lost, and inhibitory factors were bound to the promoter in a kinase-dependent manner.

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itory factors, in the prolonged repressive action of IL-1β, while physically associated with chromatin.

Effects of IL-1β in Fasting or High Glucose on Association of Factors with the Insulin Gene Promoter. Glucose induces the binding of PDX-1, BET2, MafA, and NFAT, all important for glucose-induced insulin gene transcription, to the insulin gene promoter (14, 16, 20, 22). C/EBP-β and Jun are among factors responsive to IL-1β, which interfere with insulin gene transcription. To explore the basis for the different actions of IL-1β on insulin gene transcription under normal and hyperglycemic conditions, we compared the association of these factors with the promoter in β cells preincubated in fasting or high glucose for 12 h or more. ATF2 stimulates insulin gene transcription through calcium, calmodulin kinase IV, acting through the cAMP response element (CRE) (Fig. 1C), overlapping the site reported for Jun binding to the promoter (35, 38, 39). Thus, we included ATF2 in our analysis of insulin gene promoter binding.

After preincubation in fasting or high glucose for 3 days,
human islets were treated without or with IL-1β for 4 h and exposed to fasting or high glucose for the final 20 min. MafA, BETA2, and PDX-1 bound to the promoter in islets preincubated in fasting glucose and acutely stimulated with high glucose (Fig. 3A), but not if cells were preincubated in 16 mM glucose for 3 days. Because these factors form a synergistic complex, we expected that they would be affected in a similar manner by IL-1β. Instead, rather than disrupting the interaction of a stimulatory multiprotein complex with the promoter, IL-1β affected each factor differently. IL-1β had no effect on stimulatory glucose-induced binding of PDX-1 or BETA2 but prevented MafA binding to the insulin gene promoter (Fig. 3A, lane 4). Under hyperglycemic conditions, however, IL-1β induced binding of BETA2 but not Maf or PDX-1. This suggests that BETA2 may be involved in IL-1β-induced transcription inhibition. Both ATF2 and Jun were bound to the insulin gene promoter if IL-1β was present but not in its absence. C/EBP-β bound to the promoter if cells were preincubated in and exposed to high glucose, consistent with previous findings (20), or if IL-1β was present. These changes are consistent with effects of IL-1β on nuclear run on of insulin mRNA that two of three key stimulatory factors were lost and two or more inhibitory factors were bound.

The experiments above indicated that IL-1β caused a reshuffling of factors on the insulin gene promoter to cause transcriptional inhibition. To determine when these changes took place, we examined the initial time courses of promoter interactions caused by exposure of β cells to 16 mM glucose without or with IL-1β (Fig. 3B and Fig. S2). MafA, BETA2, and PDX-1, were detected on the promoter within 10 min of exposure of cells preincubated in 4.5 mM glucose to 16 mM glucose and binding persisted over the 4-h time course (Fig. S1B and S2). Until 30 min of exposure to 16 mM glucose, binding of the glucose-sensitive factors that stimulate insulin gene transcription was comparable in the presence or absence of IL-1β (compare Fig. 3B to Fig. S2). IL-1β differentially affected the kinetics of factor binding after that time. Inclusion of IL-1β in high glucose resulted in loss of MafA after 30 min and of PDX-1 after 1 h; yet, BETA2 binding was persistent and independent of stimulatory glucose. Despite evidence for cooperative interaction of MafA/PDX-1/BETA2 as a complex with the promoter, the combination of hyperglycemia and IL-1β caused dissociation of two of the factors and at different times, while retaining the third. This suggests that the combination of glucose and IL-1β signaling selectively stabilized binding of some factors and destabilized the binding of others to the promoter.

The IL-1β-induces factors also showed distinct binding kinetics. ATF2 was detected within 10 min of IL-1β exposure. Jun binding was noted after 60 min of glucose plus IL-1β, but in contrast to ATF2, not before that time. Although Jun was not bound until 1 h of treatment with IL-1β, both JNK and ERK1/2 kinases that phosphorylate Jun, were bound immediately (Fig. 2D). This suggests that JNK did not associate with the promoter through its interaction with Jun.

We examined effects of inhibitors that block the MAPK pathways on their promoter interactions. C/EBP-β binding was blocked by U0126 or FK520 (Fig. 3C), consistent with its linkage to the ERK1/2 pathway. Both Jun and ATF2 continued to bind...
to glucose. Histone acetylation and p300 and RNA polymerase II was also observed upon exposure without IL-1 

and the related Msk effector kinases (41–44). Consistent with ERK1/2 activity on acetylation in hyperglycemic β cells most likely contributes to its inhibitory effect on insulin gene transcription under those conditions (20).

Discussion

Prolonged hyperglycemia substantially contributes to inhibition of insulin gene transcription by IL-1β. Inhibition is paralleled by loss of several factors that stimulate and a gain of factors own to inhibit insulin gene transcription (20, 22) (Fig. 5, models). The initial actions of IL-1β do not reduce glucose-stimulated insulin gene transcription. Thus, it seems likely that IL-1β produced in response to transient inflammatory events will generally have little negative impact on insulin gene transcription or perhaps other aspects of β cell function. During prolonged periods of hyperglycemia, as might occur in type II diabetes, IL-1β will rapidly suppress insulin production by β cells.

The actions of IL-1β develop through a stepwise, rather than concerted, series of events, dependent at least in part on MAPKs. In the first few minutes of exposure to IL-1β in high glucose, the glucose-sensitive factors bind to the promoter in a typical pattern, except that ATF2 is also chromatin bound within 5 min. ATF2 has been shown to be up-regulated by glucose and amino acid deprivation (47). Because ATF2 can stimulate insulin gene transcription, its immediate binding to the promoter may well enhance insulin gene transcription. Thereafter, inhibition of binding of MafA and then PDX-1, enhanced binding of Jun, and enhanced accumulation, and binding of C/EBP-β, clearly result in reduced insulin production. These changes culminate in loss of histone acetylation, the histone acetyl transferase (HAT), and coactivator activity of p300, and loss of RNA polymerase II from the promoter. Jun binding is noted at 60 min but not before, despite the fact that both JNK and ERK1/2, which can phosphorylate Jun, are ERK1/2 dependent. Although still observed after 4 h in 16 mM glucose, histone acetylation and p300 and pol II binding were no longer detected if IL-1β was also present. This pattern is temporally similar to that of PDX-1 binding. The loss of histone acetylation and interaction of pol II suggests that the promoter is inactivated by 4 h of exposure to the cytokine (Fig. 3B), consistent with nuclear run-on findings and despite the fact that the protein kinases and several other factors were still bound.

Prolonged hyperglycemia caused an unexpected change in the relationship between ERK1/2 and histone acetylation. ChIP from freshly isolated islets revealed that both modified forms of histones were detected on the promoter in stimulatory glucose and both signals were reduced by U0126 (Fig. 4A, top row). In contrast, in islets (Fig. 4A, rows 2–4) or MIN6 cells (Fig. 4B) incubated in high glucose for days, the pattern was reversed; although ERK1/2 were bound to the promoter in high glucose, histones were less highly acetylated. Hyperglycemia for as little as 1 day reduced acetylation. Under hyperglycemic conditions, acetylation increased if islets were switched to fasting glucose or treated with U0126. The effect of ERK1/2 activity on acetylation in hyperglycemic β cells most likely contributes to its inhibitory effect on insulin gene transcription under those conditions (20).

**Impact of Hyperglycemia on Acetylation of Histones on the Insulin Gene Promoter.** Modifications of histones fluctuate during cycles of transcription, some of which may occur through phosphorylation-dependent changes in promoter-bound modifying enzymes (40). ERK1/2 regulate histone modifications through Rsk and the related Msk effector kinases (41–44). Consistent with previous reports (45, 46), we found acetylation of histones 3 and 4 on the insulin gene promoter induced by glucose with or without IL-1β, using antibodies to pSer10/Ac-K14, Ac-K9/K14 H3, and K5/K8/K12/K16 H4 (Fig. 3B and Fig. S1B). Binding of p300 and RNA polymerase II was also observed upon exposure to glucose. Histone acetylation and p300 and pol II binding were in the presence of any single kinase inhibitor, although each single inhibitor prevented binding of the expected kinase in the same experiment (Fig. S3). Blockade of Jun binding required inhibition of both JNK and ERK1/2 pathways. Blockade of ATF2 binding required inhibition of p38 and JNK. These factors were responsive to two MAPK signaling pathways, and the activity of either pathway alone was apparently sufficient to retain the transcription factor on the promoter.

![Fig. 4](image-url) **Fig. 4.** ERK1/2-dependent effects on acetylation of histones on the insulin promoter in β cells during exposure to glucose. ChIP assay of Ac-H3, Ac-H4, and ERK1/2 associated with the insulin promoter in human islets cultured in 4.5 or 16 mM glucose for 3 days (A Top and B) or MIN6 cells cultured in 25 mM glucose (A Bottom). β Cells were then preincubated for 2 h in 2 mM glucose and then stimulated with 16 mM (A Top and B) or 25 mM (A Bottom) glucose for 30 min in the presence or absence of U0126 (U). (B) After the initial 3-day culture in 4.5 mM glucose, human islets were cultured for 1–3 additional days in 16 mM glucose before acute stimulation. ChIP assays were performed a minimum of three times.

![Fig. 5](image-url) **Fig. 5.** Models depicting the arrangement of factors on chromatin in acute glucose without and with IL-1 and under hyperglycemic conditions in the presence of IL-1.
bound immediately. One possible explanation for this delay is that Jun binding may require displacement of MafA complexes. Blockade of Jun binding required inhibition of both JNK and ERK1/2. Activation of either pathway alone is apparently sufficient to retain Jun on the promoter. The binding of ATF2 is also controlled by multiple pathways; blocking its chromatin binding required inhibition of p38 and JNK. This might also be due to phosphorylation of ATF2 catalyzed by both kinases or by only one together with a dependence on a distinct binding partner regulated by the other MAPK pathway.

The acetylation of H3 and H4 on the insulin gene promoter in response to acute glucose stimulation corresponded to the activation and chromatin binding of ERK1/2 and was inhibited by blocking ERK1/2, in agreement with observations in other systems (42). A similar pattern was also observed for p300, a partner regulated by the other MAPK pathway.

Under normal conditions, ERK1/2 binding of PDX-1 to the insulin promoter. PDX-1 binds to the promoter in an ERK1/2-dependent fashion and associates with p300 to assist in recruiting it to the insulin gene promoter (48–50). Our results are consistent with a relationship between PDX-1 and p300 binding. Loss of acetylation and p300 occur in the same time window as loss of PDX-1 from the promoter. Association of p300 may be enhanced through other interactions as well (51).

The altered relationship of ERK1/2 binding and histone modification in hyperglycemia suggest that ERK1/2 are not required to occupy the insulin gene promoter to influence or maintain histone acetylation state. Perhaps long-term exposure of β cells to glucose results in the recruitment of factors to the promoter by ERK1/2, which either (i) displace or exclude the acetyl transferases or (ii) recruit histone deacetylases (HDACs). C/EBP-β binds to the promoter in an ERK1/2-dependent manner under all conditions tested; C/EBP-β inhibits insulin gene transcription in response to hyperglycemia; and C/EBP-β has been shown to recruit HDAC-1 to a promoter in other cells (52). Therefore, it may be that C/EBP-β recruits an HDAC to the insulin gene promoter. HDAC is also known to bind to p300 and may recruit it under these circumstances (53).

Finally, the three MAPKs are rapidly and simultaneously chromatin bound. This finding further supports the conclusion that chromatin proteins can be phosphorylated in situ in a signal-dependent manner, just as occurs with other types of modifications. In fact, these protein kinases associate with this and other promoters whether or not the factors they phosphorylate are present. The kinetics of kinase-promoter binding suggest that the transcription factor substrates are not the primary anchors of the kinases to DNA. This leads to the supposition that the kinases associate with chromatin by mechanisms independent of their transcription factor substrates.

**Experimental Procedures**

**Materials.** RNAsin was from Promega; TRI Reagent was from Life Sciences. Antibodies to protein kinases were as described inrefs. 22, 37, and 54. Antibodies purchased from Santa Cruz Biotechnology were: NFATc2 (G1-D10), c-Maf (M-153), C/EBP-β (C-19), PDX-1 (N-18), NeuroD1 (N-19), c-Jun (H-79), ATF2 (C-19), RSK2 (C-19), RNA polymerase II (N-20), phospho-Jun (KM-1), and p300 (C-20). Antibodies to Upstate were used to recognize H3 phosphorylated on S10 and acetylated on K14 (07-081) and acetylated H4 (06-866). Antibodies to calcineurin were a gift from Claude Klee (National Institutes of Health). U0126 was from Promega, SB203580 from LC Laboratories, SP600125 from Calbiochem.

**Isolation of Human Pancreatic Islets.** Islets were isolated from human cadaveric pancreata as described in ref. 22.

**Cell Culture.** MIN6 cells were grown in DMEM or RPMI 1640 media (Gibco), with 10% FBS, 10 mM Hepes, pH 7.4, 10.2 mM l-glutamine, 50 mM sodium pyruvate, 25 mM glucose, 2.5 mM β-mercaptoethanol, penicillin (100 U/ml), streptomycin (0.1 mg/ml), at 37°C, 10% CO₂. Cells and islets were preincubated in 4.5 mM glucose in either medium or Krebs-Ringer’s solution (37°C) as indicated.

**Nuclear Run-On Assays.** MIN6 cells were washed in 10 mM Tris-HCl, pH 7.8, 10 mM KCl and were lysed by vortexing in the same solution containing 0.05% Nonidet-P-40 and placed on ice for 10 min. Nuclei were isolated by centrifugation on a 0.6 M sucrose cushion at 600 × g in a table top centrifuge. RNA was purified with phenol/CHCl₃, precipitated with ethanol, and used for PCR as described in ref. 22. For sequential ChIP, antibodies to ERK1/2 were first used to immuno precipitate material from cross-linked complexes and then antibodies to other kinases were used for a second immunoprecipitation.

**Chromatin Immunoprecipitation.** Chromatin was subjected to DNA-protein cross-linking and sonication fragmentation. The lysates were sonicated using a Fisher Scientific Sonic Dismembrator 500 to generate DNA fragments of 200- to 300-bp DNA-protein complexes were immunoprecipitated. DNA was purified with phenol/CHCl₃, precipitated with ethanol, and used for PCR as described in ref. 22. For sequential ChIP, antibodies to ERK1/2 were first used to immunoprecipitate material from cross-linked complexes and then antibodies to other kinases were used for a second immunoprecipitation. Primers: 5′-AACGTGTTTCACTAGGGCATC-3′ and 5′-AATCGTGTCCCTACACTTCTT-3′ (mism – 247 to 27), 5′-GAGGAGGAGGTGTCGACACG-3′ and 5′-CCATCTCCCTCCTGTCAA-3′ (hins – 194 to 41). 

**cDNA Synthesis.** Synthesis was carried out in the testicular was harvested with TRI Reagent (Ambion) to isolate total RNA. cDNA was prepared from 10 μg total RNA using random hexamers and the High-Capacity cDNA Archive Kit (Applied Biosystems).

**Real-Time Quantitative PCR.** PCR using TaqMan Gene Expression Assays (Applied Biosystems) was performed on an ABI 7500 DNA Sequence Detection System (Applied Biosystems) with fluorescent chemistries and cycling conditions specified by the manufacturer. 185 RNA was amplified as the control.

**Statistical Analyses.** Results are expressed as means ± SEM of three replicates, unless otherwise noted. Statistical significance was calculated by one-tailed unpaired Student’s t test.

**Immunoblot Analysis.** Cells were harvested in passive lysis buffer containing 100 mM β-glycerophosphate, 2 mM Na₃VO₄, and 100 mM NaF. Lysate protein (20 μg) was subjected to electrophoresis in 10% polyacrylamide gels in SDS. Proteins were transferred to nitrocellulose membrane (Millipore), blocked with 1% BSA, and 1% nonfat milk powder in TBS buffer containing 0.1% Tween-20 for 4–18 h at 4°C, and incubated with a primary antibody for 2 h at 4°C, followed by a horseradish peroxidase-conjugated secondary antibody diluted 1:3,000 in blocking buffer for 30 min at room temperature. Detection was by enhanced chemiluminescence.

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Fig. S1. Time course of association of proteins with the insulin gene promoter in β cells exposed to glucose. ChIP assay of transcription factors and enzymes associated with the insulin promoter in MIN6 cells cultured in 4.5 mM glucose for 3 days, then treated with 16 mM glucose for from 5 to 240 min without and with U0126 as indicated. (A) ChIP assay of protein kinases. (B) ChIP assay of transcription factors, acetylated histones, and p300. ChIP time courses were repeated a minimum of two times.
Fig. S2. Time course of association of proteins with the insulin gene promoter in β cells exposed to glucose and IL-1β. ChIP assay of transcription factors associated with the insulin promoter in human islets cultured in 4.5 mM glucose for 3 days, then treated with 16 mM glucose without or with IL-1 or 4.5 mM glucose with IL-1. ChIP time courses were repeated a minimum of two times.
ChIP assay testing inhibitor sensitivity of kinase interactions with the insulin gene promoter. ChIP assays compared sensitivity of kinase binding to inhibitors. The association of each MAPK was specifically reduced by its pathway-selective inhibitor. Like ERK1/2, Rsk binding was sensitive to inhibition by the ERK1/2 pathway inhibitor U0126 (5 μM) and the calcineurin inhibitor FK506, which prevents ERK1/2 and Rsk activation by glucose. p38 binding was selectively blocked by 5 μM SB203580, and JNK binding was selectively blocked by 5 μM SP600125. Thus, inhibiting the activity of a MAPK reduced its binding to the promoter.