Covalent Histone Modifications Underlie the Developmental Regulation of Insulin Gene Transcription in Pancreatic β Cells

Swarup K. Chakrabarti†, Joshua Francis§, Suzanne M. Ziesmann‡, James C. Garvey‡, and Raghavendra G. Mirmira‡§¶

From the †Department of Internal Medicine and the Diabetes Center, and §Department of Pharmacology, University of Virginia Health Sciences Center, Charlottesville, Virginia 22903

Histone modifying enzymes contribute to the activation or inactivation of transcription by ultimately catalyzing the unfolding or further compaction, respectively, of chromatin structure. Actively transcribed genes are typically hyperacetylated at Lys residues of histones H3 and H4 and hypermethylated at Lys-4 of histone H3 (H3–K4). To determine whether covalent histone modifications play a role in the β cell-specific expression of the insulin gene, we performed chromatin immunoprecipitation assays using anti-histone antibodies and extracts from β cell lines, non-β cell lines, and ES cells, and quantitated specific histone modifications at the insulin promoter by real-time PCR. Our studies reveal that the proximal insulin promoter is hyperacetylated at histone H3 only in β cells. This hyperacetylation is highly correlated to recruitment of the histone acetyltransferase p300 to the proximal promoter in β cells, and is consistent with the role of hyperacetylation in promoting euchromatin formation. We also observed that the proximal insulin promoter of β cells is hypermethylated at H3–K4, and that this modification is correlated to the recruitment of the histone methyltransferase SET7/9 to the promoter. ES cells demonstrate a histone modification pattern intermediate between that of β cells and non-β cells, and is consistent with their potential to express the insulin gene. We therefore propose a model in which insulin transcription in the β cell is facilitated by a unique combination of transcription factors that acts in the setting of an open, euchromatic structure of the insulin gene.

The pancreatic β cell is exclusively responsible for the synthesis and secretion of insulin. The production of insulin appears to be governed by constraints imposed at the level of transcription of the gene encoding insulin (Ins).¹ and involves an intricate interplay between transcription factors that are known to function as transactivators of the gene. In recent years, specific DNA elements within the proximal ~400 base pairs (bp) of the Ins promoter have been mapped precisely and shown to be bound by several major classes of transactivating transcription factors, including homeodomain factors (Pdx1, Lmx1.1), basic helix loop helix factors (NeuroD1, E47), and bZip factors (mMafA) (see Ref. 1 for review). In addition, acetylation, methylation, and ubiquitination (see Ref. 13 for review). Of these, acetylation and methylation of specific Lys residues of H3 and H4 have been studied extensively. Chromatin immunoprecipitation (ChIP)² assays using mammalian and yeast cells have demonstrated that transcriptionally active genes in regions of “open” chromatin (“euchromatin”) are correlated with high levels of Lys acetylation of histones H3 and H4, whereas inactive genes in regions of “closed” chromatin (“heterochromatin”) are hypoacetylated at these histones (14, 15). Lys acetylation and deacetylation are catalyzed by the action of histone acetyltransferases (HATs) and histone deacetylases, respectively;

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² The abbreviations used are: ChIP, chromatin immunoprecipitation; Ins, insulin; HAT, histone acetyltransferase; HMT, histone methyltransferase; ES, embryonic stem; RT, reverse transcriptase.

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thus, in addition to serving as a long term epigenetic marker for euchromatin, histone acetylation is also viewed as a dynamic, short term mechanism to control gene transcription (14). At least two mechanisms have been proposed to explain the effect of histone acetylation on chromatin structure and transcription. First, acetylation of Lys residues is known to diminish the positive charge in the N-terminal histone tails, and thereby mitigate electrostatic interactions between histones and DNA and promote chromatin unfolding. Second, the acetylation “mark” of these Lys residues is thought to be recognized by proteins containing the conserved bromodomain. These proteins subsequently recruit components of the basal transcriptional machinery to promote transcriptional activation (16).

Unlike acetylation, Lys methylation can have divergent effects on transcriptional activation, depending upon the specific residue modified. Thus, methylation of Lys-4 of histone H3 (H3–K4) is associated with euchromatin, whereas methylation of Lys-9 of histone H3 (H3–K9) is observed with heterochromatin (17–19). Lys methylation is catalyzed by the action of histone methyltransferases (HMTs), which demonstrate a high degree of specificity for either H3–K4 (e.g. SET7/9, Refs. 20 and 21) or H3–K9 (e.g. SUV39H1, Ref 22). Because no histone demethylases have yet been described, histone methylation is not generally believed to be dynamically regulated (as acetylation), but rather is viewed as a long term epigenetic marker for chromatin states (23). Although Lys methylation does not alter net charge of histone tails (unlike acetylation), the methyl mark is believed to either enhance or repress the binding of chromatin remodeling complexes that contain the conserved chromodomain (e.g. HP1 and NuRD deacetylase, respectively), thereby contributing to altered chromatin conformation (24, 25). Hence, regions of transcriptionally active genes are characterized by histone acetylation and/or histone H3–K4 methylation, whereas regions of inactive genes are hypoacetylated and/or contain histone H3–K9 methylation.

To explain Ins gene transcription in the β cell more fully, we hypothesized that β cells contain a characteristic set of histone modifications that lend a state of euchromatin in the region of the Ins gene. To test this hypothesis, we performed quantitative ChIP assays using anti-histone antisera and extracts from β cell lines (βTC3 and MIN6) and several non-β-cell lines (e.g., TC1.6, mPAC, NIH3T3, and ES cells) to examine the chromatin structure of the proximal and distal mouse Ins promoter. Our hypothesis proved correct, as we found that β cells display a characteristic pattern of H3 hyperacetylation in the proximal Ins promoter, and that this acetylation is highly correlated to recruitment of the HAT p300 to this promoter region. In addition, we found that the proximal Ins promoter in β cells is hypermethylated at H3–K4, and that this modification is consistent with the recruitment of the HMT SET7/9 to this region of the promoter. We therefore propose a model in which Ins transcription in the β cell is facilitated by a unique combination of transcription factors that acts in the setting of an open, euchromatic structure of the Ins gene.

MATERIALS AND METHODS

Cell Culture and Antibodies—The mouse cell lines, βTC3, TC1.6, mPAC, NIH3T3, MIN6, and embryonic stem (ES) D3 were maintained as described (26–29). Rabbit polyclonal antibodies to acetylated-H3, acetylated-H4, H3-dimethyl-K4, H3-dimethyl-K9, and SET7/9 were generously provided by Upstate Biotech, Inc. Rabbit polyclonal antibody to p300 (N-15) was from Santa Cruz Biotechnology.

Multiplex Real-time RT-PCR—Real-time RT-PCR using dual-labeled probes and total RNA from cells lines was performed as described previously (30). Forward primer, reverse primer, and probe sequences (5’ to 3’), respectively, for real-time RT-PCR were: mouse insulin, TGCTCCTTCTACACACCAAG, ACAATGCACTGCTTGCC, and (AmC6 + Texas Red)–CCCCGCCTGAAATGGAGACCC–(BHQ); actin, AGTTGATCATCATTGAGCAAG, CACCTCAGTATGGAAATGTAGT, and (6-FAM)–TGGCCACAGGTTCACATCCCCAAAGAGG–(BHQ).

Quantitative ChIP Assays—The quantitative ChIP assays were performed as described previously (26). For harvesting of ES cells, feeder embryonic fibroblasts were removed by trypsinizing cells from 10-cm plates and replating on fresh 10-cm tissue culture dishes. Approximately 30 min later, medium (containing mostly ES cells) was aspirated and replaced. This process was repeated a total of 3 times, after which the medium (containing >90% ES cells) was processed for ChIP as described (26). Co-immunoprecipitated promoter fragments were quantitated by real-time PCR using continuous SYBR Green I monitoring as detailed previously (26). Prior to ChIP, 1 μg of a plasmid containing the firefly luciferase coding sequence (pFoxLuc) was added to each cellular extract and was used to correct for differences in DNA recovery between samples after the ChIP procedure. This was accomplished by determining the quantity of recovered luciferase DNA in each sample by real-time PCR, and using this value to correct the recovery of the Ins promoter fragments. Data were expressed as fold-differences relative to control conditions, in which normal rabbit serum was used instead of specific antibody in the ChIP. In practice, DNA fragments are non-specifically and reproducibly recovered after ChIP in the absence of antibody, but are often amplified 0–6 cycles later than specifically recovered fragments. Thus, data expressed relative to these control conditions allow for (a) correction in the variation of background DNA precipitation from cell line to cell line, and (b) assessment of the absolute enrichment of specific DNA fragments after ChIP in any given cell line (26, 31, 32). ChIP assays were performed on at least 3 independent occasions; for each ChIP assay, promoter samples were quantitated in triplicate on two separate occasions. Thus, for every sample in Figs. 2–5, data represent the average of at least 6 determinations ± S.D., and are presented as fold-differences relative to control conditions (in which normal rabbit serum is used instead of specific antibody in the ChIP).

Forward and reverse primer sequences, respectively, used for PCR were (5’ to 3’): proximal Ins1, TCAGGCAAGATTGAGGAGTCTCT, TCCAAACATGTCGCTGCTG; distal Ins1, CTCTCCACACAGAAACGAGGTCACTTC, CTCACTACCCAGGGGTTAT; firefly luciferase, TGGCCAGGATAGGTCCTG, GCTTCTGCAACAGGTCTTCC. PCR products were subcloned into the T/A cloning vector pCR2.1 (Invitrogen), and several resulting clones were sequenced to confirm the identity of the amplified fragment. Primer pairs for the proximal mouse Ins1 promoter equally and effectively amplified the corresponding fragment from both the mouse Ins1 and Ins2 genes.

Immunoprecipitation-HAT Assays—Immunoprecipitation-HAT assays using anti-p300 antibody and extracts from βTC3, TC1.6, and mPAC cells were performed essentially as described (33). HAT reactions (in 30 μl total volume) consisted of immunoprecipitate or sequence samples of purified p300 catalytic subunit (positive control, Upstate Biotechnology), 10 μg of calf thymus histones (Worthington), or bovine serum albumin (negative control) and 0.2 μCi of [3H]acetyl-CoA (Amersham Biosciences). After 30 min, HAT reactions were subject either to filter binding assays (33) to determine incorporation of [3H]acetyl group into histones or to electrophoresis on a 15% SDS-polyacrylamide gel and visualized by fluorography to determine relative H3 versus H4 [3H]acetyl content. HAT data are presented as picomoles of [3H]acetate incorporated into histones or bovine serum albumin per mg, and are normalized to the total amount of protein used in each immunoprecipitation.

Western Blot Analysis—Nuclear extracts from all cell lines were prepared from single, confluent 10-cm plates of cells according to methods previously described (25, 26). Whole cell extracts were prepared from confluent 10-cm plates of cells by scraping cells into 1 ml of phosphate-buffered saline containing protease inhibitors (leupeptin, phenylmethylsulfonyl fluoride, and aprotinin) and sonicating briefly. 5 μg of nuclear or whole cell extract were subject to Western blot analysis after electrophoresis on an 8 or 15% SDS-polyacrylamide gel using anti-acetyl H3 and H3-dimethyl-K4, anti-p300, or anti-SET7/9 antibodies. Western blots were detected using the ECL-Plus™ system (Amersham Biosciences).

RESULTS

H3 and H4 Acetylation at the Ins Promoter—In these studies we used two mouse β-cell-derived lines (MIN6 and βTC3, Refs. 28 and 35), a fibroblast line (NIH3T3 cells), an α cell line (TC1.6, Ref. 36), a pancreatic ductal cell line (mPAC, Ref. 27), and ES cells. To verify the presence or absence of Ins gene expression, we isolated total RNA from each cell line and per-
formed 50 cycles of real-time RT-PCR to amplify Ins message. As demonstrated in Fig. 1, only MIN6 and βTC3 cells showed detectable transcript levels, consistent with the observation that Ins gene transcription is highly specific for β cells.

We used a real-time PCR-based ChIP assay (26) to quantitate the association of covalently modified histones with the Ins promoter in each cell line. Because only the proximal –400 bp of the Ins promoter appears to be necessary to direct exclusive expression of Ins in β cells (37), we designed PCR primer pairs to amplify a fragment of DNA within this region (bp –126 to –296 relative to the Ins1 transcriptional start site) following ChIP. For comparison, we also designed PCR primer pairs to amplify a distal genomic fragment (from bp 4653 to 4812 relative to the Ins1 transcriptional start site) in a region known not to be critical for Ins regulation (for simplicity, we refer to this region as the distal Ins promoter). The relative association of acetylated H3 and acetylated H4 with the distal and proximal Ins promoters was assessed by ChIP in βTC3, MIN6, NIH3T3, oTC1.6, mPAC, and ES cells. Fig. 2A demonstrates that β cells (βTC3 and MIN6) show striking levels of H3 acetylation at the proximal (10.5- and 18-fold, respectively, over controls) and, to a lesser extent, the distal promoter. By contrast, little or no Ins promoter H3 acetylation was observed in NIH3T3, oTC1.6, or mPAC cells. Importantly, ES cells contain a moderate level of H3 acetylation at the proximal promoter (about 5-fold over control), consistent with a more intermediate conformation of chromatin in this undifferentiated cell type. Fig. 2B demonstrates that there is little difference in H4 acetylation at the proximal promoter between β cells and the other cell types. Interestingly, ES cells contain relatively high levels of H4 acetylation (20-fold over control) at the proximal promoter, again consistent with a more open conformation of chromatin in this cell type. Overall, these data demonstrate that the Ins promoter of β cells is distinguished by hyperacetylation of H3.

The H3 and H4 acetylation patterns of the distal Ins promoter of all cell types seem to parallel the patterns observed in their corresponding proximal promoter regions (compare Fig. 2, A and B). However, hyperacetylation of H3 relative to H4 may be the more indicative of the regulatory role of the proximal Ins promoter. As shown in Fig. 2C, the ratio of H3 to H4 acetylation is significantly greater (by 5–10-fold) in the proximal Ins promoter of β cells compared with all other cell types, whereas there is no significant difference in this ratio among the cell types in the distal Ins promoter. Thus, these data demonstrate that the proximal Ins promoter is hyperacetylated at H3 relative to H4 in β cells, suggesting a more open chromatin conformation of the promoter in these cells.

p300 Directs Proximal Ins Promoter H3 Hyperacetylation in β Cells—It has been shown previously that the coactivator p300 physically interacts with several important β cell transcription factors (Pdx1, NeuroD1, and HNF6), and is capable of augmenting transactivation of the Ins promoter by these factors (2, 3, 38). Because p300 contains intrinsic HAT activity (39), we sought to determine by ChIP if p300 is directly recruited to the proximal Ins promoter of β cells and whether its catalytic activity could account for the relative H3 hyperacetylation we observed. For these studies, we used 3 representative pancreatic cell lines: oTC1.6, mPAC, and βTC3. As shown in

Fig. 1. RT-PCR demonstrating β cell-specific expression of the Ins gene. The cell lines indicated were grown in the presence of high glucose in the medium, and harvested for total RNA, which served as template for reverse transcription with random hexamers, and subsequent PCR for either insulin or actin message, as described under “Materials and Methods.” A, after 30 cycles of PCR, samples were analyzed on 2% ethidium bromide-stained agarose gels. B, after reverse transcription, samples from each cell line were subject to 50 cycles of multiplex real-time PCR in the presence of dual-labeled probes for insulin and actin, as detailed under “Materials and Methods.” Data represent quantities of insulin message relative to the quantities of actin message in each cell line (to correct for variations in input quantity of RNA). UN, undetectable.
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Fig. 3. *p300 is recruited to the proximal Ins promoter in βTC3 cells and accounts for H3 hyperacetylation of the promoter.* A, quantitative ChIP assay showing association of p300 with the proximal promoter only of βTC3 cells, and not with either promoter region in αTC1.6 and mPAC cells. Data are expressed as fold-differences relative to control, in which normal rabbit serum was used in the ChIP instead of anti-p300 antibody. **+** indicates that the value was statistically different (*p < 0.01*) from control. Each ChIP assay was performed on at least three independent occasions; for each ChIP assay, promoter samples were quantitated in triplicate on two separate occasions. B, Western blot (after electrophoresis on an 8% SDS-polyacrylamide gel) showing the presence of p300 protein in βTC3, αTC1.6, and mPAC nuclear extracts. C, immunoprecipitation-HAT assay demonstrating that p300 HAT specific activity can be immunoprecipitated from βTC3, αTC1.6, and mPAC cells using anti-p300 antibody. D, HAT assay samples were subject to electrophoresis on a 15% SDS-polyacrylamide gel and visualized by fluorography to determine acetylation distribution patterns. The acetylation distribution pattern of recombinant p300 catalytic subunit using calf thymus histones (lane 1) or bovine serum albumin (lane 2) demonstrates the near equal pattern of acetylation of H3 and H4. The acetylation distribution pattern of p300 immunoprecipitated from βTC3 cells using calf thymus histones (lane 3) or bovine serum albumin (lane 4) demonstrates that native p300 displays catalytic specificity for H3 over H4, H2A, and H2B.

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Fig. 3A, p300 is directly associated only with the proximal Ins promoter in βTC3 cells (but not αTC1.6 or mPAC cells), notwithstanding that p300 protein and HAT activity are detectable in all cell types examined (Fig. 3, B and C, respectively). Fig. 3D demonstrates that whereas the recombinant p300 catalytic subunit acetylates H3 and H4 equally in *vitro* (lane 1), full-length p300 immunoprecipitated from βTC3 cells preferentially acetylates H3 (lane 3). This finding is similar to other *in vitro* studies showing preferential acetylation of H3 by p300 (20, 39), and is therefore consistent with the concept that p300 recruitment is responsible for H3 hyperacetylation at the proximal Ins promoter.

**H3 Methylation at the Ins Promoter—**H3–K4 methylation indicates regions of euchromatin and active genes, whereas H3–K9 methylation is associated with regions of heterochromatin and inactive genes. To determine whether H3 methylation is linked to activity of the Ins promoter, we performed quantitative ChIP assays using anti-H3-dimethyl-K4 and anti-H3-dimethyl-K9 antibodies and extracts from β cells and non-β-cells. As illustrated in Fig. 4A high levels of H3–K4 methylation are present in the proximal *Ins* promoter of βTC3 and MIN6 cells (36- and 22-fold, respectively, relative to controls), whereas all non-insulin-producing cell types showed 2–6-fold relative methylation. H3–K4 methylation was significantly lower in the distal promoter of βTC3 and MIN6 cells (9- and 12-fold, respectively, over controls), but was still statistically greater than that observed for the other cell types in the distal promoter (3–4-fold over controls). There was no difference in H3–K9 methylation in the proximal promoter among any of the cell types studied (Fig. 4B). However, the distal promoter in MIN6 cells contains significantly higher H3–K9 methylation (11-fold over control) than all other cell types (1–3-fold over controls). This latter finding suggests a progressive opening of chromatin structure from the distal to proximal promoter in MIN6 cells.

Just as with H3 and H4 acetylation, we observed striking differences between distal and proximal promoter regions when the ratio of H3–K4 to H3–K9 methylation was considered (Fig. 4C). This ratio may reflect the relative “propensity” for a given chromatin segment to occupy a euchromatin conformation (18). Thus, Fig. 4C demonstrates that β cells display a much higher H3–K4/H3–K9 methylation ratio (by up to 14-fold) in the proximal *Ins* promoter than in non-insulin-producing cells. The same ratio in the distal *Ins* promoter reveals little cell-type heterogeneity. Therefore, consistent with the H3 acetylation data, the H3 methylation patterns suggest that the proximal *Ins* promoter of β cells adopts a euchromatin conformation contributing to a more active state of *Ins* transcription.

**SET7/9 Is Recruited to the Proximal Ins Promoter of β Cells—**The remarkable level of H3–K4 methylation at the proximal *Ins* promoter in β cells led us to consider the possibility that the HMT SET7/9 might be responsible for this enzymatic modifi-
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**Fig. 4.** H3–K4 and H3–K9 methylation patterns of the distal and proximal Ins promoter in β cell and non-β cell lines. This figure is similar to Fig. 2 except that the specific antibody used in the ChIP was either anti-H3-dimethyl-K4 (A) or anti-H3-dimethyl-K9 (B). Data are also expressed as the ratio of H5–K4 to H5–K9 methylation (C), which represents the data in panel A divided by the corresponding value in panel B. Each ChIP assay was performed on at least three independent occasions; for each ChIP assay, promoter samples were quantitated in triplicate on two separate occasions.

**Fig. 5.** SET7/9 is recruited to the proximal Ins promoter of βTC3 cells. A, Western blot (after electrophoresis on a 15% SDS-polyacrylamide gel) demonstrating the presence of SET7/9 protein (50 kDa) in HeLa, βTC3, mPAC, and αTC1.6 nuclear extracts. B, quantitative ChIP assay showing association of SET7/9 with the proximal Ins promoter only of βTC3 cells, and not with either promoter region in αTC1.6 and mPAC cells. Data are expressed as fold-differences relative to control, in which normal rabbit serum was used in the ChIP instead of anti-SET7/9 antibody. * indicates that the value was statistically different (p < 0.01) from control. Each ChIP assay was performed on at least three independent occasions; for each ChIP assay, promoter samples were quantitated in triplicate on two separate occasions.

**Fig. 6.** Analysis of global H3 acetylation and H3–K4 methylation in β cells and non-β cells. 5 μg of whole cell extracts from all cell lines were subject to electrophoresis on a 15% SDS-polyacrylamide gel and then subject to Western blotting. The upper panel shows the results of the Western blot after probing with anti-acetylated H3 antibody. This membrane was then stripped and reprobed using anti-H3-dimethyl-K4 antibody (lower panel).

**DISCUSSION**

The finding that distinct histone modifications can lead either to direct alterations in chromatin structure or to the recruitment of chromatin modifying complexes has led to the proposal of the “histone code” hypothesis (13). This hypothesis predicts that the various covalent histone modifications at any given gene are interrelated, and that in combination they contribute to a mechanism that can alter the chromatin structure and transcriptional rate of that gene. Unlike the genetic code,
the histone code has the capacity to exhibit heterogeneity depending upon the organism or even the specific gene in question. In this report, we provide the first evidence for the existence of a histone code for Ins gene transcription.

Acetylation is perhaps the most extensively studied histone modification. Several Lys residues in H3 and H4 are known to be acetylated in response to the action of various HATs. In some systems, important differences have been noted in the relative acetylation of H3 versus H4, suggesting that acetylation of these two histones can be independently regulated (14). For example, activation of the steroidogenic acute regulatory protein gene in mouse Leydig tumor cells is associated with rapid acetylation of H3 in the proximal promoter, with no accompanying changes in either the distal promoter or in H4 acetylation (31). In this regard, our studies suggest that relative H3 hyperacetylation occurs in the proximal Ins promoter region of b cells. This finding is consistent with the well-established regulatory role of this promoter region in Ins transcription (37). Our studies demonstrate a pattern of H3 acetylation and p300 binding that strongly implicates a direct role for the HAT activity of p300 in maintaining an euchromatin environment in the proximal Ins promoter of b cells. Consistent with this finding, recent studies have shown the direct interaction of p300 with the b cell factors Pdx1, NeuroD1, and HNF1a (2, 3, 38) as a possible explanation for how p300 might be recruited specifically to the Ins promoter.

An equally important finding in our studies is the relative absence of H3 acetylation at the Ins promoter of most non-b cells. This hypoacetylation may contribute to a state of inaccessible, “silent” chromatin (heterochromatin) in these cell types (41–43), and might explain why expression of b cell transcription factors in heterologous cell types does not always lead their binding and activation of the endogenous Ins promoter (26). ES cells are a notable exception, because they have the potential to express the endogenous Ins gene (6, 29, 44, 45). In this regard, we observed both H3 acetylation and high level H4 acetylation at the proximal Ins promoter in this highly undifferentiated cell type. It is likely that histone acetylation, particularly of H4, in ES cells may prevent chromatin from adopting a closed state until such time that transcription factors and other chromatin modifying enzymes gain access to make more permanent adjustments in chromatin conformation (14). A similar mechanism involving H4 acetylation has been demonstrated for maintenance of the active X chromosome in differentiating ES cells (46).

H3 methylation can also promote the formation of either euchromatin or heterochromatin, depending upon which residues are methylated. Several Lys residues of H3 and H4 have been demonstrated to be targets for methylation (47). It is believed that H3–K4 methylation promotes transcriptional activation and euchromatin formation by enhancing the acetylation of neighboring histones by HATs and by simultaneously preventing the binding of heterochromatin-forming proteins (e.g. NuRD deacetylase complex) (20, 47). By contrast, H3–K9 methylation is believed to promote transcriptional repression and heterochromatin formation through both the recruitment of heterochromatin-forming proteins (e.g. HP1) and the prevention of histone acetylation by HATs (47). In the latter regard, H3–K9 is also a known target for acetylation; thus, methylation of this residue would preclude its acetylation, and vice versa (48). Recent biochemical and ChIP data (18, 20, 21) suggest that methylation of H3–K4 and H3–K9 antagonize one another, implying that co-occurrence of euchromatin is mutually exclusive. Our data on the histone methylation and acetylation patterns of the Ins promoter support these observations. We found that (a) cell types with active Ins transcription (bTC3 and MIN6) demonstrate a pattern of H3–K4 hypermethylated, H3 hyperacetylated, and H3–K9 hypomethylated at the proximal Ins promoter, (b) cell types with inactive Ins transcription (aTC1.6, NIH3T3, and mPAC) are characterized by a pattern of H3–K4 hypomethylation and H3 hypoacetylation, and (c) a cell type with the potential for Ins transcription (ES cells) demonstrates an intermediate level of H3-K4 methylation and histone hyperacetylation.

To explain the H3–K4 hypermethylated pattern of the Ins promoter in b cells, we propose that the H3–K4-specific methyltransferase SET7/9 is specifically recruited to the proximal Ins promoter. Because we observe SET7/9 expression in all cell types examined, it is unclear exactly how SET7/9 is exclusively recruited to the promoter in b cells. Although it is possible that the interaction of SET7/9 with b-cell-specific transcription factors (such as Pdx1, NeuroD1, etc.) might lead to its recruitment to the proximal Ins promoter (as with p300), recent ChIP analysis of the collagenase gene in glioblastoma cells suggests that SET7/9 might be recruited to active genes by components of the pre-initiation complex. It is hypothesized that the recruitment of SET7/9 serves to stabilize the pre-initiation complex by histone methylation and thereby promote transcription (49).

Taken together, our data point to the existence of a histone code underlying Ins gene transcription in b cells. Although our studies have focused on general H3 and H4 acetylation and H3–K4 and H3–K9 methylation patterns, we propose this code will likely involve an intricate network of several types of histone modifications that is established early in development and becomes more refined as differentiation of b cells proceeds. This epigenetic mechanism likely complements the well-established genetic mechanism of transcription factor-mediated activation that is believed to regulate Ins transcription. Accordingly, we propose the preliminary model for Ins gene activation shown in Fig. 7. This model makes the assumption that activation of the Ins promoter in a previously inactive environment is accompanied by specific changes in histone modifications. This assumption would appear to be a reasonable one, as dynamic changes in histone modifications have clearly been observed in several other systems (31, 32, 46, 50–52), and even recently in the case of the Ins gene upon induction with glucose (53). We propose that chromatin exists in a precursor state similar to that observed in undifferentiated ES cells, and is characterized predominantly by H4 hyperacetylation. This
state might be envisioned to be euchromatin in nature, because ES cells can be induced to express the Ins gene under specific conditions (6, 29, 44, 45). However, this precursor state is likely to be transient, as stem cells rapidly differentiated, and two possible fates can be envisioned. In the non-β cell fate, the activity of histone deacetylases may predominate, such that deacetylation occurs and chromatin is condensed to the heterochromatin form (right arm of Fig. 7). This condensed chromatin impedes accessibility of DNA to transcription factors, so that the gene remains silenced even in the face of heterologous expression of β cell transcription factors (Pdx1, NeuroD1, etc.). In the β cell fate (left arm of Fig. 7), the activity of HATs (e.g. p300) and HMTs (e.g. SET7/9) predominate, thus stabilizing the euchromatin state and allowing access to transactivating transcription factors. Although Fig 7 indicates that the action of HATs and HMTs precedes the action of transcription factors, it is possible that both events are variably interrelated, because HATs or HMTs might be recruited to the promoter by transcription factors. We anticipate that this model will serve as a framework for further studies to define more precisely the histone code of the Ins gene and to identify additional enzymes and chromatin remodeling complexes participating in the establishment of Ins chromatin states.

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