Epidemiological studies show that a poor intrauterine environment induced by unbalanced maternal diet or body composition, placental insufficiency or endocrine factors induces an offspring phenotype that is characterised by an increased risk of developing chronic non-communicable diseases such as cardiovascular disease and the metabolic syndrome in later life [1]. These findings have been replicated in animal models where restricted nutrition during pregnancy induces dyslipidaemia, obesity, hypertension, hyperinsulinaemia and hyperleptinaemia in the offspring [2]. This association between poor intrauterine growth and increased risk of disease in later life may result from a predictive adaptive response where the fetus responds to environmental cues during development with permanent adjustments in its development and homeostatic systems to aid later survival and reproductive fitness. However, if these adaptations are inappropriate for the postnatal environment, they may ultimately lead to an increased risk of disease because its homeostatic capacity is mismatched to that environment [3]. The mechanism by which cues about nutrient availability in the postnatal environment are transmitted to the fetus and the process by which different, stable phenotypes are induced are beginning to be understood. The purpose of this chapter is to discuss the results of recent studies on the role of epigenetics in the induction of an altered fetal phenotype by maternal nutrition during pregnancy.

**Phenotype Induction and Gene Transcription**

The induction of changes to the phenotype of the offspring that persist throughout the lifespan of the organism implies stable changes to gene transcription which result in altered activities of metabolic pathways and homeostatic control processes. Feeding a protein-restricted (PR) diet during pregnancy induces reduced expression of
11β-hydroxysteroid dehydrogenase type 2 and increased expression of the glucocorticoid receptor (GR) in liver, lung, kidney and brain of the offspring during fetal, neonatal and adult life. In the liver, increased GR activity upregulates phosphoenolpyruvate carboxykinase expression and activity and so increases capacity for gluconeogenesis [2].

Restricting maternal protein intake during pregnancy and/or lactation in rats also alters the expression of genes involved in lipid homeostasis. The offspring of rats fed a PR diet during pregnancy show increased blood triacylglycerol (TAG) and non-esterified fatty acid concentrations [4]. Peroxisomal proliferator-activated receptor-α (PPARα) expression was increased in the liver of the offspring of rats fed a PR diet during pregnancy and was accompanied by upregulation of its target gene acyl-CoA oxidase, while PPARγ1 expression was unchanged. In contrast, in adipose tissue the expression of the PPARγ adipose-specific isoform PPARγ2 was reduced [4, 5]. Increased PPARα expression would be expected to increase TAG clearance. However, increased hepatic TAG synthesis may result from increased flux of non-esterified fatty acid from adipose tissue as a result of reduced expression of PPARγ expression [5] and may have exceeded the capacity of fatty acid clearance pathways regulated by PPARα. Overall, the offspring of dams fed a PR diet during pregnancy show impaired lipid homeostasis.

Thus, these studies demonstrate that maternal nutrient restriction during pregnancy induces long-term stable effects on transcription and, importantly, in many cases the genes which show altered expression following prenatal undernutrition are transcription factors which regulate multiple pathways in development and metabolism. Maternal nutrition thus by modifying the expression of a few key transcription factors may alter metabolic and developmental pathways leading ultimately to an altered phenotype and increased disease susceptibility.

### Epigenetic Mechanisms and Regulation of Transcription

One mechanism by which maternal nutrient restriction may lead to long-term changes in gene expression within the offspring is through altered epigenetic gene regulation. As epigenetic processes are integral in determining when and where specific genes are expressed, alterations in the epigenetic regulation of genes may lead to profound phenotypic effects. The word ‘epigenetics’ literally means on top of genetics and refers to heritable processes which modulate gene expression potential without altering DNA sequence. The major epigenetic processes are DNA methylation, histone modification and microRNAs. As most is understood about DNA methylation, this will be the main focus of this chapter.

Methylation at the 5′ position of cytosine in DNA within a CpG dinucleotide (the p denotes the intervening phosphate group) is a common modification in mammalian genomes and constitutes a stable epigenetic mark that is transmitted through cell division [6]. CpG dinucleotides are found clustered at the 5′ ends of genes/promoters in
regions known as CpG islands. Hypermethylation of these CpG islands is associated with transcriptional repression, while hypomethylation of CpG islands is associated with transcriptional activation. DNA methylation can induce transcriptional silencing by blocking the binding of transcription factors and/or through promoting the binding of the methyl CpG-binding protein (MeCP2). The latter binds to methylated cytosines and, in turn, recruits histone-modifying complexes to the DNA [7]. MeCP2 recruits both histone deacetylases (HDACs), which remove acetyl groups from the histones, and histone methyl transferases which methylate lysine 9 on H3, resulting in a closed chromatin structure and transcriptional silencing [7]. Covalent modifications to histones, such as acetylation and methylation, alter chromatin structure [8] and hence the ability of the transcriptional machinery to gain access to the DNA (fig. 1).

DNA methylation is important for asymmetrical silencing of imprinted genes, X chromosome inactivation and silencing of retrotransposons [6]. DNA methylation also plays a key role in cell differentiation by silencing the expression of specific genes during the development and differentiation of individual tissues. The methylation of CpGs is largely established during embryogenesis or in early postnatal life. Following fertilisation, maternal and paternal genomes undergo extensive demethylation. Demethylation is an active process that strips the male genome of methylation within hours of fertilisation; by contrast the maternal genome is only passively demethylated during subsequent cleavage divisions. Thus embryonic DNA becomes hypomethylated, which correlates with the pluripotency of these embryonic cells. Imprinted genes escape this erasure. This period of demethylation is followed by global de novo methylation just prior to blastocyst implantation during which 70% of CpGs are methylated, mainly in repressed heterochromatin regions and in repetitive sequences such as retrotransposable elements [6]. In addition, during development and early postnatal life de novo methylation also occurs of tissue specific

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**Fig. 1.** Regulation of transcription by DNA methylation. When CpG dinucleotides are unmethylated, transcription factors bind, recruiting histone acetyl transferases (HATs); this leads to RNA polymerase binding and transcription of the gene. Methylation of CpGs by the activity of Dnmt blocks transcription factor binding and leads to the recruitment of MeCP2, which in turn recruits the HDAC/histone methyl transferase (HMT) complex. The overall effect of DNA and histone methylation is to induce long-term silencing of transcription.
genes, limiting gene expression and the developmental fates of differentiating cells. For example, the expression of Oct-4, a key regulator of cellular pluripotency in the early embryo, is permanently silenced by hypermethylation around E6.5 in the mouse [9], while HoxA5 and HoxB5 which are required for later stages of development are methylated and silenced in early postnatal life [10]. However, once these methylation patterns have been established during development, these epigenetic markers are in most cases maintained with high fidelity throughout life. The periods during development when these methylation patterns are being established are likely to be susceptible to early life environmental influences.

**Early Life Environment and Epigenetic Gene Regulation**

There is increasing evidence that prenatal and early postnatal environments can modify the epigenetic regulation of specific genes. Pham et al. [11] have shown that ligation of a uterine artery in the rat leads to increased p53 methylation and decreased p53 expression in the kidney of the offspring, which is associated with increased apoptosis and reduced nephron number. Variations in maternal behaviour have also been shown to lead to epigenetic changes in rats. Weaver et al. [12] showed that pups raised by rat dams which showed poorer nurturing had an increased stress response. The effect was due to hypermethylation of a specific CpG within the promoter of the GR gene in the hippocampus of the offspring. These changes persisted into adulthood and were associated with altered histone acetylation and reduced binding of the transcription factor NGF1A to the GR promoter. Central infusion of the HDAC inhibitor trichostatin A removed the differences in histone acetylation, DNA methylation, NGF1A binding and the hypothalamus-pituitary-adrenal axis stress response.

The effects of early nutrition on the epigenetic regulation of imprinted genes and intracisternal A particle (IAP) retrotransposons have also been reported. Mouse embryos cultured in Whitten’s medium without amino acids showed bi-allelic expression of the imprinted H19 gene, while those cultured in medium containing amino acids showed mono-allelic expression [13]. In humans, assisted reproductive technologies are associated with increased risk of Angelman’s syndrome and Beckwith-Weidemann syndrome which are caused by decreased methylation of the regulatory regions of the UBE3A, and H19 and IGF-2 genes [13]. Alterations to the epigenetic regulation of imprinted genes produce dramatic alterations to the phenotype of the offspring which are evident in early life and so contrast with the phenotypes induced by variations in maternal nutrition throughout pregnancy which become clinically apparent in later life [3].

Differences in the maternal intake of micronutrients during pregnancy in the agouti mouse have been shown to induce differences in the coat colour of the offspring. The murine A^{ag} mutation results from the insertion of an IAP retrotransposon