Sleeping Beauty, Awake! Regulation of Insulin Gene Expression by Methylation of Histone H3

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In all cell types of the body, save one, the insulin gene is quiescent and is ensconced in a compact and protected environment of chromatin. How the insulin gene is coaxed out of its chromatin slumber in the pancreatic β-cell is unknown. Moreover, this question is of practical concern to current efforts to transform stems cells or other cells types to become insulin-secreting cells as means for treating type 1 diabetes. The DNA of all eukaryotic organisms including humans is wrapped around an octamer of the histone proteins H2A, H2B, H3, and H4 and garnished with a large array of proteins that collectively orchestrate the higher-order packaging, DNA replication, chromosomal division and recombination, and gene transcription. In recent years, molecular biologists have discovered that an essential step of teasing a gene out of dormancy is to modify the histones that lie in direct contact with the regulatory regions of genes. The two most important enzymatic histone modifications in this process are acetylation and methylation (1–3), which act together to open the chromatin structure and allow access of the promoter regions of genes to RNA polymerase and other transcription factors. Methylation of a specific lysine residue (Lys4) in the H3 histone (H3K4) is the most common modification in the gene promoter region of "poised" or actively transcribing genes (4,5). The methylation of H3K4 can vary between one to three methyl groups being added to this single lysine residue.

Mirmira and coworkers (6,7) had previously shown that the insulin promoter exhibited a high degree of H3K4 dimethylation in β-cells but not in other cell types. Moreover, H3K4 methylation of the insulin promoter is largely dependent upon the recruitment of the Set7/9 methyltransferase to the promoter in the presence of the pancreatic duodenal homeobox-1 (PDX-1, a.k.a. insulin promoter factor-1 [IPF-1]), an all important transcription factor that is required to specify and maintain β-cell development as well as promote insulin gene transcription (8).

The functional importance of H3K4 dimethylation of the insulin promoter was not previously explicitly addressed. In the current issue of *Diabetes*, Deering et al. (9) reveal the role of the Set7/9 methyltransferase in regulating the transcription of the insulin gene as well as other important β-cell genes. As a prelude to the functional studies, they found that Set7/9 is highly expressed in the islet β- and α-cells and is likely to owe its high expression to transcriptional activation mediated by PDX-1. To assess the functional importance of Set7/9, the Set7/9 protein was depleted in the β-cell line βTC3 using siRNA transfection. Three genes—*Ins1/2*, *Mafa*, and *Glut2* (*Slc2a2*)—were consistently downregulated when Set7/9 was depleted, whereas several other important β-cell genes (e.g., *Nkx6.1*, the sulfonylurea receptor, a subunit of the ATP-sensitive K^+ channel, glucokinase, and *Pdx1*) were unaffected. Reduction of *Ins1/2* and *Glut2* mRNAs was also found to be associated with reduced H3K4 dimethylation in the promoter region of these two genes.

To confirm the relevance of these findings, Deering et al. depleted Set7/9 by directly injecting chemically stabilized siRNA into mice. Although this unusual strategy does not result in exclusive targeting of siRNA to the β-cells, it can be effective when the targeted gene is highly expressed in the tissue under study and the tissue is particularly competent for siRNA transfection —and in this case it worked. *Ins1/2*, *Mafa*, and *Glut2* were again downregulated in the islets of the injected mice by two independent siRNAs directed against Set7/9. Moreover, the physiology of the islets was altered as shown by a decrease in glucose-stimulated calcium mobilization and insulin secretion.

What specific role does Set7/9 and H3K4 dimethylation play in transcription of the insulin gene in the context of other transcription factors? PDX-1 appears to be required at the insulin promoter to transition the RNA polymerase from transcription initiation to elongation but is insufficient by itself to recruit RNA polymerase to the promoter (Fig. 1). Deering et al. show that depleting Set7/9 results in decreased recruitment of RNA polymerase II to the insulin and glut2 promoter. PDX-1 is required for Set7/9 to bind to these promoters, whereas PDX-1 can bind them without Set7/9. Not addressed is whether Set7/9 only acts to methylate H3K4 or is also required to be bound at the promoter to maintain the methylated state, i.e., does the prince kiss and run or hang around for a stable relationship?

Future studies are needed to determine whether H3K4 dimethylation by Set7/9 is required to establish an active insulin promoter during the initial differentiation of the endocrine progenitor cells into β-cells during embryonic development. Once the differentiated state is established, it must be maintained during cell division when many tissue-specific expressed genes are transiently turned off (2,5). The epigenetic memory of transcriptionally active genes during mitosis appears to involve a complex set of modifications comprised of methylation and acetylation of both histones and DNA. The establishment and regulation of chromatin interactions at the insulin gene as well as key regulators of β-cell development are pertinent to ongoing efforts to develop surrogate sources of insulin in the treatment of type 1 diabetes. Recently Zhou et al. (10) demonstrated that exocrine pancreatic acinar cells could

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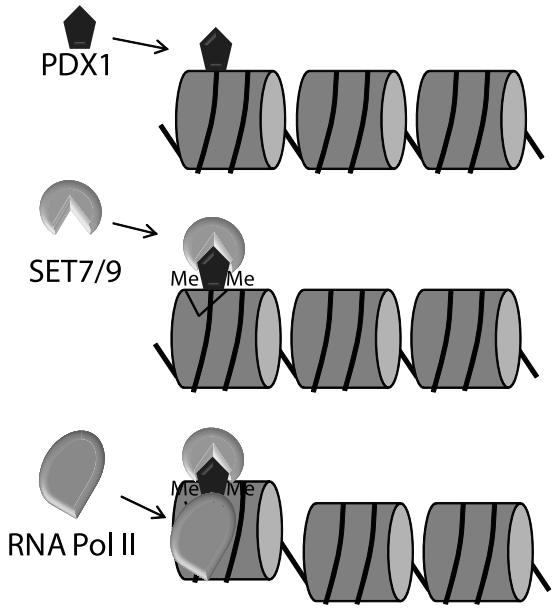

FIG. 1. Proposed model for activation of transcription of the insulin gene mediated by H3K4 methylation. PDX-1 is proposed to bind first to the insulin promoter, allowing the recruitment of Set7/9 followed by dimethylation of H3K4. Methylation H3K4 opens the chromatin structure of the promoter and facilitates recruitment of RNA polymerase II and subsequent transcription of the insulin gene. Numerous other transcription factors and modifying enzymes participate in these reactions (not shown), but their interactions with Set7/9 and PDX-1 are largely unknown.

be reprogrammed to become insulin-secreting β-like cells by injecting three genes (*Pdx1*, *Ngn3*, and *Mafa*) into adult pancreata of mice. Although chromatin modification was not investigated in this latter study, we can surmise that Set7/9 may have been activated in these cells by virtue of the increased levels of PDX-1. Introduction of these three genes in other tissues did not result in reprogramming, suggesting that the exocrine pancreas, which shares a common developmental origin with the endocrine pancreas, is predisposed to reprogramming to β-cells. This predisposition of exocrine pancreatic acinar cells may be related to epigenetic memory of chromatin from early stages of pancreatic development before the separation of endocrine and exocrine pancreatic lineages. Changes in the H3 methylation status are also correlated with gene silencing, and Park et al. (11) have shown that type 2 diabetes induced by intrauterine growth retardation is associated with silencing of *Pdx1* through demethylation of H3K4 and methylation of H3K9. Further research is clearly warranted to confirm the cause-and-effect relationship of chromatin modification and regulation of gene expression in β-cells and to extend these studies to humans as related to both the etiology and treatment of diabetes.

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