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TOPIC HIGHLIGHT

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Epigenetic mechanisms involved in developmental nutritional programming

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Abstract

The ways in which epigenetic modifications fix the effects of early environmental events, ensuring sustained responses to transient stimuli, which result in modified gene expression patterns and phenotypes later in life, is a topic of considerable interest. This review focuses on recently discovered mechanisms and calls into question prevailing views about the dynamics, position and functions of epigenetic marks. Most epigenetic studies have addressed the long-term effects on a small number of epigenetic marks, at the global or individual gene level, of environmental stressors in humans and animal models. In parallel, increasing numbers of studies based on high-throughput technologies and focusing on humans and mice have revealed additional complexity in epigenetic processes, by highlighting the importance of crosstalk between the different epigenetic marks. A number of studies focusing on the developmental origin of health and disease and metabolic programming have identified links between early nutrition, epigenetic processes and long-term illness. The existence of a self-propagating epigenetic cycle has been demonstrated. Moreover, recent studies demonstrate an obvious sexual dimorphism both for programming trajectories and in response to the same environmental insult. Despite recent progress, we are still far from understanding how, when and where environmental stressors disturb key epigenetic mechanisms. Thus, identifying the original key marks and their changes throughout development during an individual's lifetime or over several generations remains a challenging issue.

Key words: DNA methylation; Developmental origin of health and disease; Epigenetics; Histone modifications; Nutrition

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INTRODUCTION

Epigenetic marks are candidate memories of early life events. All the cells in the body have identical genomes. However, each cell has one of many "epigenomes", unique sets of epigenetic instructions for establishing and maintaining lineage-specific expression profiles[1]. The genome is programmed to express appropriate sets

of genes, in particular tissues, at specific time points during the individual's life. Epigenetic events create a memory of cell identity, maintaining genomic functions such as the maintenance of cell identity after differentiation, the propagation of essential features of chromosomal architecture and dosage compensation[2].

Unlike genetic information, which is extremely stable, epigenetic events are reversible, responding to endogenous and exogenous (environmental) signals. There is convincing experimental evidence to suggest that epigenetic marks serve as a memory of exposure, in early life, to inappropriate environments. These marks induce long-term changes in gene expression, potentially leading to disease in later life, the "developmental origin of health and disease" (DOHaD) hypothesis[3,4].

We focus here on the most recently discovered mechanisms. Significant advances in analytical technologies have led to epigenome characterization becoming a key element in increasing numbers of investigations[5-10]. These recent data challenge prevailing views about the dynamics, relevant position and functions of many epigenetic marks and their complex patterns of crosstalk. We highlight improvements in our understanding of the relationships between epigenetic processes and environmental factors, such as maternal nutrition, and discuss the gaps in our knowledge that remain to be filled. The reversibility of the chromatin modification states determining gene expression status is essential for interaction between the environment and the dynamic epigenome. However, some epigenetic marks laid down early in development, under the influence of environmental factors, must remain stable, acting as a memory of the event long after exposure has ceased. The basis of this paradox, the need for both reversibility and stability, remains unclear.

RECONSIDERING DNA METHYLATION DOGMAS

Cytosine methylation is the only epigenetic modification directly affecting the DNA molecule. It is required for correct embryonic development in mammals. The DNA of most vertebrates is depleted in CpG dinucleotides, the main target for DNA methylation. Furthermore, the role of DNA methylation in genome regulation, other than in genomic imprinting and X inactivation, remains unclear. CpG islands (CGIs) and promoters have been studied in detail because they are easily accessible in terms of the techniques available and sequence specificity. However, other sequences should be taken into consideration (Figure 1).

Mammalian genomes are punctuated by CGIs, DNA sequences with an unusually high frequency of CpG sites[11]. There is considerable evidence for a functional role of CGI-promoter methylation in transcription, but the correlation between CGI methylation and transcription status is poor for many genes. Recently, "CGI shores" were defined as sequences up to 2 kb around CGI and their methylation are highly conserved, tissue-specific and strongly related to gene expression[12,13]. Several large-scale methylation studies have called into question some of the prevailing views about the dynamics and function of DNA methylation. Weber and co-workers investigated the function of DNA methylation in *cis*-regulatory regions and its impact on gene expression by mapping DNA methylation throughout the genome with a methylated DNA immuno precipitation-chip approach and defined three classes of promoters in terms of CpG frequency[14]. They showed that (1) the methylation of CpG-poor promoters did not prevent gene expression; (2) DNA methylation was not a general mechanism of gene repression, as most CGI promoters remain unmethylated even when inactive; and (3) DNA methylation was principally involved in regulating key developmental genes. Thus, promoter CpG density and gene function are the main predictors of promoter methylation state. Shen and co-workers reported that a subset of CGIs within the promoters of key developmental genes were subject to tissue-specific methylation during development. Such methylation had previously been reported only for imprinted and X-inactivated genes. This observation suggests the existence of a programmed mechanism of DNA methylation[15]. Unmethylated regions, recently identified as non-promoter CGIs, become methylated during development in a tissue-specific manner, potentially modifying gene expression[16]. Thus, the methylation of other regulatory elements may also be important for transcriptional regulation. Moreover, as first observed for the active X chromosome, gene-body methylation may be a hallmark of active genes in the whole genome[11,17,18].

Most studies have focused on the methylation of CpG nucleotides, but a potential role of non-CpG methylation has been demonstrated in embryonic cells and adult tissues. In non-CpG contexts, methylation is observed principally in gene bodies, being much rarer at protein-binding sites and enhancers and entirely absent after the induction of differentiation in embryonic stem (ES) cells[19-21]. In a pathological context, Barrès *et al*[20] showed that non-CpG methylation was readily detectable in the skeletal muscles of patients with type 2 diabetes (T2D). They found that the peroxisome proliferator-activated receptor g coactivator-1a (PGC-1a) gene displayed hypermethylation in diabetic subjects, which was negatively correlated with PGC-1a mRNA and mitochondrial DNA levels. Bisulfite sequencing revealed the proportion of non-CpG methylation to be highest. Exposure to tumor necrosis factor α or free fatty acids resulted in a short-term increase in non-CpG methylation in human myotubes. Thus, non-CpG methylation, previously reported almost exclusively in plants and ES cells, may have a physiological role in human skeletal muscle[21,22].

Finally, hydroxymethylation of cytosine was recently identified in mouse ES and neuronal cells[23,24]. Altogether, these new findings highlight the complexity of DNA methylation and the importance of not focusing solely on CGIs and promoters, which should be taken into account in future studies.

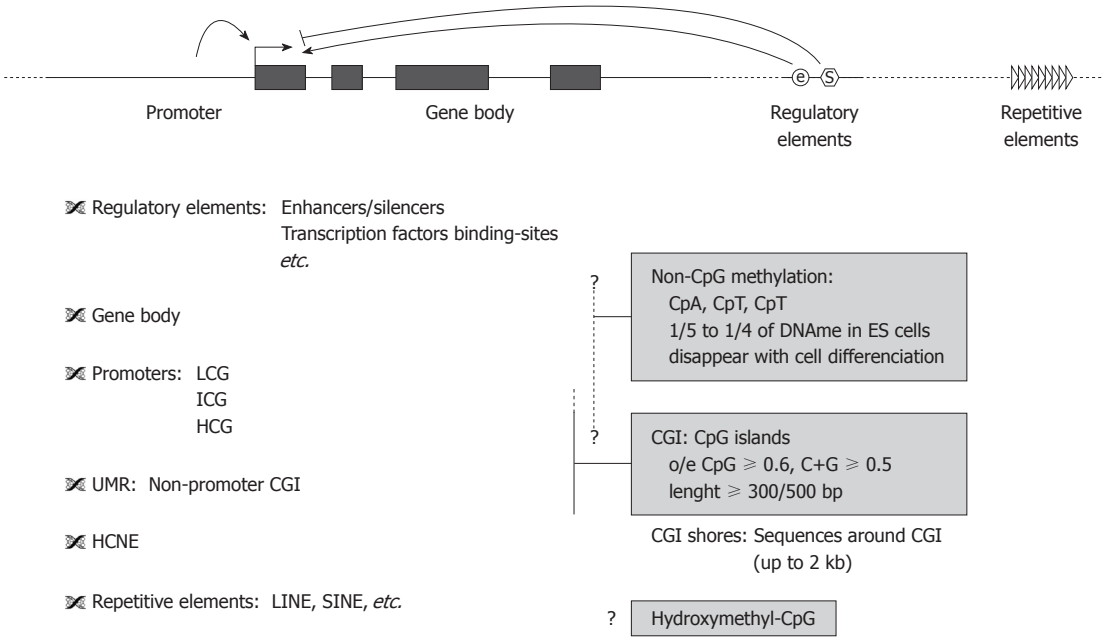

Figure 1 Target sequences for DNA methylation studies. The majority of DNA methylation concerns methylcytosine on CpG dinucleotides but recently hydroxymethylcytosine and methylation on non-CpG sites were identified. Non-CpG methylation was reported in gene body, promoters and repetitive elements; its expanse needs to be further investigated. CpG islands (CGI) and gene promoters are preferred targets in many studies as they correspond to a tractable fraction of the genome with obvious regulatory potential. CGIs are defined algorithmically, as sequences with an observed-to-expected ratio of CpG greater than 0.6, a G+C content greater than 0.5 and, in most cases, a length of more than 500 bp. Three classes of promoters were defined according to their CpG content: LCP have the highest probability to be methylated but methylation correlates poorly to transcription, HCP have low probability to be methylated but this correlates with gene expression. However, transcriptional regulation of genes depends also on distal regulatory elements such as enhancers, insulators, locus control regions and silencing elements. In addition, recent studies show that gene-bodies in active transcription sites are enriched in DNA methylation. Moreover, non-promoter CGIs unmethylated regions (UMR) were recently identified, initially unmethylated they become methylated during development in a tissue-specific manner. "CGI shores" sequences were described around CGI, their methylation in normal tissues is highly conserved, tissue-specific and strongly related to gene expression and were highly sensitive to DNA alterations in colon cancer, as opposed to promoters or CGIs. Highly methylated repetitive elements and highly conserved non-coding elements can also be interesting targets for DNA methylation studies. LCG: Low-CpG promoters; ICG: Intermediate-CpG promoters; HCG: High-CpG promoters; HCNE: Highly conserved non-coding elements.

EVOLVING WORLD OF HISTONE MODIFICATIONS

Recent studies have greatly modified our understanding of histone modifications. Histone modifications lead to the recruitment and binding of critical DNA-regulatory proteins controlling transcription, replication, recombination and repair. Each modification constitutes a signal that is read alone or in combination with other marks on the same or neighboring histones, constituting a "histone code". Histone protein tails display at least 9 different types of post-translational modifications (e.g., acetylation, methylation, ubiquitination, phosphorylation…) with many target sites and at least 50 different modifications having been identified[25]. Histone-modifying enzymes, such as histone methyltransferases or histone demethylases, histone acetyl transferases or histone deacetylases (HDACs) add or remove epigenetic marks on histone tails[26]. Their presence on histones may induce a higher-order chromatin structure and may co-ordinate the ordered recruitment of enzyme complexes for DNA manipulation. For example, acetylation is associated exclusively with active chromatin states, whereas lysine and arginine methylation may be associated with active transcription or repression. Histone modifications may thus influence many fundamental biological processes and may be epigenetically inherited[25].

A complex picture is emerging in which DNA methylation and histone modifications act in concert in an epigenetic program integrating gene-silencing networks within the cell[27,28]. Crosstalk occurs between DNA methylation and histone modifications and is mediated by methyl- or histone-binding proteins, which decipher the regulatory information encoded by the DNA methylation and histone marks[29].

Ambiguous chromatin structure of ES cells

After fertilization, the acquisition of pluripotency involves the epigenetic resetting of the gamete genome to allow the activation of essential genes, such as pluripotency-associated genes. ES cells have an open chromatin structure that is essential for pluripotency and allows the transcription of developmentally regulated genes. The gene expression program of ES cells keeps these cells in a pluripotent state, but also allows them to differentiate into more specialized cells in response to appropriate signals.

One particular group of transcription factors, "pioneer factors", is essential early in development. Pioneer factors binding to promoters and enhancers enable chromatin access for other tissue-specific transcription factors. Such proteins, including members of the fork head

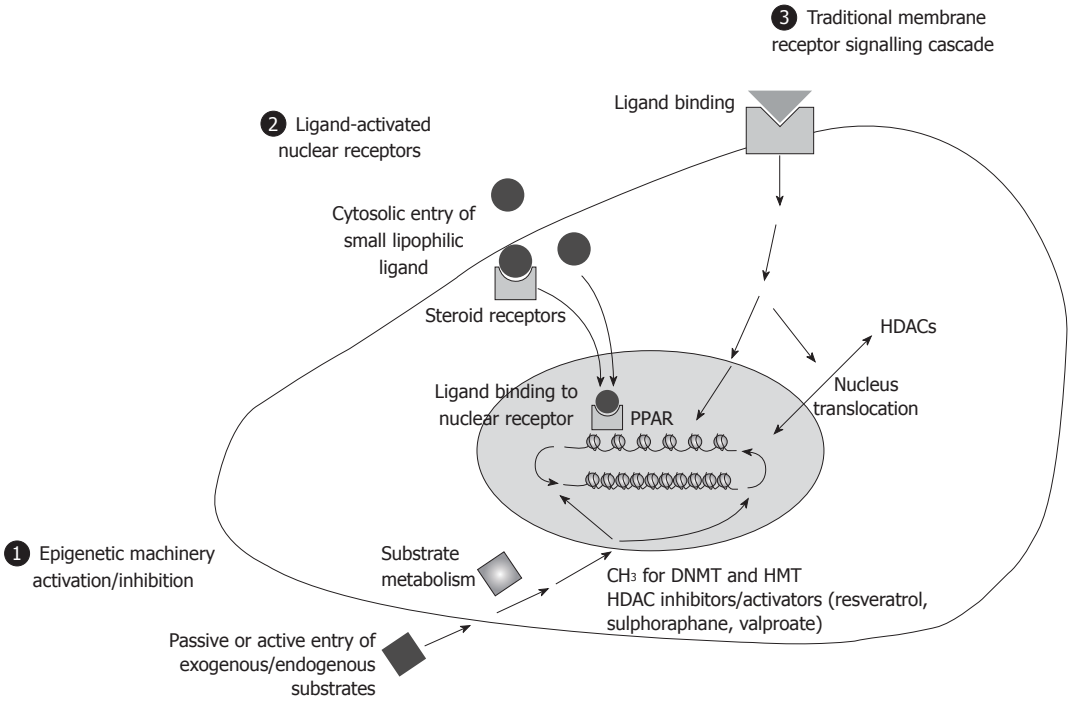

Figure 2 Mechanistic pathways for environmental factors involved in epigenetic reprogramming. There are three ways to link environmental factors such as nutrients or drugs from the cell membrane to the chromatin structure: (1) Some environmental factors, aging and gender may target chromatin modifying enzymes or their substrate availability. Exogenous/endogenous substrates [methyl donors such as folates, histone deacetylase (HDAC) inhibitors such as TSA, *etc.*], after passive or active entry through the cell membrane, undergo cell specific metabolism. Thus, endogenous or exogenous compounds may lead to the alteration of a critical balance of chromatin remodelling enzymes, at the whole genome level, or to specific regions targeted by specific enzymes, e.g. HDACs; (2) Some other compounds (like endocrine disruptors) specifically bind to nuclear receptors, like steroid receptors, may be present in the cytoplasm, bind to their ligand, undergo several modifications and be subsequently translocated to the nucleus where they bind to their responsive elements (RE). The binding of other nuclear receptors, like PPARs and retinoid X receptor (RXR), with their natural polyunsaturated fatty acids ligands or drugs like fibrates lead to the recruitment of co-activators and chromatin remodelling factors. The appropriate modifications of the epigenetic marks at PPAR/RXR RE in target gene promoters modulate the expression of a particular set of genes, in a tissue-specific manner depending on the presence of appropriate co-factors; and (3) Traditional membrane receptor-signalling cascades may be involved. It is possible, depending on the type of ligand, that different pathways could be used. The maintenance of epigenetic patterns is dependent on the preservation of the balance of factors such as DNMTs, Histone acetyl transferases/HDACs or histone methyltransferases/histone demethylases and on the translocation of these enzymes into the nucleus. Extra- or intracellular signalling pathways could trigger activation of one of these factors and result in loci-specific modifications. PPAR: Peroxisome proliferator-activated receptor; DNMT: DNA methyltransferase.

family Foxa, have been found to play important roles at many stages of mammalian life, in early development, organogenesis and, finally, metabolism and homeostasis in the adult[30].

Bivalent chromatin domains, with overlapping repressive H3K27me3 and activating H3K4me3 histone modifications, have recently been shown to mark the promoters of more than 2000 silent regulatory genes involved in developmental processes[7,28,31-36]. Although not unique to ES cells, these bivalent marks seem to play a special role in differentiating cells, keeping developmental genes poised for expression during differentiation. Moreover, inactive unmethylated CGI promoters have high H3K4me2 levels, which may protect DNA from methylation[14]. In addition, Guenther *et al*[37] found that nucleosomes with H3K4me3 and H3K9/K14ac modifications, together with RNA polymeraseII, occupied the promoters of most protein-coding genes in human ES cells. Only a subset of these genes produces detectable full-length transcripts and is occupied by nucleosomes with H3K36me3 modifications, a hallmark of elongation. The others display transcription initiation but not elongation, consistent with regulation principally at post-initiation steps. The genes encoding most developmental regulators fall into this group.

These data suggest a model in which epigenetic marks restrict and define differentiation potential during development[14,38,39]. Identification of the markers involved in establishing transcriptional competence in pluripotent cells should make it possible to explore potential disturbance due to environmental factors. If modulation by environmental stressors occurs at these early stages, the resulting epigenetic marks should escape the resetting process, allowing them to manifest during adulthood[40,41].

How early nutrition sculpts our epigenomes

Throughout evolution, organisms have been faced with the challenge of sensing changes in their environment, such as food depletion and stress, and adapting to them, to ensure their survival. These responses implicitly involve mechanisms, such as chromatin targeting, for adapting the expression of fundamental genes and ensuring genome integrity. Environmental factors, such as diet, nutrients, drugs or the social environment, can be linked

to chromatin structure in several ways (Figure 2)[42].

There is emerging evidence that the environmental changes, triggered at different stages of development, lead to the self-propagation of epigenetic marks associated with changes in gene expression and an adult-onset phenotype[43-55] (for review see[56]). Most studies have dealt with DNA methylation, histone modifications or chromatin occupancy by components of the epigenetic machinery, rarely with combinations of these factors. Such combined studies would provide (1) a better definition of the respective roles of the different epigenetic alterations; (2) an understanding of the consequences of crosstalk between different modifications; (3) identification of the initiating mark; or (4) the matrix for subsequent marks as the individual progresses from an asymptomatic, latent state towards full-blown disease.

Environmental conditions before conception and implantation

Preimplantation development in mammals has recently been shown to be sensitive to environmental conditions, both *in vivo* and *in vitro,* modifying blastocyst potential and leading to long-term changes in fetal and postnatal health and physiology. Similarly, the environment inhabited by a breeding female before conception and early in pregnancy has striking effects on the oocytes developing in the ovarian follicle and embryos in the early stages of development in the reproductive tract. Environmental conditions at these stages may also alter behavior, cardiovascular function and reproductive function throughout postnatal life[57-64]. Low maternal protein consumption or vitamin B and methionine status leads to behavioral and cardiovascular abnormalities in offspring, sex-specific changes in hepatic gene expression in rat fetuses and changes in imprinted gene expression in the rat embryo-fetal axis[65-68]. It has recently been shown that *in vitro* culture conditions, as found in assisted reproduction technology, may affect global patterns of DNA methylation and gene expression. Gametes or early embryos from couples undergoing treatment for infertility may therefore display epigenetic modifications. An association was indeed observed between *in vitro* conception and changes in DNA methylation, potentially affecting the long-term pattern of expression of genes involved in chronic metabolic disorders, such as obesity and T2D[45]. Thus, identifying the specific features and functions of the epigenetic build-up at these stages and determining the mechanisms by which environmental factors may affect them in the long term will be a major milestone in the domain of DOHaD investigation[69,70].

Post-translational histone modifications have been implicated in the complex changes in gene expression driving early mammalian development. Optimization of the chromatin immunoprecipitation technique enables analysis of histone modifications in mouse embryos in culture, from the 8-cells stage to blastocysts. An increase in H4ac and H3K4me in the promoters of *Hoxb1* and *Hoxb9* was observed after the exposure of embryos to the HDAC inhibitor valproic acid. These changes are heritable, even after removal of the inhibitor, at least until the blastocyst stage. These findings illustrate the way in which an environmental signal can generate an inherited epigenetic modification during early development with potential long-term consequences[40].

Ontogeny of chromatin remodeling: An ongoing process

Recent epigenomic profiling and functional studies have provided insights into the dynamics and regulatory complexity of the transcriptional repression mediated by histone-modifying enzymes and other chromatin-associated proteins. These machineries clearly function in a sequential manner. Furthermore, the repressed chromatin state is dynamic rather than static and reflects the balance between antagonistic enzyme activities[41]. A full understanding of the role of chromatin in transcriptional regulation will require knowledge of the relative levels of antagonistic histone modifications and their spatial distributions with respect to transcription factor binding sites and RNA polII[71].

Pinney *et al*[72] studied epigenetic events at the promoter of the gene encoding Pdx1, a critical transcription factor for β cell function and development, the expression of which is reduced in intrauterine growth retardation (IUGR), promoting the development of diabetes in adulthood. They demonstrated that IUGR induces a self-propagating epigenetic cycle, in which the mSin3A/HDAC complex is first recruited to the Pdx1 promoter at the fetal stage, leading to histone deacetylation and a loss of binding of major transcription factors to the Pdx1 promoter, resulting in transcriptional repression. In the postnatal period, as histone deacetylation progresses, active H3K4me3 levels decrease and repressive H3K9me2 accumulates. This epigenetic process is still reversible at this stage, which may be an important developmental window for therapeutic approaches. H3K9me2 accumulation promotes the recruitment of DNMT3A, initiating *de novo* DNA methylation and locking *pdx1* in a silent state in the pancreas of adults born with IUGR.

Similarly, Raychaudhuri *et al*[53] focused on the sequence of epigenetic mechanisms responsible for the weak expression of Glut4 in the skeletal muscle of subjects with IUGR[53]. Different DNMTs bound the Glut4 promoter at different ages: DNMT1 bound postnatally, whereas DNMT3a and 3b bound in adults. DNA methylation was unaffected in subjects with IUGR, but they displayed greater binding of DNMTs to the Glut4 promoter, resulting in higher levels of methyl CpG-binding protein (MeCP2). H3K14 deacetylation mediated by HDAC1 recruitment and enhanced HDAC4 binding were observed. This set the stage for Suv39H1 methylase-mediated dimethylation of H3K9 and an increase in the recruitment of heterochromatin protein 1, which partially inactivates postnatal and adult Glut4 gene transcription in subjects with IUGR. This study demonstrated that perinatal nutrient restriction resulting in IUGR leads to histone modifications in skeletal muscle that directly de-

crease Glut4 gene expression. This effectively creates a metabolic knockdown of this important regulator of peripheral glucose transport and insulin resistance, thereby contributing to the adult T2D phenotype.

Finally, two groups recently demonstrated that mice with disrupted H3K9 demethylase Jhdm2a gene develop adult-onset obesity, hypertriglyceridemia, hypercholesterolemia, hyperinsulinemia and hyperleptinemia, hallmarks of metabolic syndrome. Thus, this H3K9 demethylase is a crucial regulator of genes involved in energy expenditure and fat storage[73-75]. The disruption of epigenetic components may therefore play a key role in the progression of obesity and metabolic syndrome.

Thus, histone modifications can be stably inherited while giving rise to additional alterations, and the epigenetic landscapes established under the influence of an environmental factor at a given stage, in a specific chromatin context, may evolve with time. The epigenetic landscapes observed subsequently may therefore not fully reflect the mechanisms initially involved.

Physiological "hyperglycemic metabolic memory" is based on epigenetic modifications

Diabetic patients continue to develop inflammation and vascular complications, even when glycemia is controlled. This poorly understood metabolic memory phenomenon poses major challenges for diabetes treatment. Recent studies have highlighted the persistent and dramatic effects of short-term hyperglycemic spikes on vascular cells in animal models and humans. They have demonstrated a link between epigenetic changes (H3K9me and H3K4me) and the expression of transcription factors, such as NFkB, involved in modulating inflammatory gene expression[76-78]. Brasacchio *et al*[77] reported that hyperglycemia induced dynamic cooperativity between histone methylase and demethylases, associated with gene-activating epigenetic marks on the H3 lysine tail. Thus, an increase in NFkB gene expression is associated with the persistence of epigenetic marks after the removal of a cell from its hyperglycemic environment, providing evidence that epigenetic modifications contribute to changes in gene expression, potentially forming the basis of a physiological "hyperglycemic memory".

Malprogramming other than that associated with early nutrition is beyond the scope of this review. The long-term outcomes of epigenetics alterations in malprogramming to other later-onset diseases or to short-term outcomes of epigenetic changes are progressively declined. However the similitude of the mechanisms involved, whatever the environmental factor (i.e. circadian, nutritional, hormonal or exercise-induced changes) is striking and should help complete our understanding of the picture[55,70,79-84].

SEXUAL DIMORPHISM OF GENE EXPRESSION AND EPIGENETICS

The vast majority of common diseases, including atherosclerosis, diabetes, osteoporosis, asthma, neuropsychological and autoimmune diseases which often take root in early development, display sex bias. Moreover, the risk of developing complex disease in offspring often depends on the sex of the affected parent. The relevance of epigenetic mechanisms underlying the physiological differences between sexes, particularly in drug metabolism, fits well into the epigenetic theory of complex disease (reviewed in[85]).

This bias could be explained by the role of sex chromosomes, the different regulatory pathways underlying sexual development of most organs and finally, lifelong fluctuating impact of sex hormones. Many tissues exhibit sexual dimorphism for a substantial proportion of the genes that they express[42,86]. In fact, sex-specific expression appears to be under the control of sex-specific epigenetic marks. Environmental factors such as social behavior, nutrition or chemical compounds can influence, in a sex-related manner, these flexible epigenetic marks during particular temporal windows of life. For example, modifications of histone H3 are sexually dimorphic in the developing mouse brain and patterns of acetylation, but not methylation, are masculinized in females by testosterone in utero[87]. There are many examples of sex differences in the effects of prenatal and early postnatal life exposures on the risks of subsequent metabolic dysfunction[42,88-92].

It's not all hormones: Roles of sex chromosomes

Sexual dimorphism has been explained traditionally by the regulatory pathways that underlie sexual development of the gonads, brain and other organs, and the impact of lifelong fluctuations in the circulating level of sex hormones. Mammalian sexual differentiation was assumed to be initiated by the presence or absence of the testis-determining factor SRY, encoded on the Y chromosome, in a very narrow spatiotemporal window restricted to the Sertoli cells between 6 and 7 wk of gestation. However, recent findings propose that sexual dimorphism precedes gonadal development. Recently, it was found that the sexual dimorphism between male and female cells in their response to chemical exposure to either ethanol or camptothecin apparently occurred at fetal stages that preceded the production of sex hormones and, accordingly, could be directly attributed to a sex chromosome effect[93,94]. Sex-determining genes on sex-chromosomes can influence not only the development of non-gonadal secondary sexual organs but also of organs outside of the reproductive system, such as brain[95]. Indeed, at the level of the whole body, the sex-chromosomes are crucial for establishment of sex-dimorphism of cellular functions[42]. All male cells possess a single X chromosome of maternal origin and a Y chromosome of paternal origin. Female cells consist of two populations, both of which possess two X chromosomes: one population with inactive maternally inherited X and the second population with inactive paternally inherited X. As a consequence of this random female mosaicism, it is possible that certain

traits, such as cognitive traits, show a greater degree of variability amongst females than amongst males[96].

Extent of global sexual dimorphism

A substantial proportion of dimorphic gene expression might be under the control of sex-specific epigenetic marks. The regulatory pathways underlying sexual differentiation clearly result in extensive differences in gene expression in adults. The genetic and transcriptional mechanisms regulating differences between the sexes have intensively been investigated in the liver but dimorphic gene expression have also been reported in mouse kidney, blastocysts, lacrimal gland, placenta and brain[96-104], and more global differences[105-109]. A recent microarray analysis of 23 574 transcripts by Yang *et al*[86] revealed the extent of sexual dimorphism in gene expression to be much greater than previously recognized. The degree of sexual dimorphism ranged from 14% of active genes in the brain to 70% in the liver. These genes displayed highly tissue-specific patterns of expression, correlated with high levels of activity of distinct pathways. Differences in expression level of a factor of less than 1.2 between tissues were observed for 70% of the sexually dimorphic genes. Interestingly, these genes displayed evidence of clustering, not only on the sex chromosomes, but also on several autosomes.

Sexual dimorphism of gene expression in the liver

Gene expression in somatic cells and tissues can be influenced by external factors, such as the extracellular hormonal milieu. A good example of hormonal regulation is the effect of growth hormone (GH) on gene expression in the liver, which leads to sex-differences in many metabolic processes, such as steroid and fatty acid metabolism, cholesterol homeostasis and drug metabolism[110]. Important sex differences also characterize responses to various hepatic stresses in both rodent models and humans. For example, alcohol-induced liver fibrosis is more prevalent in women than in men, whereas sepsis and hepatitis virus-induced liver fibrosis, hepatic ischemia/reperfusion injury and hepatocellular carcinoma are more prevalent in men than in women; and these sex differences are at least in part due to hormonal factors[110,111]. Support for the involvement of chromatin features in the regulation of genes showing sex differences in liver comes from the discovery of short genomic regions that show sex-dependent and GH-regulated differences in chromatin accessibility ("hypersensitivity sites") in liver tissue, as probed using the enzyme DNase I. Thus, increased hypersensitivity to DNase I cleavage in the male liver tissue compared to that of female liver tissue is seen in the promoter regions of two male-specific genes, *Ca/Slp*, sex-limited protein, and *Cypc,* which catalyzes testosterone hydroxylation[110-113]. DNase hypersensitive chromosomal regions, such as these, have increased access to transcription factors and other DNA-binding proteins, and include promoters, enhancers, silencers and insulators. These findings of sex differences in DNase hypersensitivity are indicative of a sex-specific liver chromatin organization, which is presumably established and/or maintained by the sexually dimorphic patterns of pituitary GH secretion that emerge at puberty, and through their downstream signaling, which leads directly to the sex-dependent patterns of nuclear STAT5b activity[110,114-117].

Sexual dimorphism of placenta

The placenta has long been considered to be an asexual organ, with most placental studies consistently pooling data for male and female placentae into a single group[96]. However, predisposition to metabolic disease differs between the sexes, with women more likely to develop obesity and men cardiovascular disease. This sexual dimorphism may already exist during development. Indeed, there is mounting evidence to suggest that the sex of the embryo, through the embryo-derived tissues of the placenta, plays a significant role in determining fetal size, nutrition, morbidity and survival[96,118]. Only a handful of studies have reported differences between the sexes, in terms of the expression of individual genes or pathways in male and female human and rodent placentae. These studies also addressed the impact of differences in the quality of the maternal diet on placental gene expression, with a systematic investigation of the relationship between diet and the expression of sexually dimorphic genes, providing insight into the different sensitivities of male and female fetuses to what the mother eats[96,101-105,108,109].

We have data showing that gene expression and DNA methylation are sexually dimorphic in male and female placentae under control conditions. Surprisingly, in stressful conditions, say at high fat or low calorie diet, or maternal overweight/obesity, the placentae from male and female fetuses do not use the same gene pathways and networks to cope with the stress. Does that lead to different outcomes? Maybe this leads to sex-dependent differences in the outcome of programming with long lasting effects. Alternatively, males may "climb the mountain" taking the north face while females take the south face but they ultimately reach the same peak after using different paths.

CONCLUSION

The DOHaD science is still accumulating proof of evidence of fetal programming: a developmental insult (diet, drugs, lifestyle, social interventions, *etc.*) leading to long-term consequences (metabolic syndrome, psychiatric diseases, *etc.*). A new field is emerging, aiming to identify epigenetic targets to improve our understanding of the ontology of chronic diseases in response to environmental factors. Experiments in this area must be carefully designed.

How should such studies be carried out? Investigations should first be carried out in appropriate animal models exposed to specific environmental factors during critical developmental windows. Many analytical procedures are available; each with its own biases and limita-

tions and the choice depends on the question posed. We may need genome-wide or gene-specific approaches targeting regulatory regions (promoters, enhancers, gene body or elsewhere) and assessing functional significance[9]. New high-throughput tools are becoming available and may soon be applied more widely as DNA sequencing costs drop, to studies of the epigenomic changes associated with developmental shifts, environmental changes, and disease states[14,15,18,38,119-123].

Where should we look for epigenomic effects? Each environmental factor may target specific cell types, leading to a unique, specific epigenome identifiable only in the appropriate tissue[124], which will often contain mixed cell populations. However, the appropriate tissues are generally not available for study in humans. Fortunately, recent data suggest that the traces left by specific environmental factors can be visualized in leukocytes, at least for dietary factors[81]. However, the question remains as to whether surrogate tissues obtained by minimally invasive procedures, such as the placenta or cord blood, truly reflect early programming *in utero*, cataloguing intrauterine environmental events, or whether adult tissues and cells, such as lymphocytes, monocytes or buccal smears, reflect the lifelong metabolic memory[43,76,77,125].

When should epigenetic effects be studied? Circadian and seasonal rhythms are important components. Sampling at the right time may unmask pertinent marks important for determining both the nature of the challenge and the extent of the effect[126].

What are we actually studying? Are the marks observed the cause or just a consequence? It would be very interesting to carry out studies at several time points, to unravel the sequence of epigenetic events and to distinguish between causal changes and the resulting epigenetic landscape.

Who should be studied? It should be borne in mind that men and women have different programming trajectories[42]. Different recent studies show an obvious sexual dimorphism in response to the same environmental insult.

Why not? The inherent reversibility of epigenetic marks is promising for treatment approaches. However, one major potential problem is that epigenetic processes associated with the disturbance of programming by early environmental events may disappear during differentiation, may be leaky, leading to irreparable changes in the number of nephrons, β cells of the pancreas, or changes in the function of target tissues, or may remain dormant until the appropriate environmental stimulus comes along to activate them[127].

All epigenetic changes are, in theory, flexible, but can interventions really modify them? Without side effects[128]? A growing number of studies have demonstrated the potential reversibility or compensation of misprogramming with appropriate nutrients or epigenetic drugs[52,129-132].

This should make it possible to identify the specific epigenetic marks induced by specific environmental factors and to study their changes during the individual's life and potential reversibility, using appropriate epigenetic tools.

REFERENCES

1 **Jenuwein T**. The epigenetic magic of histone lysine methylation. *FEBS J* 2006; **273**: 3121-3135

2 **Probst AV**, Dunleavy E, Almouzni G. Epigenetic inheritance during the cell cycle. *Nat Rev Mol Cell Biol* 2009; **10**: 192-206

3 **Gluckman PD**, Hanson MA, Bateson P, Beedle AS, Law CM, Bhutta ZA, Anokhin KV, Bougnères P, Chandak GR, Dasgupta P, Smith GD, Ellison PT, Forrester TE, Gilbert SF, Jablonka E, Kaplan H, Prentice AM, Simpson SJ, Uauy R, West-Eberhard MJ. Towards a new developmental synthesis: adaptive developmental plasticity and human disease. *Lancet* 2009; **373**: 1654-1657

4 **McAllister EJ**, Dhurandhar NV, Keith SW, Aronne LJ, Barger J, Baskin M, Benca RM, Biggio J, Boggiano MM, Eisenmann JC, Elobeid M, Fontaine KR, Gluckman P, Hanlon EC, Katzmarzyk P, Pietrobelli A, Redden DT, Ruden DM, Wang C, Waterland RA, Wright SM, Allison DB. Ten putative contributors to the obesity epidemic. *Crit Rev Food Sci Nutr* 2009; **49**: 868-913

5 **Meissner A**, Mikkelsen TS, Gu H, Wernig M, Hanna J, Sivachenko A, Zhang X, Bernstein BE, Nusbaum C, Jaffe DB, Gnirke A, Jaenisch R, Lander ES. Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature* 2008; **454**: 766-770

6 **Mikkelsen TS**, Hanna J, Zhang X, Ku M, Wernig M, Schorderet P, Bernstein BE, Jaenisch R, Lander ES, Meissner A. Dissecting direct reprogramming through integrative genomic analysis. *Nature* 2008; **454**: 49-55

7 **Mikkelsen TS**, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim TK, Koche RP, Lee W, Mendenhall E, O'Donovan A, Presser A, Russ C, Xie X, Meissner A, Wernig M, Jaenisch R, Nusbaum C, Lander ES, Bernstein BE. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* 2007; **448**: 553-560

8 **Fouse SD**, Shen Y, Pellegrini M, Cole S, Meissner A, Van Neste L, Jaenisch R, Fan G. Promoter CpG methylation contributes to ES cell gene regulation in parallel with Oct4/Nanog, PcG complex, and histone H3 K4/K27 trimethylation. *Cell Stem Cell* 2008; **2**: 160-169

9 **Shen Y**, Matsuno Y, Fouse SD, Rao N, Root S, Xu R, Pellegrini M, Riggs AD, Fan G. X-inactivation in female human embryonic stem cells is in a nonrandom pattern and prone to epigenetic alterations. *Proc Natl Acad Sci USA* 2008; **105**: 4709-4714

10 **Pauler FM**, Sloane MA, Huang R, Regha K, Koerner MV, Tamir I, Sommer A, Aszodi A, Jenuwein T, Barlow DP. H3K27me3 forms BLOCs over silent genes and intergenic regions and specifies a histone banding pattern on a mouse autosomal chromosome. *Genome Res* 2009; **19**: 221-233

11 **Illingworth RS**, Bird AP. CpG islands--'a rough guide'. *FEBS Lett* 2009; **583**: 1713-1720

12 **Irizarry RA**, Ladd-Acosta C, Wen B, Wu Z, Montano C, Onyango P, Cui H, Gabo K, Rongione M, Webster M, Ji H, Potash JB, Sabunciyan S, Feinberg AP. The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. *Nat Genet* 2009; **41**: 178-186

13 **Doi A**, Park IH, Wen B, Murakami P, Aryee MJ, Irizarry R, Herb B, Ladd-Acosta C, Rho J, Loewer S, Miller J, Schlaeger T, Daley GQ, Feinberg AP. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and

fibroblasts. *Nat Genet* 2009; **41**: 1350-1353
14 **Weber M**, Hellmann I, Stadler MB, Ramos L, Pääbo S, Rebhan M, Schübeler D. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat Genet* 2007; **39**: 457-466
15 **Shen L**, Waterland RA. Methods of DNA methylation analysis. *Curr Opin Clin Nutr Metab Care* 2007; **10**: 576-581
16 **Straussman R**, Nejman D, Roberts D, Steinfeld I, Blum B, Benvenisty N, Simon I, Yakhini Z, Cedar H. Developmental programming of CpG island methylation profiles in the human genome. *Nat Struct Mol Biol* 2009; **16**: 564-571
17 **Hellman A**, Chess A. Gene body-specific methylation on the active X chromosome. *Science* 2007; **315**: 1141-1143
18 **Ball MP**, Li JB, Gao Y, Lee JH, LeProust EM, Park IH, Xie B, Daley GQ, Church GM. Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. *Nat Biotechnol* 2009; **27**: 361-368
19 **Lister R**, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, Nery JR, Lee L, Ye Z, Ngo QM, Edsall L, Antosiewicz-Bourget J, Stewart R, Ruotti V, Millar AH, Thomson JA, Ren B, Ecker JR. Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* 2009; **462**: 315-322
20 **Barrès R**, Osler ME, Yan J, Rune A, Fritz T, Caidahl K, Krook A, Zierath JR. Non-CpG methylation of the PGC-1alpha promoter through DNMT3B controls mitochondrial density. *Cell Metab* 2009; **10**: 189-198
21 **Grandjean V**, Yaman R, Cuzin F, Rassoulzadegan M. Inheritance of an epigenetic mark: the CpG DNA methyltransferase 1 is required for de novo establishment of a complex pattern of non-CpG methylation. *PLoS One* 2007; **2**: e1136
22 **Ramsahoye BH**, Biniszkiewicz D, Lyko F, Clark V, Bird AP, Jaenisch R. Non-CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a. *Proc Natl Acad Sci USA* 2000; **97**: 5237-5242
23 **Tahiliani M**, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, Rao A. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* 2009; **324**: 930-935
24 **Kriaucionis S**, Heintz N. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. *Science* 2009; **324**: 929-930
25 **Kouzarides T**. Chromatin modifications and their function. *Cell* 2007; **128**: 693-705
26 **Allis CD**, Berger SL, Cote J, Dent S, Jenuwien T, Kouzarides T, Pillus L, Reinberg D, Shi Y, Shiekhattar R, Shilatifard A, Workman J, Zhang Y. New nomenclature for chromatin-modifying enzymes. *Cell* 2007; **131**: 633-636
27 **Fuks F**. DNA methylation and histone modifications: teaming up to silence genes. *Curr Opin Genet Dev* 2005; **15**: 490-495
28 **Ram EV**, Meshorer E. Transcriptional competence in pluripotency. *Genes Dev* 2009; **23**: 2793-2798
29 **Cedar H**, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. *Nat Rev Genet* 2009; **10**: 295-304
30 **Friedman JR**, Kaestner KH. The Foxa family of transcription factors in development and metabolism. *Cell Mol Life Sci* 2006; **63**: 2317-2328
31 **Azuara V**, Perry P, Sauer S, Spivakov M, Jørgensen HF, John RM, Gouti M, Casanova M, Warnes G, Merkenschlager M, Fisher AG. Chromatin signatures of pluripotent cell lines. *Nat Cell Biol* 2006; **8**: 532-538
32 **Lee TI**, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, Chevalier B, Johnstone SE, Cole MF, Isono K, Koseki H, Fuchikami T, Abe K, Murray HL, Zucker JP, Yuan B, Bell GW, Herbolsheimer E, Hannett NM, Sun K, Odom DT, Otte AP, Volkert TL, Bartel DP, Melton DA, Gifford DK, Jaenisch R, Young RA. Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell* 2006; **125**: 301-313
33 **Bernstein BE**, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* 2006; **125**: 315-326
34 **Ku M**, Koche RP, Rheinbay E, Mendenhall EM, Endoh M, Mikkelsen TS, Presser A, Nusbaum C, Xie X, Chi AS, Adli M, Kasif S, Ptaszek LM, Cowan CA, Lander ES, Koseki H, Bernstein BE. Genomewide analysis of PRC1 and PRC2 occupancy identifies two classes of bivalent domains. *PLoS Genet* 2008; **4**: e1000242
35 **Pietersen AM**, van Lohuizen M. Stem cell regulation by polycomb repressors: postponing commitment. *Curr Opin Cell Biol* 2008; **20**: 201-207
36 **Rodriguez J**, Muñoz M, Vives L, Frangou CG, Groudine M, Peinado MA. **Bivalent domains enforce transcriptional** memory of DNA methylated genes in cancer cells. *Proc Natl Acad Sci USA* 2008; **105**: 19809-19814
37 **Guenther MG**, Lawton LN, Rozovskaia T, Frampton GM, Levine SS, Volkert TL, Croce CM, Nakamura T, Canaani E, Young RA. Aberrant chromatin at genes encoding stem cell regulators in human mixed-lineage leukemia. *Genes Dev* 2008; **22**: 3403-3408
38 **Weber M**, Schübeler D. Genomic patterns of DNA methylation: targets and function of an epigenetic mark. *Curr Opin Cell Biol* 2007; **19**: 273-280
39 **Mohn F**, Weber M, Rebhan M, Roloff TC, Richter J, Stadler MB, Bibel M, Schübeler D. Lineage-specific polycomb targets and de novo DNA methylation define restriction and potential of neuronal progenitors. *Mol Cell* 2008; **30**: 755-766
40 **VerMilyea MD**, O'Neill LP, Turner BM. Transcription-independent heritability of induced histone modifications in the mouse preimplantation embryo. *PLoS One* 2009; **4**: e6086
41 **Gamble MJ**, Frizzell KM, Yang C, Krishnakumar R, Kraus WL. The histone variant macroH2A1 marks repressed autosomal chromatin, but protects a subset of its target genes from silencing. *Genes Dev* 2010; **24**: 21-32
42 **Gabory A**, Attig L, Junien C. Sexual dimorphism in environmental epigenetic programming. *Mol Cell Endocrinol* 2009; **304**: 8-18
43 **Tobi EW**, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD, Slagboom PE, Heijmans BT. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum Mol Genet* 2009; **18**: 4046-4053
44 **Steegers-Theunissen RP**, Obermann-Borst SA, Kremer D, Lindemans J, Siebel C, Steegers EA, Slagboom PE, Heijmans BT. Periconceptional maternal folic acid use of 400 microg per day is related to increased methylation of the IGF2 gene in the very young child. *PLoS One* 2009; **4**: e7845
45 **Katari S**, Turan N, Bibikova M, Erinle O, Chalian R, Foster M, Gaughan JP, Coutifaris C, Sapienza C. DNA methylation and gene expression differences in children conceived in vitro or in vivo. *Hum Mol Genet* 2009; **18**: 3769-3778
46 **Fu Q**, Yu X, Callaway CW, Lane RH, McKnight RA. Epigenetics: intrauterine growth retardation (IUGR) modifies the histone code along the rat hepatic IGF-1 gene. *FASEB J* 2009; **23**: 2438-2449
47 **Goyal R**, Goyal D, Leitzke A, Gheorghe CP, Longo LD. Brain renin-angiotensin system: fetal epigenetic programming by maternal protein restriction during pregnancy. *Reprod Sci* 2010; **17**: 227-238
48 **Park JH**, Stoffers DA, Nicholls RD, Simmons RA. Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of Pdx1. *J Clin Invest* 2008; **118**: 2316-2324
49 **Lillycrop KA**, Phillips ES, Torrens C, Hanson MA, Jackson AA, Burdge GC. Feeding pregnant rats a protein-restricted diet persistently alters the methylation of specific cytosines in the hepatic PPAR alpha promoter of the offspring. *Br J*

Nutr 2008; **100**: 278-282
50 **Aagaard-Tillery KM**, Grove K, Bishop J, Ke X, Fu Q, McKnight R, Lane RH. Developmental origins of disease and determinants of chromatin structure: maternal diet modifies the primate fetal epigenome. *J Mol Endocrinol* 2008; **41**: 91-102
51 **Coupé B**, Amarger V, Grit I, Benani A, Parnet P. Nutritional programming affects hypothalamic organization and early response to leptin. *Endocrinology* 2010; **151**: 702-713
52 **Burdge GC**, Lillycrop KA, Phillips ES, Slater-Jefferies JL, Jackson AA, Hanson MA. Folic acid supplementation during the juvenile-pubertal period in rats modifies the phenotype and epigenotype induced by prenatal nutrition. *J Nutr* 2009; **139**: 1054-1060
53 **Raychaudhuri N**, Raychaudhuri S, Thamotharan M, Devaskar SU. Histone code modifications repress glucose transporter 4 expression in the intrauterine growth-restricted offspring. *J Biol Chem* 2008; **283**: 13611-13626
54 **Plagemann A**, Harder T, Brunn M, Harder A, Roepke K, Wittrock-Staar M, Ziska T, Schellong K, Rodekamp E, Melchior K, Dudenhausen JW. Hypothalamic proopiomelanocortin promoter methylation becomes altered by early overfeeding: an epigenetic model of obesity and the metabolic syndrome. *J Physiol* 2009; **587**: 4963-4976
55 **McGowan PO**, Sasaki A, D'Alessio AC, Dymov S, Labonté B, Szyf M, Turecki G, Meaney MJ. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci* 2009; **12**: 342-348
56 **Attig L**, Gabory A, Junien C. Early nutrition and epigenetic programming: chasing shadows. *Curr Opin Clin Nutr Metab Care* 2010; **13**: 284-293
57 **Thompson JG**, Gardner DK, Pugh PA, McMillan WH, Tervit HR. Lamb birth weight is affected by culture system utilized during in vitro pre-elongation development of ovine embryos. *Biol Reprod* 1995; **53**: 1385-1391
58 **Sinclair KD**, Allegrucci C, Singh R, Gardner DS, Sebastian S, Bispham J, Thurston A, Huntley JF, Rees WD, Maloney CA, Lea RG, Craigon J, McEvoy TG, Young LE. DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. *Proc Natl Acad Sci USA* 2007; **104**: 19351-19356
59 **Ecker DJ**, Stein P, Xu Z, Williams CJ, Kopf GS, Bilker WB, Abel T, Schultz RM. Long-term effects of culture of preimplantation mouse embryos on behavior. *Proc Natl Acad Sci USA* 2004; **101**: 1595-1600
60 **Fernández-Gonzalez R**, Moreira P, Bilbao A, Jiménez A, Pérez-Crespo M, Ramírez MA, Rodríguez De Fonseca F, Pintado B, Gutiérrez-Adán A. Long-term effect of in vitro culture of mouse embryos with serum on mRNA expression of imprinting genes, development, and behavior. *Proc Natl Acad Sci USA* 2004; **101**: 5880-5885
61 **Fleming TP**, Kwong WY, Porter R, Ursell E, Fesenko I, Wilkins A, Miller DJ, Watkins AJ, Eckert JJ. The embryo and its future. *Biol Reprod* 2004; **71**: 1046-1054
62 **Sinclair KD**, Singh R. Modelling the developmental origins of health and disease in the early embryo. *Theriogenology* 2007; **67**: 43-53
63 **Kwong WY**, Wild AE, Roberts P, Willis AC, Fleming TP. Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. *Development* 2000; **127**: 4195-4202
64 **Ashworth CJ**, Toma LM, Hunter MG. Nutritional effects on oocyte and embryo development in mammals: implications for reproductive efficiency and environmental sustainability. *Philos Trans R Soc Lond B Biol Sci* 2009; **364**: 3351-3361
65 **Rooke JA**, McEvoy TG, Ashworth CJ, Robinson JJ, Wilmut I, Young LE, Sinclair KD. Ovine fetal development is more sensitive to perturbation by the presence of serum in embryo culture before rather than after compaction. *Theriogenology* 2007; **67**: 639-647
66 **Watkins AJ**, Ursell E, Panton R, Papenbrock T, Hollis L, Cunningham C, Wilkins A, Perry VH, Sheth B, Kwong WY, Eckert JJ, Wild AE, Hanson MA, Osmond C, Fleming TP. Adaptive responses by mouse early embryos to maternal diet protect fetal growth but predispose to adult onset disease. *Biol Reprod* 2008; **78**: 299-306
67 **Kwong WY**, Miller DJ, Ursell E, Wild AE, Wilkins AP, Osmond C, Anthony FW, Fleming TP. Imprinted gene expression in the rat embryo-fetal axis is altered in response to periconceptional maternal low protein diet. *Reproduction* 2006; **132**: 265-277
68 **Kwong WY**, Miller DJ, Wilkins AP, Dear MS, Wright JN, Osmond C, Zhang J, Fleming TP. Maternal low protein diet restricted to the preimplantation period induces a gender-specific change on hepatic gene expression in rat fetuses. *Mol Reprod Dev* 2007; **74**: 48-56
69 **Corry GN**, Tanasijevic B, Barry ER, Krueger W, Rasmussen TP. Epigenetic regulatory mechanisms during preimplantation development. *Birth Defects Res C Embryo Today* 2009; **87**: 297-313
70 **Aranda P**, Agirre X, Ballestar E, Andreu EJ, Román-Gómez J, Prieto I, Martín-Subero JI, Cigudosa JC, Siebert R, Esteller M, Prosper F. Epigenetic signatures associated with different levels of differentiation potential in human stem cells. *PLoS One* 2009; **4**: e7809
71 **Hublitz P**, Albert M, Peters AH. Mechanisms of transcriptional repression by histone lysine methylation. *Int J Dev Biol* 2009; **53**: 335-354
72 **Pinney SE**, Simmons RA. Epigenetic mechanisms in the development of type 2 diabetes. *Trends Endocrinol Metab* 2010; **21**: 223-229
73 **Inagaki T**, Tachibana M, Magoori K, Kudo H, Tanaka T, Okamura M, Naito M, Kodama T, Shinkai Y, Sakai J. Obesity and metabolic syndrome in histone demethylase JHDM2a-deficient mice. *Genes Cells* 2009; **14**: 991-1001
74 **Okada Y**, Tateishi K, Zhang Y. Histone demethylase JHDM2A is involved in male infertility and obesity. *J Androl* 2010; **31**: 75-78
75 **Tateishi K**, Okada Y, Kallin EM, Zhang Y. Role of Jhdm2a in regulating metabolic gene expression and obesity resistance. *Nature* 2009; **458**: 757-761
76 **Villeneuve LM**, Reddy MA, Lanting LL, Wang M, Meng L, Natarajan R. Epigenetic histone H3 lysine 9 methylation in metabolic memory and inflammatory phenotype of vascular smooth muscle cells in diabetes. *Proc Natl Acad Sci USA* 2008; **105**: 9047-9052
77 **Brasacchio D**, Okabe J, Tikellis C, Balcerczyk A, George P, Baker EK, Calkin AC, Brownlee M, Cooper ME, El-Osta A. Hyperglycemia induces a dynamic cooperativity of histone methylase and demethylase enzymes associated with gene-activating epigenetic marks that coexist on the lysine tail. *Diabetes* 2009; **58**: 1229-1236
78 **El-Osta A**, Brasacchio D, Yao D, Pocai A, Jones PL, Roeder RG, Cooper ME, Brownlee M. Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. *J Exp Med* 2008; **205**: 2409-2417
79 **Ernst C**, McGowan PO, Deleva V, Meaney MJ, Szyf M, Turecki G. The effects of pH on DNA methylation state: In vitro and post-mortem brain studies. *J Neurosci Methods* 2008; **174**: 123-125
80 **Ateeq B**, Unterberger A, Szyf M, Rabbani SA. Pharmacological inhibition of DNA methylation induces proinvasive and prometastatic genes in vitro and in vivo. *Neoplasia* 2008; **10**: 266-278
81 **Delage B**, Dashwood RH. Dietary manipulation of histone structure and function. *Annu Rev Nutr* 2008; **28**: 347-366
82 **McGee SL**, Fairlie E, Garnham AP, Hargreaves M. Exercise-induced histone modifications in human skeletal muscle. *J Physiol* 2009; **587**: 5951-5958

83 **Waterland RA**. Early environmental effects on epigenetic regulation in humans. *Epigenetics* 2009; **4**: 523-525
84 **Guerrero-Bosagna CM**, Skinner MK. Epigenetic transgenerational effects of endocrine disruptors on male reproduction. *Semin Reprod Med* 2009; **27**: 403-408
85 **Kaminsky Z**, Wang SC, Petronis A. Complex disease, gender and epigenetics. *Ann Med* 2006; **38**: 530-544
86 **Yang X**, Schadt EE, Wang S, Wang H, Arnold AP, Ingram-Drake L, Drake TA, Lusis AJ. Tissue-specific expression and regulation of sexually dimorphic genes in mice. *Genome Res* 2006; **16**: 995-1004
87 **Tsai HW**, Grant PA, Rissman EF. Sex differences in histone modifications in the neonatal mouse brain. *Epigenetics* 2009; **4**: 47-53
88 **Flanagan DE**, Moore VM, Godsland IF, Cockington RA, Robinson JS, Phillips DI. Fetal growth and the physiological control of glucose tolerance in adults: a minimal model analysis. *Am J Physiol Endocrinol Metab* 2000; **278**: E700-E706
89 **Sugden MC**, Holness MJ. Gender-specific programming of insulin secretion and action. *J Endocrinol* 2002; **175**: 757-767
90 **Wilcoxon JS**, Redei EE. Prenatal programming of adult thyroid function by alcohol and thyroid hormones. *Am J Physiol Endocrinol Metab* 2004; **287**: E318-E326
91 **Gallou-Kabani C**, Vigé A, Junien C. Lifelong circadian and epigenetic drifts in metabolic syndrome. *Epigenetics* 2007; **2**: 137-146
92 **Owens JA**, Thavaneswaran P, De Blasio MJ, McMillen IC, Robinson JS, Gatford KL. Sex-specific effects of placental restriction on components of the metabolic syndrome in young adult sheep. *Am J Physiol Endocrinol Metab* 2007; **292**: E1879-E1889
93 **Penaloza C**, Estevez B, Orlanski S, Sikorska M, Walker R, Smith C, Smith B, Lockshin RA, Zakeri Z. Sex of the cell dictates its response: differential gene expression and sensitivity to cell death inducing stress in male and female cells. *FASEB J* 2009; **23**: 1869-1879
94 **Bermejo-Alvarez P**, Rizos D, Rath D, Lonergan P, Gutierrez-Adan A. Sex determines the expression level of one third of the actively expressed genes in bovine blastocysts. *Proc Natl Acad Sci USA* 2010; **107**: 3394-3399
95 **Blecher SR**, Erickson RP. Genetics of sexual development: a new paradigm. *Am J Med Genet A* 2007; **143A**: 3054-3068
96 **Clifton VL**. Review: Sex and the human placenta: mediating differential strategies of fetal growth and survival. *Placenta* 2010; **31** Suppl: S33-S39
97 **Tullis KM**, Krebs CJ, Leung JY, Robins DM. The regulator of sex-limitation gene, rsl, enforces male-specific liver gene expression by negative regulation. *Endocrinology* 2003; **144**: 1854-1860
98 **Waxman DJ**, Celenza JL. Sexual dimorphism of hepatic gene expression: novel biological role of KRAB zinc finger repressors revealed. *Genes Dev* 2003; **17**: 2607-2613
99 **Wiwi CA**, Gupte M, Waxman DJ. Sexually dimorphic P450 gene expression in liver-specific hepatocyte nuclear factor 4alpha-deficient mice. *Mol Endocrinol* 2004; **18**: 1975-1987
100 **Clodfelter KH**, Holloway MG, Hodor P, Park SH, Ray WJ, Waxman DJ. Sex-dependent liver gene expression is extensive and largely dependent upon signal transducer and activator of transcription 5b (STAT5b): STAT5b-dependent activation of male genes and repression of female genes revealed by microarray analysis. *Mol Endocrinol* 2006; **20**: 1333-1351
101 **Lehavi O**, Aizenstein O, Evans MI, Yaron Y. 2nd-trimester maternal serum human chorionic gonadotropin and alpha-fetoprotein levels in male and female fetuses with Down syndrome. *Fetal Diagn Ther* 2005; **20**: 235-238
102 **Steier JA**, Bergsjø PB, Thorsen T, Myking OL. **Human chorionic gonadotropin in maternal serum in relation to fetal gender and utero-placental blood flow. *Acta Obstet Gynecol Scand* 2004; **83**: 170-174
103 **Brown MJ**, Cook CL, Henry JL, Schultz GS. Levels of epidermal growth factor binding in third-trimester and term human placentas: elevated binding in term placentas of male fetuses. *Am J Obstet Gynecol* 1987; **156**: 716-720
104 **Sood R**, Zehnder JL, Druzin ML, Brown PO. Gene expression patterns in human placenta. *Proc Natl Acad Sci USA* 2006; **103**: 5478-5483
105 **Mao J**, Zhang X, Sieli PT, Falduto MT, Torres KE, Rosenfeld CS. Contrasting effects of different maternal diets on sexually dimorphic gene expression in the murine placenta. *Proc Natl Acad Sci USA* 2010; **107**: 5557-5562
106 **Bishai JM**, Penninga L, Nijland R, Meulenaar R, Gheorghe CP, Zhao Y, Buchholz JN, Zhang L, Longo LD. Pre- and postjunctional alpha(2)-adrenergic receptors in fetal and adult ovine cerebral arteries. *Am J Physiol Regul Integr Comp Physiol* 2002; **282**: R1654-R1662
107 **Goyal R**, Galffy A, Field SA, Gheorghe CP, Mittal A, Longo LD. Maternal protein deprivation: changes in systemic renin-angiotensin system of the mouse fetus. *Reprod Sci* 2009; **16**: 894-904
108 **Gheorghe CP**, Goyal R, Holweger JD, Longo LD. Placental gene expression responses to maternal protein restriction in the mouse. *Placenta* 2009; **30**: 411-417
109 **Gheorghe CP**, Mohan S, Oberg KC, Longo LD. Gene expression patterns in the hypoxic murine placenta: a role in epigenesis? *Reprod Sci* 2007; **14**: 223-233
110 **Waxman DJ**, Holloway MG. Sex differences in the expression of hepatic drug metabolizing enzymes. *Mol Pharmacol* 2009; **76**: 215-228
111 **Yokoyama Y**, Nimura Y, Nagino M, Bland KI, Chaudry IH. Current understanding of gender dimorphism in hepatic pathophysiology. *J Surg Res* 2005; **128**: 147-156
112 **Hemenway C**, Robins DM. DNase I-hypersensitive sites associated with expression and hormonal regulation of mouse C4 and Slp genes. *Proc Natl Acad Sci USA* 1987; **84**: 4816-4820
113 **Ström A**, Eguchi H, Mode A, Legraverend C, Tollet P, Strömstedt PE, Gustafsson JA. Characterization of the proximal promoter and two silencer elements in the CYP2C11 gene expressed in rat liver. *DNA Cell Biol* 1994; **13**: 805-819
114 **Wauthier V**, Sugathan A, Meyer RD, Dombkowski AA, Waxman DJ. Intrinsic sex differences in the early growth hormone responsiveness of sex-specific genes in mouse liver. *Mol Endocrinol* 2010; **24**: 667-678
115 **Waxman DJ**, O'Connor C. Growth hormone regulation of sex-dependent liver gene expression. *Mol Endocrinol* 2006; **20**: 2613-2629
116 **Choi HK**, Waxman DJ. Growth hormone, but not prolactin, maintains, low-level activation of STAT5a and STAT5b in female rat liver. *Endocrinology* 1999; **140**: 5126-5135
117 **Choi HK**, Waxman DJ. Plasma growth hormone pulse activation of hepatic JAK-STAT5 signaling: developmental regulation and role in male-specific liver gene expression. *Endocrinology* 2000; **141**: 3245-3255
118 **Clifton VL**, Hodyl NA, Murphy VE, Giles WB, Baxter RC, Smith R. Effect of maternal asthma, inhaled glucocorticoids and cigarette use during pregnancy on the newborn insulin-like growth factor axis. *Growth Horm IGF Res* 2010; **20**: 39-48
119 **Waterland RA**, Kellermayer R, Rached MT, Tatevian N, Gomes MV, Zhang J, Zhang L, Chakravarty A, Zhu W, Laritsky E, Zhang W, Wang X, Shen L. Epigenomic profiling indicates a role for DNA methylation in early postnatal liver development. *Hum Mol Genet* 2009; **18**: 3026-3038
120 **Deng J**, Shoemaker R, Xie B, Gore A, LeProust EM, Antosiewicz-Bourget J, Egli D, Maherali N, Park IH, Yu J, Daley GQ, Eggan K, Hochedlinger K, Thomson J, Wang W, Gao Y, Zhang K. Targeted bisulfite sequencing reveals changes in DNA methylation associated with nuclear reprogramming. *Nat Biotechnol* 2009; **27**: 353-360
121 **Irizarry RA**, Ladd-Acosta C, Carvalho B, Wu H, Brandenburg SA, Jeddeloh JA, Wen B, Feinberg AP. Comprehensive

high-throughput arrays for relative methylation (CHARM). *Genome Res* 2008; **18**: 780-790

122 **van Straten EM**, Bloks VW, Huijkman NC, Baller JF, van Meer H, Lütjohann D, Kuipers F, Plösch T. The liver X-receptor gene promoter is hypermethylated in a mouse model of prenatal protein restriction. *Am J Physiol Regul Integr Comp Physiol* 2010; **298**: R275-R282

123 **Berman BP**, Weisenberger DJ, Laird PW. Locking in on the human methylome. *Nat Biotechnol* 2009; **27**: 341-342

124 **Brown SE**, Weaver IC, Meaney MJ, Szyf M. Regional-specific global cytosine methylation and DNA methyltransferase expression in the adult rat hippocampus. *Neurosci Lett* 2008; **440**: 49-53

125 **Heijmans BT**, Tobi EW, Lumey LH, Slagboom PE. The epigenome: archive of the prenatal environment. *Epigenetics* 2009; **4**: 526-531

126 **Vollmers C**, Gill S, DiTacchio L, Pulivarthy SR, Le HD, Panda S. Time of feeding and the intrinsic circadian clock drive rhythms in hepatic gene expression. *Proc Natl Acad Sci USA* 2009; **106**: 21453-21458

127 **Barker DJ**. Fetal programming of coronary heart disease. *Trends Endocrinol Metab* 2002; **13**: 364-368

128 **Csoka AB**, Szyf M. Epigenetic side-effects of common pharmaceuticals: a potential new field in medicine and pharmacology. *Med Hypotheses* 2009; **73**: 770-780

129 **Szyf M**, Weaver IC, Champagne FA, Diorio J, Meaney MJ. Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. *Front Neuroendocrinol* 2005; **26**: 139-162

130 **Lillycrop KA**, Phillips ES, Jackson AA, Hanson MA, Burdge GC. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J Nutr* 2005; **135**: 1382-1386

131 **Lillycrop KA**, Slater-Jefferies JL, Hanson MA, Godfrey KM, Jackson AA, Burdge GC. Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. *Br J Nutr* 2007; **97**: 1064-1073

132 **Srinivasan M**, Aalinkeel R, Song F, Mitrani P, Pandya JD, Strutt B, Hill DJ, Patel MS. Maternal hyperinsulinemia predisposes rat fetuses for hyperinsulinemia, and adult-onset obesity and maternal mild food restriction reverses this phenotype. *Am J Physiol Endocrinol Metab* 2006; **290**: E129-E134

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