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Role of metabolic programming in the pathogenesis of β-cell failure in postnatal life

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Abstract Intrauterine growth retardation (IUGR) has been linked to later development of type 2 diabetes in adulthood. Human studies indicate that individuals who were growth retarded at birth have impaired insulin secretion and insulin resistance. Multiple animal models of IUGR demonstrate impaired β-cell function and development. We have developed a model of IUGR in the rat that leads to diabetes in adulthood with the salient features of most forms of type 2 diabetes in the human: progressive defects in insulin secretion and insulin action prior to the onset of overt hyperglycemia. Decreased β-cell proliferation leads to a progressive decline in β-cell mass. Using this model, we have tested the hypothesis that uteroplacental insufficiency disrupts the function of the electron transport chain in the fetal β-cell and leads to a debilitating cascade of events: increased production of reactive oxygen species, which in turn damage mitochondrial (mt) mtDNA and causes further production of reactive oxygen species (ROS). The net result is progressive loss of β-cell function and eventual development of type 2 diabetes in the adult. Studies in the IUGR rat also demonstrate that an abnormal intrauterine environment induces epigenetic modifications of key genes regulating β-cell development; experiments directly link chromatin remodeling with suppression of transcription. Future research will be directed at elucidating the mechanisms underlying epigenetic modifications in offspring.

Keywords Intrauterine growth retardation · Type 2 diabetes · Fetal origins of adult disease · Epigenetics · Mitochondria · Oxidative stress

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Abbreviations

IUGR	intrauterine growth retardation
SGA	small for gestational age
LP	low protein
HDAC1	histone deacetylase 1

1 Introduction

The period from conception to birth is a time of rapid growth, cellular replication and differentiation, as well as functional maturation of organ systems. These processes are very sensitive to alterations in the intrauterine milieu. Programming describes the mechanisms whereby a stimulus or insult at a critical period of development has lasting or lifelong effects. This review will discuss the human and animal data supporting the Developmental Origin's of Adult Disease hypothesis and some of the underlying cellular and molecular mechanisms responsible for the observed defects in β-cell function and development.

2 Low birth weight

It is becoming increasingly apparent that the *in utero* environment in which a fetus develops may have long-term effects on subsequent health and survival [1, 2]. The landmark cohort study of 300,000 men by Ravelli et al. showed that exposure to the Dutch famine of 1944–1945 during the first one-half of pregnancy resulted in significantly higher obesity rates at age 19 years [3]. Subsequent studies of English men demonstrated a relationship between low birth weight and the later development of cardiovascular disease [4] and impaired glucose tolerance [5–8]. Other studies of populations in the United States [9–11],

Sweden [12], France [13, 14], Norway [15], and Finland [16], have demonstrated a significant correlation between low birth weight and the later development of adult diseases. The associations with low birth weight and increased risk of coronary heart disease, stroke, and type 2 diabetes remain strong, even after adjusting for lifestyle factors (e.g., smoking, physical activity, occupation, income, dietary habits, childhood socio-economic status) and occur independent of the current level of obesity or exercise [17].

3 High birth weight

An increased birth weight is associated with an enhanced body mass index (BMI) and an elevated prevalence of adulthood obesity [18]. Those individuals who are obese as adults tend to have been heavier at birth and to have had an accelerated gain in body mass through childhood and adolescence. Factors in early childhood may lead to obesity through metabolic programming (discussed below) or establishment of lifestyle behaviors. During infancy, breastfeeding may protect against the development of excess weight during childhood. Most, but not all, epidemiological studies demonstrate this protective effect, which could be mediated by behavioral and/or physiological mechanisms. Confounding cultural factors associated with both the decision to breastfeed and later obesity, however, are possible. Recent data also suggest that rapid weight gain during infancy is associated with obesity later in childhood, perhaps reflecting a combination of genetically determined catch-up growth and postnatal environmental factors [18].

4 Low birth weight and insulin secretion

It remains controversial as to whether the adverse effects of intrauterine growth retardation on glucose homeostasis are mediated through programming of the fetal endocrine pancreas [1]. Growth-retarded fetuses and newborns have been reported to have both a reduced population of pancreatic β-cells [19] or a normal percentage of pancreatic area occupied by β-cells [20]. Both of these studies were observational, morphometric analyses were not optimal, and only a small number of fetuses/newborns were examined. It is likely that a significant proportion, but not all growth-retarded fetuses will have reduced β-cell numbers. A more clinically relevant consideration is the impact of fetal growth retardation upon β-cell function.

Intrauterine growth retarded (IUGR) fetuses have been found to exhibit lower insulin and glucose levels and higher G/I ratio in the third trimester as measured by cordocentesis [21]. Two recent studies showed that IUGR infants display decreased pancreatic β-cell function, but increased insulin sensitivity at birth [22, 23]. Low birth weight has been associated with reduced insulin response after glucose ingestion in young non-diabetic men, whereas, other studies have found no impact of low birth weight upon insulin secretion [17, 18]. However, none of these earlier studies adjusted for the corresponding insulin sensitivity, which has a profound impact upon insulin secretion. Therefore, Jensen et al. measured insulin secretion and insulin sensitivity in a well-matched Caucasian population of 19-year-old glucose tolerant men with birth-weights of either below the 10th percentile (small for gestational age—SGA) or between the 50th and 75th percentile (controls) [24]. To eliminate the major confounders such as "diabetes genes", none of the participants had a family history of diabetes, hypertension, or ischemic heart disease. There was no difference between the groups with regard to current weight, body mass indes (BMI), body composition, and lipid profile. When controlled for insulin sensitivity, insulin secretion was reduced by 30%. Insulin sensitivity, however, was normal in the SGA subjects. The investigators hypothesized that defects in insulin secretion may precede defects in insulin action and that once SGA individuals accumulate body fat, they will develop insulin resistance [24].

5 Insulin secretion in offspring of diabetic mothers

Several epidemiological studies show that the risk for diabetes is significantly higher in the offspring of mothers who have type 2 diabetes [25–28]. It is likely that impaired β-cell function contributes to this increased risk. Islet hypertrophy and β-cell hyperplasia are typical features of fetuses and newborns of diabetic mothers [29]. Most studies have focused on altered glucose homeostasis during the newborn period; two clinical investigations done at older ages, however, demonstrate altered insulin secretion [28, 30]. Thus, both a deficiency and a surfeit of nutrient availability to the fetus during development have profound lasting effects upon β-cell function.

6 Genetics versus environment

Several epidemiological and metabolic studies of twins and first-degree relatives of patients with type 2 diabetes have demonstrated an important genetic component of diabetes [31–34]. The association between low birth weight and risk of type 2 diabetes in some studies could theoretically be explained by a genetically-determined reduced fetal growth rate. In other words, the genotype responsible for type 2 diabetes may itself cause retarded fetal growth *in utero*. This forms the basis for the fetal insulin hypothesis, which

suggests that genetically-determined insulin resistance could result in low insulin-mediated fetal growth *in utero* as well as insulin resistance in childhood and adulthood [35]. Insulin is one of the major growth factors in fetal life and monogenic disorders that affect fetal insulin secretion or fetal insulin resistance also affect fetal growth. Mutations in the gene encoding glucokinase have been identified that result in low birth weight and maturity onset diabetes of the young [36, 37].

The recently described type 2 diabetes susceptibility gene Transcription Factor7-like 2 (*TCF7L2*) confers a risk-allele frequency of approximately 30% [38]. Studies of non-diabetic subjects show that *TCF7L2* diabetes-risk genotypes alter insulin secretion [39–41]. A large study of 24,053 subjects from 6 studies demonstrated that *TCF7L2* is the first type 2 diabetes gene to be reproducibly associated with altered birth weight. Each maternal copy of the T allele at *re7903146* increased offspring birth weight by 30 g, and the investigators suggest that the most likely mechanism is through reduced maternal-insulin secretion resulting in maternal hyperglycemia and increased insulin-mediated fetal growth [42].

Recent genetic studies suggest that the increased susceptibility to type 2 diabetes of subjects who are born SGA also results from the combination of both genetic factors and an unfavorable fetal environment. Polymorphisms of Peroxisome Proliferator-Activated Receptor-γ2 (PPARγ2), a gene involved in the development and in the metabolic function of adipose tissue, have been shown to modulate the susceptibility of subjects who are born SGA to develop insulin resistance later in life [43, 44]. The polymorphism is only associated with a higher risk of type 2 diabetes if birth weight is reduced [43, 44]. There is obviously a close relationship between genes and the environment. Not only can maternal gene expression alter the fetal environment, the maternal intrauterine environment also affects fetal gene expression and both influence birth weight.

7 What animal models can tell us

Animal models have a normal genetic background upon which environmental effects during gestation or early postnatal life can be tested for their role in inducing diabetes. Ontogeny of β-cell development in the rodent approximates what has been observed in the human [45, 46]. The most commonly used animal models for IUGR are caloric or protein restriction, glucocorticoid administration, or induction of uteroplacental insufficiency in the pregnant rodent. In the rat, maternal dietary protein restriction (approximately 40–50% of normal intake, termed LP) throughout gestation and lactation has been reported to alter insulin secretory capacity and reduce β-cell mass through a reduction in β-cell proliferation rate and an increase in apoptosis [47–55]. Expression of Pdx-1 (pancreatic duodenal homeobox-1), a homeodomain-containing transcription factor that regulates early development of both endocrine and exocrine pancreas, and later differentiation and function of β-cells [56], is also reduced in islets from pups of LP mothers [57]. In adulthood, rats born from LP mothers still have reductions in β-cell mass and insulin secretion and show glucose intolerance, but usually not overt diabetes [47, 48, 55]. In old age, LP offspring develop fasting hyperglycemia associated with insulin resistance [58–62].

Total caloric restriction during the last week of pregnancy and throughout lactation also reduces β-cell mass and impairs insulin secretion in the offspring [63, 64]. When maternal undernutrition is prolonged until weaning and normal nutrition is given to the offspring from weaning onwards, growth retardation and β-cell mass reduction persists into adulthood [64].

Treatment of pregnant rats with dexamethasone during the last week of gestation retards fetal growth [65]. Insulin content of fetal β-cells is reduced and is associated with a reduction in Pdx-1 [65].

An ovine model of IUGR induced by placental insufficiency (heat-induced) results in a significant reduction in β-cell mass in fetuses near term (0.9 of gestation) from decreased rates of β-cell proliferation and neogenesis [66]. Plasma insulin concentrations in the IUGR fetuses are lower at baseline and glucose-stimulated insulin secretion is impaired. Similar deficits occur with arginine-stimulated insulin secretion. A deficiency in islet glucose metabolism also occurs in the rate of islet glucose oxidation at maximal stimulatory glucose concentrations. Thus, pancreatic islets from nutritionally-deprived IUGR fetuses caused by chronic placental insufficiency have impaired insulin secretion caused by reduced glucose-stimulated glucose oxidation rates, insulin biosynthesis, and insulin content. This impaired glucose stimulated insulin secretion (GSIS) occurs despite an increased fractional rate of insulin release from a greater proportion of releasable insulin as a result of diminished insulin stores [67].

To extend these experimental studies of growth retardation, we developed a model of IUGR in the rat that restricts fetal growth [68–70]. Growth-retarded fetal rats have critical features of a metabolic profile characteristic of growth-retarded human fetuses: decreased levels of glucose, insulin, IGF-I, amino acids, and oxygen [68–72]. Birth weights of IUGR animals are significantly lower than those of controls until approximately 7 weeks of age, when IUGR rats catch up to controls. Between 7 and 10 weeks of age, the growth of IUGR rats accelerates and surpasses that of controls, and by 26 weeks of age, IUGR rats are obese

[69]. No significant differences are observed in blood glucose and plasma insulin levels at 1 week of age. Between 7 and 10 weeks of age, however, IUGR rats develop mild fasting hyperglycemia and hyperinsulinemia. IUGR animals are glucose-intolerant and insulin-resistant at an early age. First-phase insulin secretion in response to glucose is also impaired early in life in IUGR rats, before the onset of hyperglycemia. There are no significant differences in β-cell mass, islet size, or pancreatic weight between IUGR and control animals at 1 and 7 weeks of age. In 15-week-old IUGR rats, however, the relative β-cell mass is 50% that of controls, and by 26 weeks of age, β-cell mass is less than one-third that of controls. This loss of β-cell mass is accompanied by a reduction in Pdx-1 expression that is greater than that in β-cell mass [73]. By 6 months of age, IUGR rats develop diabetes with a phenotype remarkably similar to that observed in the human with type 2 diabetes: progressive dysfunction in insulin secretion and insulin action [69]. Thus, despite different animal models of IUGR, these studies support the hypothesis that an abnormal intrauterine milieu can induce permanent changes in β-cell function after birth and lead to type 2 diabetes in adulthood.

8 Cellular mechanisms: Mitochondrial dysfunction and oxidative stress

Uteroplacental insufficiency, caused by such disorders as preeclampsia, maternal smoking and abnormalities of uteroplacental development, is one of the most common causes of fetal growth retardation. The resultant abnormal intrauterine milieu restricts the supply of crucial nutrients to the fetus, thereby limiting fetal growth. Multiple studies have shown that intrauterine growth retardation is associated with increased oxidative stress in the human fetus [73–80]. A major consequence of limited nutrient availability is an alteration in the redox state in susceptible fetal tissues leading to oxidative stress. In particular, low levels of oxygen, evident in growth-retarded fetuses, will decrease the activity of complexes of the electron transport chain, which will generate increased levels of reactive oxygen species (ROS). Overproduction of ROS initiates many oxidative reactions that lead to oxidative damage not only in the mitochondria but also in cellular proteins, lipids, and nucleic acids. Increased ROS levels inactivate the iron–sulfur centers of the electron transport chain complexes, and tricarboxylic acid cycle aconitase, resulting in shutdown of mitochondrial energy production.

A key adaptation enabling the fetus to survive in a limited energy environment may be the reprogramming of mitochondrial function. However, these alterations in mitochondrial function can have deleterious effects, especially in cells that have a high-energy requirement, such as the β-cell. The β-cell depends upon the normal production of ATP for nutrient-induced insulin secretion [81–88] and proliferation [89]. Thus, an interruption of mitochondrial function can have profound consequences for the β-cell.

Mitochondrial dysfunction can also lead to increased production of ROS, which causes oxidative stress if the defense mechanisms of the cell are overwhelmed. β-cells are especially vulnerable to ROS because expression of antioxidant enzymes in pancreatic islets is very low [90, 91], and β-cells have a high oxidative energy requirement. Increased ROS impair glucose-stimulated insulin secretion [92, 93], decrease gene expression of key β-cell genes [94–98], and induce cell death [98–103].

We have examined the causal role of mitochondrial dysfunction in the impairment of β-cell function and development in IUGR offspring [104]. Reactive oxygen species production and oxidative stress gradually increase in IUGR islets. ATP production is impaired and continues to deteriorate with age. The activities of complex I and III of the electron transport chain progressively decline in IUGR islets. Mitochondrial DNA point mutations accumulate with age and are associated with decreased mitochondrial DNA content and reduced expression of mitochondria-encoded genes in IUGR islets. Mitochondrial dysfunction results in impaired insulin secretion. These results demonstrate that IUGR induces mitochondrial dysfunction in the fetal β-cell, leading to increased production of ROS, which in turn damage mitochondrial DNA. A self-reinforcing cycle of progressive deterioration in mitochondrial function leads to a corresponding decline in β-cell function. Finally, a threshold in mitochondrial dysfunction and ROS production is reached, and diabetes ensues [104].

9 Molecular mechanisms: Epigenetic regulation of β-cell genes

An adverse intrauterine milieu impacts the development of the fetus by modifying gene expression in both pluripotential cells and terminally differentiated, poorly replicating cells such as the β-cell. The long-range effects on the offspring [into adulthood] depend upon the cells undergoing differentiation, proliferation, and/or functional maturation at the time of the disturbance in maternal fuel economy. Permanent alterations to the phenotype of the offspring suggest that fetal growth retardation is associated with stable changes in gene expression.

Epigenetic modifications of the genome provide a mechanism that allows the stable propagation of gene activity states from one generation of cells to the next. Excellent reviews on this topic appear frequently, reflecting the rapid advances of knowledge in the field [105–108]. Epigenetic states can be modified by environmental factors,

which may contribute to the development of abnormal phenotypes. There are at least two distinct classes of epigenetic information that can be inherited with chromosomes. One class of epigenetic control of gene expression involves changes in chromatin proteins, usually involving modifications of histone tails. The amino termini of histones can be modified by acetylation, methylation, sumoylation, phosphorylation, glycosylation, and ADP ribosylation. The most common modifications involve acetylation and methylation of lysine residues in the amino termini of H3 and H4. Increased acetylation induces transcription activation, whereas decreased acetylation usually induces transcription repression. Methylation of histones is associated with both transcription repression and activation.

The second class of epigenetic regulation is DNA methylation, in which a cytosine base is modified by a DNA methyltransferase at the C5 position of cytosine, a reaction that is carried out by various members of a single family of enzymes. Approximately 70% of CpG (cytosine–guanine) dinucleotides in human DNA are constitutively methylated, whereas most of the unmethylated CpGs are located in CpG islands. CpG islands are CG-rich sequences located near coding sequences, and serve as promoters for the associated genes. Approximately half of mammalian genes have CpG islands. Methylation of CpG sites is also maintained by DNA methyltransferases. DNA methylation is commonly associated with gene silencing and contributes to X-chromosomal inactivation, genomic imprinting, as well as transcriptional regulation of tissue-specific genes during cellular differentiation [108].

Most CpG islands remain unmethylated in normal cells, however, under some circumstances such as cancer [109–114] and oxidative stress (see below), they can become methylated *de novo*. This aberrant methylation is accompanied by local changes in histone modification and chromatin structure, such that the CpG island and its embedded promoter acquire a repressed conformation that is incompatible with gene transcription. It is not known why particular CpG islands are susceptible to aberrant methylation. A recent study by Feltus et al. suggests that there is a "sequence signature associated with aberrant methylation" [115]. Of major significance to type 2 diabetes is their finding that Pdx-1 is one of only 15 CpG genes (a total of 1749 genes with CpG islands were examined) that is susceptible to increased methylation from over-expression of a DNA methyltransferase.

Hypermethylation of specific genes has also been observed in tissues of aging individuals [113]. As an age-related disease, type 2 diabetes increases in prevalence in older populations as the metabolic profile of individuals deteriorates with time. DNA methylation errors that accumulate with increasing age could explain this phenomenon, perhaps through induction of oxidative stress.

Reactive oxygen species can also lead to alterations in DNA methylation, without changing the DNA base sequence [117]. Such changes in DNA methylation patterns have been shown to affect the expression of multiple genes [116]. Replacement of guanine with the oxygen radical adduct 8-hydroxyguanine profoundly alters methylation of adjacent cytosines [116]. Histones, because of their abundant lysine residues, are also very susceptible to oxidative stress [117–119].

A number of studies have suggested that uteroplacental insufficiency induces epigenetic modifications in the offspring [120–124]. Genome-wide DNA hypomethylation has been found in postnatal IUGR liver and is associated with an increase in total H3 acetylation [120]. Acetylation of histone H3 and acetylation of H3 lysine-9 (H3/K9), lysine-14 (H3/K14), and lysine-18 (H3/K18) is increased at the promoters of PPAR-coactivator-1 (PGC-1) and Carnitine palmitoyltransferase 1 (CPTI), respectively, in IUGR liver [122]. At day 21 of life, the neonatal pattern of H3 hyperacetylation persists only in the IUGR males. Whether hyperacetylation at these sites actually causes increased transcription of PGC-1 or CPT1 and how these findings relate to a phenotype in the offspring remains to be determined.

We have examined epigenetic regulation of Pdx-1 in β-cells of IUGR rats [125]. As early as 24 h after the onset of growth retardation, Pdx-1 mRNA levels are reduced by more than 50% in IUGR fetal rats [73]. Suppression of Pdx-1 expression persists after birth and progressively declines in the IUGR animal, implicating an epigenetic mechanism. The proximal promoter of Pdx-1 is obligate for transcription of the gene and the histones H3 and H4 are heavily acetylated in normal β-cells [126]. In islets of IUGR animals, however, H3 and H4 in this region of the Pdx-1 promoter are deacetylated. Histone deacetylation is catalyzed by histone deacetylases (HDACs) and HDAC1 is strongly associated with the proximal Pdx-1 promoter in IUGR β-cells. Reversal of deacetylation by an HDAC inhibitor normalizes Pdx-1 expression in islets of IUGR animals, demonstrating that histone deacetylation contributes to the observed Pdx-1 transcription suppression [125].

Unlike acetylation, histone H3 methylation can be equally associated with either transcriptional activation or repression. Methylation of the lysine residue Lys4 H3 (H3-K4) correlates with activation of gene expression, whereas H3Lys9 (H3-K9) methylation is involved in the establishment and maintenance of silent heterochromatin regions [105–108]. Lysine methylation is catalyzed by the action of histone methyltransferases (SET7/9), which demonstrate a high degree of specificity for H3-K4. There is a loss of binding of SET7/9 to the proximal promoter of Pdx-1 in β-cells from IUGR animals, which results in a marked reduction of methylation of H3K4 in this region of Pdx-1.

These observations demonstrate that the level of H3 acetylation is linked to the degree of H3K4 methylation.

Transcriptional repression is also facilitated by methyl-CpG binding proteins that bind to promoter-proximal methylated DNA sequences, thereby maintaining the condensed nucleosome structure [127]. However, one methyl-CpG-binding domain protein-MeCP2 also mediates transcription repression through histone deacetylation [128–130]. MeCP2 contains a transcriptional repression domain which functions by recruitment of the co-repressor Sin3A, a histone deacetylase [128–130], and a histone 3 lysine 9 methyltransferase (Suv39h) [131, 132]. MeCP2 binding occurs in IUGR fetal pancreas as early as 24 h after uterine artery ligation. Association of MeCP2 with the proximal promoter of Pdx-1 precipitates Sin3A binding at day 1 of life in IUGR islets. The repressor complex consisting of MeCP2, Sin3A, HDAC1 and Suv39h induces H3 deacetylation and methylation of H3K9 [125]. Thus, a cascade of epigenetic events is triggered by IUGR resulting in permanent suppression of Pdx-1 expression. The sequence of epigenetic events (Fig. 1) that occurs in IUGR islets leading to suppression of Pdx-1 transcription appears to be the following: MeCP2 binds to methylated DNA in the CpG island at the Pdx-1 promoter. This results in recruitment of a repressor complex, which catalyze the deacetylation of H3 and methylation of H3K9, respectively. Deacetylation of H3 in turn promotes the loss of H3K4 methylation, further suppressing Pdx-1 transcription. As the IUGR animals age, DNA methylation of the CpG island progresses, thereby permanently silencing Pdx-1 expression [125].

How do these events lead to diabetes? Targeted homozygous disruption of Pdx-1 in mice results in pancreatic agenesis [133], and homozygous mutations yield a similar phenotype in humans [134]. Milder reductions in Pdx-1 protein levels, as occurs in the *Pdx*+/− mice, allow for the development of a normal mass of β-cells [135], but impair several events in glucose-stimulated insulin secretion [136]. These results indicate that Pdx-1 plays a critical role, distinct from its developmental role, in the normal function of β-cells [137]. This may be the reason why humans with heterozygous missense mutations in Pdx-1 exhibit early and late onset forms of type 2 diabetes [137, 138].

10 Conclusions

The human and animal studies described above clearly show that an adverse intrauterine environment associated with fetal growth retardation or fetal overgrowth results in impaired function and development of the β-cell, which in turn leads to the development of type 2 diabetes. Animal models demonstrate that the cellular and molecular mechanisms underlying altered β-cell development are related to abnormal mitochondrial function and epigenetic alterations of key β-cell genes.

11 Key unanswered questions

Much of the recent progress in understanding epigenetic phenomena is directly attributable to technologies that allow researchers to pinpoint the genomic location of proteins that package and regulate access to the DNA. The advent of DNA microarrays and inexpensive DNA sequencing has allowed many of those technologies to be applied to the whole genome. It is possible that epigenetic profiling of CpG islands in the human genome can be used as a tool to identify genomic loci that are susceptible to DNA methylation or loss of DNA methylation.

The genome-wide mapping of histone modifications by ChIP-chip has led to important insights regarding the mechanism of transcriptional and epigenetic memory, and how different chromatin states are propagated through the

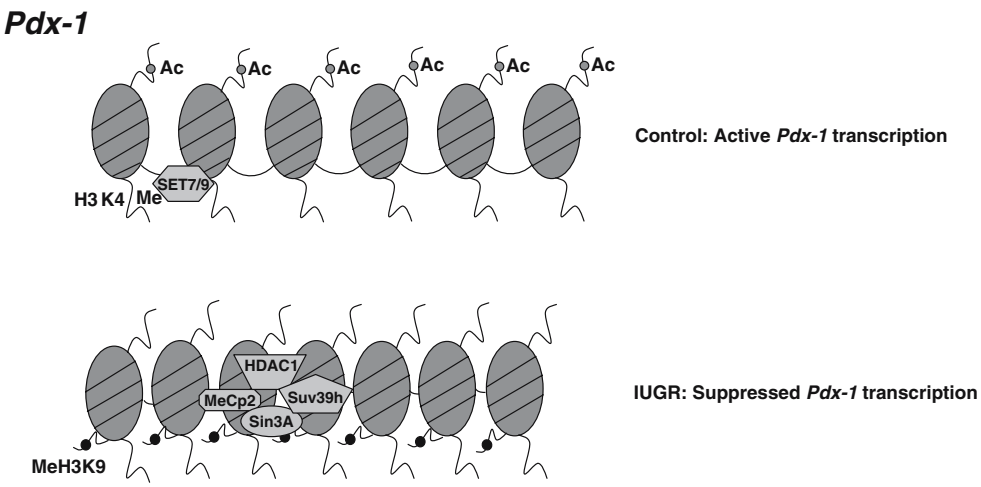

Fig. 1 Schematic of histone acetylation and methylation of the proximal promoter of Pdx-1. In control animals, H3 is heavily acetylated and H3K4 is methylated in islets from control animals. H3K4 methylation is catalyzed by SET7/9. In IUGR animals, acetylation of H3 and methylation of H3K4 of Pdx-1 are lost and methylation of H3K9 is gained. These histone modifications are mediated by HDAC1 (histone deacetylase 1), Sin3A, and Suv39h

genome [139]. In the near future it is likely that technologies will be developed that will allow genome-wide epigenetics studies, especially applied to the limited numbers of cells that can be isolated to a high degree of purity by techniques such as laser capture microscopy. Epigenetic modifications can then be used as biomarkers for disease.

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