Nutrition, Epigenetics, and Developmental Plasticity: Implications for Understanding Human Disease

Graham C. Burdge[1] and Karen A. Lillycrop[2]

[1]Institute of Human Nutrition, University of Southampton School of Medicine, Southampton, SO16 6YD, United Kingdom; email: g.c.burdge@southampton.ac.uk

[2]Development and Cell Biology, University of Southampton School of Biological Sciences, Southampton, SO16 7PX, United Kingdom; email: k.a.lillycrop@southampton.ac.uk

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Abstract

There is considerable evidence for induction of differential risk of noncommunicable diseases in humans by variation in the quality of the early life environment. Studies in animal models show that induction and stability of induced changes in the phenotype of the offspring involve altered epigenetic regulation by DNA methylation and covalent modifications of histones. These findings indicate that such epigenetic changes are highly gene specific and function at the level of individual CpG dinucleotides. Interventions using supplementation with folic acid or methyl donors during pregnancy, or folic acid after weaning, alter the phenotype and epigenotype induced by maternal dietary constraint during gestation. This suggests a possible means for reducing risk of induced noncommunicable disease, although the design and conduct of such interventions may require caution. The purpose of this review is to discuss recent advances in understanding the mechanism that underlies the early life origins of disease and to place these studies in a broader life-course context.

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INTRODUCTION

Genetic variation is only able to explain a small proportion of the risk of a range of noncommunicable diseases (100). Technical constraints and the complexities of polygenic disease traits may account for some of this limitation. It is becoming clear that persistent changes in tissue structure and physiology that are induced during the development of the embryo and fetus also play an important role in determining risk of noncommunicable diseases. A substantial number of studies have shown that constraint in the early life environment is associated with increased risk of cardio-metabolic disease, affective disorders and cognitive decline, osteoporosis and sarcopenia, allergy and

inflammation, and specific cancers (51). These associations are marked by variation within the normal range of anthropometric markers at birth or in the first years of life. This process has been termed fetal programming, but perhaps is better described as phenotype induction because "programming" has connotations of the genetic program for development and is deterministic (48). Rather, the process that underlies induction of differential risk of disease by variation in the prenatal environment reflects environmental cues acting through developmental plasticity, which generate a range of genotypes from a single genome. The manifestation of disease is contingent on interactions with the environment throughout the life course (48). Recent findings show that altered epigenetic regulation of specific genes is central to the process by which different phenotypes are generated and hence differential risk of disease. This review focuses on the role of altered epigenetic processes in induction and stability of altered phenotypes and of differential disease risk by nutrition in early life, the mechanisms that underlie such epigenetic changes, and the extent to which they may be prevented by dietary interventions.

DEVELOPMENTAL PLASTICITY

Development represents a period of rapid change in the expression of the genome during which environmental cues may induce persistent changes in the phenotype of an organism. The developmental program tends to follow a path, termed canalization, in which the characteristics of the wild type or typical phenotype are buffered against genetic and epigenetic change (143). However, many organisms undergo adaptations during development in response to cues about the future environment that alter the developmental program in a manner that generates novel phenotypes. Such deviation from canalized development allows production of different phenotypes from a single genome more rapidly than could be achieved by mutation. For example, crowding of adult desert locusts (*Scistocerca gregaria*) induces the production of offspring that are gregarious, diurnal, and migratory in contrast to the nocturnal, sedentary forms that are produced under low population density (113). The offspring of *Daphnia* are born with a defensive "helmet" structure if their mother has been exposed to chemicals produced by predators (81). The duration of daylight to which meadow voles (*Microtus pennsylvanious*) are exposed during pregnancy determines coat thickness in the offspring in anticipation of winter or spring temperatures (88). Such rapid changes in phenotype may facilitate short-term survival but may also be assimilated and so produce stable phenotypes on which natural selection may act (144). Gluckman & Hanson (47) have suggested that the changes induced in mammals, including humans, by poor prenatal nutrition or maternal stress reflect an adaptive responses to environmental cues acting through developmental plasticity which induce phenotypes which predict the future environment and this confer a fitness advantage. For example, poor nutrition of the pregnant mother may signal to the fetus that nutrients are scarce in the postnatal environment and so induce metabolic adaptations in the offspring that reduce energy demands. However, an incorrect prediction, such as may occur if maternal nutrition is adequate but placental function is suboptimal, would result in mismatch between the physiology of the offspring and the future environment. Such mismatch has been suggested to underlie cardio-metabolic disease in humans (47). One important feature of adaptive changes during development is that different phenotypes are generated from the same genome. In order for truly adaptive phenotypes to be induced, cues about the future environment would need to interact with the genome in a manner that results in specific changes in gene expression and hence tissue function. We return to this point later. It is becoming clear that such persistent changes in the expression of the genome involve altered epigenetic regulation of specific genes.

THE EARLY LIFE ORIGINS OF HUMAN CHRONIC DISEASE

Evidence from Human Studies

There is substantial evidence that increased risk of cardio-metabolic diseases such as type 2 diabetes mellitus, hypertension, obesity, and osteoporosis are associated with relative thinness and small size at birth or in infancy (3, 55, 59, 110). Conversely, risk of specific cancers, including breast cancer and hepatoblastoma, are associated with birth weight at the higher end of the typical range of birth weight (22). The association between risk of cardio-metabolic disease and size at birth was initially identified in western populations, although these observations have been replicated in several ethnic groups. Placental volume is also negatively associated with risk of cardio-metabolic disease (54). These associations mark variation in the quality of the intrauterine environment such that reduced growth reflects prenatal constraint. As well as the limiting effects of small uterine size, constrained growth may reflect other aspects of the intrauterine environment such as nutrition, oxygen supply, and hormonal exposure. Importantly, the associations between prenatal growth and future risk of noncommunicable disease occur across the range of infant size typical for each population. This is in contrast to intrauterine growth retardation, which may be induced by adverse events during gestation, such as infection, and leads to birth weight below the normal range. Intrauterine growth retardation is also associated with increased risk of impaired glucose homeostasis, as is premature birth (68, 69).

Although the effects of the prenatal environment on the future health of the offspring have been demonstrated repeatedly, the precise nature of the environmental cues is less clear. Our focus is specifically on the role of nutrition in inducing altered phenotypes. Although prenatal nutrition is presumed to play a central role in the induction of differential disease risk, direct evidence is limited to a few studies. One key example is the famine that occurred in the Netherlands as a result of the Nazi blockade during the winter of 1944, which resulted in a decrease in daily calorie intake from 1800 kcal to between 400 and 800 kcal (119). Nevertheless, women continued to become pregnant and give birth during this period. Because the duration and severity of the famine were carefully documented, it has been possible to investigate the effects of nutritional constraint and the timing in gestation of exposure to famine on future risk of disease in the offspring. The findings of these studies show that exposure to famine was associated with increased risk of obesity, mood disorders, impaired glucose and lipid homeostasis, and reduced renal function in a manner contingent on gestational age at the time of exposure to famine (119). Furthermore, adult children of women who took part in a dietary intervention study in Motherwell, Scotland, in which they were instructed to consume 0.45 kg meat and to avoid carbohydrate throughout their pregnancy, had increased blood pressure (124) and blood cortisol concentration (66). Antenatal supplementation with folic acid has been reported to increase birth and placental weights (132). Thus, intakes of specific nutrients during pregnancy alter the development and future disease risk of the fetus.

A small number of studies have shown that nongenomic transmission of induced phenotypes between generations may be an important mechanism in human disease (50). Patterns of nutrition during the prepubertal growth period in children in Överkalix, Sweden during the nineteenth century are associated with differential risk of early death in their grandchildren (112). Poor maternal nutrition has also been associated with increased risk of type 2 diabetes mellitus over several generations in North American Indians (10). Individuals whose grandparents were in utero during the Dutch Hunger Winter had lower birth weight (126). Evidence for induced epigenetic changes in nongenomic inheritance of disease risk is discussed below.

Nutrition Models of the Early Life Origins of Human Chronic Disease

A number of models of the effect of undernutrition or overnutrition during pregnancy have been described that have proved valuable in demonstrating the casual relationship between nutrition in early life and the phenotype of the offspring and in identifying the underlying mechanisms (1, 2, 13). These include maternal protein restriction, global dietary restriction, or a high-saturated-fat diet fed over specific periods including preconception, pregnancy, and lactation, or a combination of these.

Global undernutrition during lactation or for 21 days after weaning or after puberty reduced cell number in a range of tissues, which was only reversed by return to adequate nutrition in the postweaning and postpubertal groups (154). Woodall and coworkers showed that reducing global nutrient intake in pregnant rats to 30% of ad libitum resulted in intrauterine growth retardation (156). This model is also characterized by higher systolic blood pressure, hyperinsulinemia, hyperleptinemia, hyperphagia, reduced locomotion, and obesity in the offspring, which were exacerbated by feeding a high-fat diet after weaning (141). It is a matter for debate whether such severe nutrient restriction is directly relevant to understanding the effects on the offspring attributed to variations in maternal nutrition within normal ranges, as described by Barker & Osmond (3). Nevertheless, this model is highly relevant to more severe maternal nutrient restriction such as that which occurred during the Dutch Hunger Winter, when nutrient intake was reduced to 22% of prefamine calorie intake (119). Modest global nutrient restriction during pregnancy also induces alterations in metabolism and the hypothalamic-pituitary-adrenal (HPA) axis. Feeding guinea pigs 85% of ad libitum diet throughout gestation induced impaired cholesterol homeostasis in the male offspring (76). In sheep, 15% global nutrient restriction during the first half of pregnancy induced in the offspring lower adrenocorticotrophin hormone (ACTH) and cortisol responses to exogenous corticotropin-releasing hormone and arginine vasopressin administration and blunted cortisol response to ACTH (63).

Dietary protein restriction during pregnancy in rodents has been used extensively to study the effect of maternal undernutrition on the offspring. Perinatal protein restriction led to a permanent impairment of insulin secretion (150). Offspring of dams fed a diet with a modest reduction in protein during pregnancy show a number of the features similar to cardio-metabolic disease in humans, including graded hypertension that is inversely related to maternal protein intake (83), impaired lipid (23) and glucose homeostasis (41), vascular dysfunction (135), impaired immunity (27), increased susceptibility to oxidative stress (87), increased fat deposition, and altered feeding behavior (6, 7). Subtle differences in apparently similar animal models induce different phenotypes in the offspring. For example, Burdge et al. (23) reported raised blood triacylglycerol (TAG) but no change in blood cholesterol concentration in male and female offspring of rats fed a protein-restricted (PR) diet during pregnancy, whereas Lucas et al. (98) reported lower TAG and cholesterol concentrations when dams were fed a PR diet during pregnancy and lactation. These studies differed in both the developmental stages exposed to the PR diet and in the lipid, protein, and energy content of the respective diets. Furthermore, the direction of induced change in lipid and glucose metabolism is dependent upon the relative amounts of protein and folic acid in the maternal diet (23). In guinea pigs, female offspring born to dams fed a PR diet in the first half of pregnancy (1–35 days) had raised mean arterial blood pressure that was associated with an increased intraventricular septum and anterior left ventrical wall thickness. These offspring did not exhibit growth restriction. In contrast, the offspring from dams fed the same PR diet in late gestation (36–70 days) were growth restricted but did not display altered blood pressure or left ventricular structure (11). These findings show that the timing and duration of exposure to undernutrition and variations in the precise macro- and micronutrient contents of the

HPA: hypothalamic-pituitary-adrenal

ACTH: adrenocorticotrophin hormone

TAG: triacylglycerol

PR: protein restricted

maternal diet together determine the phenotype induced in the offspring.

Recent studies have reported that maternal undernutrition during pregnancy is associated with greater susceptibility to cancer in rodents. In comparison with controls, offspring of rats fed a high-fat diet had structural changes to mammary tissue, higher estrogen receptor-α expression, increased levels of activated mitogen-activated protein kinase, and increased sensitivity to tumor induction with 7,12-dimethylbenz[a]anthracene (31). The offspring of rats fed a PR diet during pregnancy and lactation had a twofold increase in sensitivity to mammary tumor induction by nitrosomethylurea (40). Thus, although these studies used opposing maternal dietary insults, the overall outcome was to increase the sensitivity of mammary tissue to tumorigenic agents.

There is emerging evidence from studies in small animal models that induced phenotypes can be passed to more than one generation by a nongenomic mechanism. In rats, feeding a PR diet to the F_0 generation during pregnancy resulted in elevated blood pressure and endothelial dysfunction (136) and insulin resistance (103, 159) in the F_1 and F_2 generations, despite adequate nutrition during pregnancy in the F_1 generation. The adverse effects on glucose homeostasis of feeding a PR diet during pregnancy in the F_0 generation have been found in the offspring up to the F_3 generation (9). The administration of dexamethasone to dams in late pregnancy induced an increased expression of the glucocorticoid receptor (GR) and its target gene phosphoenolpyruvate carboxykinase (PEPCK) in the liver of the F_1 and F_2, but not F_3, offspring (35). These findings raise an important issue about the mechanism by which induced phenotypes are transmitted between generations. Because cells that give rise to the F_1 germ line, and hence F_2 generation, are present in F_1 embryos, they may be altered directly by environmental cues from the grandmother. Alternatively, transmission to the F_2 generation may represent changes induced in the F_1 generation passing to the F_2 generation. Thus, assessment of true nongenomic transmission between generations requires studies that continue to at least the F_3 generation (125). Loss of altered phenotype between the F_2 and F_3 generations as reported by Drake et al. (35) suggests that the phenotype present in the F_2 generation may have resulted from exposure of the F_1 germ line to dexamethasone. Whether the effects of maternal nutrient constraint are passed to the F_3 generation is unclear. Benyshek et al. (9) reported transmission of impaired glucose homeostasis to the F_3 generation with increasing severity in each generation when F_0 dams were fed a PR diet during pregnancy and lactation. Harrison & Langley-Evans (61) reported transmission of hypertension and altered nephron number in the male and females lines to the F_2, but not F_3, generation. One important difference between studies using maternal PR models is that transmission to the F_3 generation was induced when maternal dietary constraint was maintained during pregnancy and lactation, whereas transmission stopped at F_2 when the PR diet was fed during pregnancy alone. This implies that the number of generations to which an altered phenotype is transmitted is contingent on the severity of the nutritional constraint of the F_0 dams. The mechanism by which induced phenotypes are transmitted between generations is a matter for debate. Two mechanisms have been proposed that are not mutually exclusive. Female offspring expressing an altered phenotype as a result of environmental constraint before birth may exhibit altered physiological adaptations to pregnancy, thus altering the intrauterine environment and producing a series of altered phenotypes over successive generations (**Figure 1**). It has also been proposed that induced changes in the epigenetic regulation of genes could be passed between generations (see below).

A capacity to transmit an altered phenotype to multiple generations may be important in species such as rats, in which several generations are produced in one year, and all could be exposed to an environmental challenge, such as unusually low temperature or drought, that would adversely affect food availability.

GR: glucocorticoid receptor

PEPCK: phosphoenolpyruvate carboxykinase

However, one question that remains to be answered is how the capacity to transmit an induced phenotype between generations produces increased fitness in humans, where development and the start of reproduction are separated by more than a decade. One possible explanation may lie in the role of migration in human evolution (117). Migration of human ancestors out of Africa to colonize a wide range of environments would have resulted in exposure to marked differences in the type and abundance of food. Based on current knowledge, which shows that modest variations in nutrient availability induce an altered phenotype in the offspring, it is possible that colonization of new geographical regions may also have induced phenotypic change. If these changes were adaptive, and provided the population resided in a particular area for several decades, then transmission of a phenotype that predicted the new environment might have conveyed an advantage while retaining phenotypic flexibility to undertake further migration.

EPIGENETIC REGULATION OF GENES

The term epigenetics refers to processes that induce heritable changes in gene expression potential without altering the gene sequence (15). The major epigenetic processes in mammalian cells are methylation of carbon 5 of cytosine residues in CpG nucleotides; covalent modifications of histones such as methylation, acetylation, phosphorylation, and ubiquitination; and the activities of small interfering RNAs (15, 74). Because of their role in the early life origins of disease, this review concentrates principally on the first two mechanisms. The roles of DNA methylation and histone modifications in regulating transcription are closely interlinked, and there is some debate as to which is the primary process. CpG dinucleotides may be clustered in regions known as CpG islands. In general terms, methylation of CpG within gene promoters is associated with transcriptional inactivation, whereas unmethylated promoters are potentially transcriptionally active. However, there are exceptions. If the methylated CpG falls within the response element of a transcriptional repressor, this may induce expression of the gene. Approximately 70% of CpG dinucleotides are methylated, principally in heterochromatin and repetitive sequences such as retrotransposons (158). Most studies have focused on the effect of methylation of CpG dinucleotides within the 5′ regulatory regions of genes on transcription. Some genes, for example, apolipoprotein E (145), contain CpG islands toward the 3′ end, although the regulatory function is uncertain.

CpG: cytosine and guanine nucleotides linked by phosphate

Epigenetics and Development

Patterns of DNA methylation are established early in development. In mammals, the zygote undergoes rapid demethylation of the male genome within a few hours of fertilization (104, 111), although the mechanism is not known. The female genome is passively demethylated during subsequent mitotic divisions (89). The overall effect of genome-wide demethylation is to produce pluripotent cells in which all genes are potentially transcriptionally active. Loss of pluripotency and cell differentiation and the establishment of adult tissue function are dependent in changes in the methylation status of individual gene promoters at different times in development. Between formation and implantation of the blastocyst and gastrulation, the genome of the embryo undergoes de novo methylation, which is followed by gene-specific demethylation and methylation de novo during cell differentiation (74). For example, the pluripotency-associated gene Oct-4 is permanently silenced by hypermethylation of its promoter around E6.5 in mice (46), whereas HoxA5 and HoxB5, which are involved in pattern development, are not silenced until early postnatal life (67). Other genes undergo graded changes in the level of promoter methylation and transcription during development. Δ-Crystallin II and PEPCK promoters are methylated in the early embryo but undergo progressive demethylation during development and are expressed in adult tissues (8, 57). In

Dnmt: DNA methyltransferase

MeCP: methyl CpG-binding protein

MBD: methyl-CpG-binding domain

addition to gene silencing by promoter methylation, differential methylation of individual CpG dinucleotides can induce more subtle modulation of transcriptional activity. For example, telomerase activity is down-regulated in most cells during terminal differentiation in embryogenesis as a result of methylation of the CpG-rich promoter region, but it is often reactivated in cancer cells. It has been proposed that activation of telomerase in preneoplastic cells is due to a shift in regulation between the activator c-Myc and the suppressor WT1 by changes in the methylation status of specific CpG within the binding domains of these transcription factors in the promoter of the catalytic subunit (134).

Genomic Imprinting

Genomic imprinting describes the monoallelic expression of specific gene loci in a manner dependent upon the parental origin (116). The majority of the 53 human genes that are imprinted are located in CpG-rich domains, termed imprinting centers, in which methylation of CpG dinucleotides results in repression of either the maternal or paternal allele (32, 107). Because these methylation patterns are established in the gamete before fertilization, they are excluded from the genome-wide demethylation that occurs after fertilization (18, 82). Impaired imprinting leading to biallelic expression is causally associated with a number of congenital diseases, including Angelman's, Prader-Willi, and Beckwith-Weidemann syndromes. These conditions are characterized by specific anatomical abnormalities and impaired neurodevelopment, which are apparent at birth (115, 130, 138). The effects of the environment experience by the early embryo on imprinting is discussed below.

Enzymes and Proteins Associated with Epigenetic Regulation

DNA methylation is induced and maintained in mammals by three DNA methyltransferases. Deletion or mutation of the genes encoding these enzymes results in embryonic lethality or severe disruption of development and loss of imprinting (74). DNA methyltransferase (Dnmt)-1 is responsible for maintaining patterns of CpG dinucleotides methylation through replication cycles. Two major isoforms have been identified. Dnmt1o is expressed in oocytes and early embryos, where it is important for maintaining methylation of imprinted genes (70) but is absent from adult tissues and sperm due to hypermethylation of its promoter (77). Dnmt1s is expressed in oocytes and early embryos but is also present in all adult tissues, although at lower levels in postmitotic cells (77, 90). Dnmt1o and -1s are expressed in newly fertilized zygotes but show a rapid reduction in the level of mRNA by the two-cell stage, which may contribute to demethylation of the maternally derived genome. In mice, Dnmt1o and -1s are undetectable between two-cell embryos and the blastocyst stage, but Dnmt1s is re-expressed in gastrulating embryos (77). Variations in Dnmt1o expressed during development are associated with changes in the methylation status of the Dnmt1o promoter (77). DNA methylation de novo is catalyzed by Dnmt3a and 3b (109). Like Dnmt1, Dnmt3a and 3b are not expressed in two-cell- to eight-cell-stage embryos. Dnmt3a is re-expressed in morula and Dnmt3a in gastrula, which is associated with genome-wide methylation de novo (77). Dnmt3L does not appear to have catalytic activity, but it is involved in coordinating Dnmt 3a and 3b activities (62).

A group of proteins that bind methylated CpGs provides a link between DNA methylation and histone modifications. Methyl CpG-binding protein (MeCP)-2 and methyl-CpG-binding domain (MBD) proteins 1 to 3 bind to methylated DNA sequences and recruit histone-modifying enzymes and other proteins, including polycomb, that induce repressive heterochromatin and gene silencing (58, 65). Transcriptionally active euchromatin is associated with acetylated lysine residues on histones that, together with methylation of histone H3 at K4, facilitate transcriptionally active euchromatin structure (80, 127, 137,

160). Methylation of cytosine in promoter regions and binding of MeCP2 to methylated DNA recruits histone deacetylases (HDACs) and histone methyltransferases that in turn induce di- and trimethylation of K9 on histone H3 (43, 96, 108), thus converting euchromatin to heterochromatin. Recent studies have shown, however, that Dnmt1 is recruited by a number of histone-modifying enzymes, such as HDAC1, HDAC2, and the histone methyl transferases SUV39 and EZH2 (42, 43, 120, 142), which suggests that chromatin structure may also determine DNA methylation status and that there is a reciprocal relationship between these two processes.

Although DNA methylation is generally regarded as a stable epigenetic mark, this does not explain the presence of Dnmt-1 in postmitotic cells such as neurones (56), where it would be expected to be redundant. Szyf (129) has proposed that one possible role for the presence of Dnmt-1 in postmitotic cells is to remethylate CpGs demethylated by demethylases. The identity of such demethylases remains uncertain. Several candidates have been proposed, including MBD2b (14), MBD4 (161), the DNA repair endonucleases XPG (Gadd45a) (4), and a G/T mismatch repair DNA glycosylase (75), although the evidence that they fulfill this role is limited. However, there is strong evidence that cells contain demethylase activity. For example, the myosin gene is actively demethylated during muscle development (97), interferon-γ and IL-4 promoters are reciprocally hypomethylated by IL-12 or IL-4 signaling, respectively, during differentiation of naïve T cells into Th1 or Th2 phenotypes (121), the GR is demethylated in the presence of the HDAC inhibitor tricostatin-A (149), and paternal DNA undergoes active demethylation in newly fertilized zygotes (100). Thus, the methylation status of CpG in postmitotic cells may represent an equilibrium state dependent upon the relative activities of Dnmt1 and demethylases (129). If so, this provides a mechanism by which environmental exposures that activate or inhibit these enzymes may induce a shift in the methylation/demethylation equilibrium, leading to a change in the methylation status and, in turn, the level of transcriptional activation of a gene. Such processes may be important in loss of differentiation and reactivation of cell cycling in cancer (129).

HDACs: histone deacetylases

PPAR: peroxisomal proliferator-activated receptor

INDUCED CHANGES IN THE EPIGENETIC REGULATION OF GENES

Induction of Altered Transcription by Nutrition in Early Life

The induction of changes to the phenotype of the offspring that persist throughout the lifespan implies stable changes to gene transcription resulting in altered activities of metabolic pathways and homeostatic control processes and differences in the structure of tissues. The latter may result from changes in stem cell allocation to various lineages, variations in the rate and/or number of mitosis, and the extent of apoptosis. Together, these processes provide cellular and molecular explanations for variation between individuals in body structure and functional capacity to respond to environmental challenge (47). In women, differences in body structure and metabolic capacity induced by the environment experienced before birth may, in turn, influence the timing of their sexual maturation (49) and reproductive success (30) and the birth weight of their children (29), which represents one mechanism by which phenotypic changes may be passed to successive generations.

There have not been any studies that have specifically investigated the effect of the nutrition of pregnant women on gene expression in their offspring. Eriksson et al. (39) showed that increased risk of insulin resistance in adults was associated with lower birth weight only in individuals who had the Pro12Ala to Ala12Ala mutation in the peroxisomal proliferator-activated receptor (PPAR) γ2 gene, where substitution of proline with alanine reduced the transcriptional activity.

Recent studies have investigated the specificity of induced changes in the transcriptome

using microarray analysis in animal models. Gheorghe et al. (44) showed by transcriptome-wide analysis that feeding mice a PR diet between gestational days 10.5 and 17.5 altered the expression of 235 genes (91 genes up-regulated). These findings show that the effects of maternal diet on the expression of the fetal genome are specific to a relatively small proportion of the 22,690 examined by the array (~1%). Increased expression of genes was involved in the p53 pathway, apoptosis, negative regulators of cell growth, negative regulators of cell metabolism, and genes related to epigenetic control. Genes involved in nucleotide metabolism had lower expression. Crucially, this study does not show, for obvious reasons, the number of genes that are altered persistently in the offspring. Our recent data show that in the liver of adult male offspring of dams fed a PR diet during pregnancy, 311 genes differed significantly (of which 222 were increased) from offspring of control dams (94). One hundred ninety-one genes differed significantly (of which 45 were increased) between offspring of dams fed the PR diet supplemented with 5mg/kg feed folic acid (PRF) compared to offspring of control dams. Only 16 genes were significantly altered in both PR and PRF offspring compared to controls. Thus, feeding an altered maternal diet during pregnancy induced persistent changes in a relatively small proportion of the transcriptome, and the pattern of induced changes was contingent on the protein and folic acid of the maternal diet.

Studies that have used a candidate gene approach have provided important insights into the mechanisms that underlie different phenotypes. Because of the researchers' interests in understanding human disease, the genes studied have tended to be those involved in macronutrient metabolism and HPA axis and cardiovascular function. Feeding a PR diet to pregnant rats induced increased GR expression and reduced 11β-hydroxysteroid dehydrogenase type II expression in liver, lung, kidney, and brain in the offspring (12, 92). Increased GR expression has also been reported in the lung, liver, adrenal gland, and kidney of the offspring of sheep fed a restricted diet during pregnancy (20, 53, 152). Feeding a PR diet to pregnant and/or lactating rats also up-regulated the expression of glucokinase (16), acetyl-CoA carboxylase (102), PPARα, acyl-CoA oxidase and carnitine palmitoyltransferase-1, but not PPARγ1, (25, 26, 92, 95) in the liver of the offspring. In contrast, PPARγ2 expression was reduced in adipose tissue (25).

Together, these findings show that induction of an altered phenotype involves persistent changes to the expression of a subset of genes that, for those characterized so far, are associated with the altered epigenetic regulation of their promoters or of the promoters of transcription factors that regulate their activity.

Epigenetic Change Is Critical for Induction of Alternative Phenotypes by Nutrition in the Honey Bee

Differentiation in the expression of the genome of the honey bee (*Apis mellifera*) is probably the clearest example of induction of alternative phenotypes and epigenotypes by nutrition in early life. Female bees are genetic clones. However, queens are distinct from workers in their morphology, capacity to reproduce, behavior, and longevity. The difference between the queen and worker castes lies in the exposure of the genetically identical larvae to royal jelly, an as yet incompletely defined mixture of proteins, amino acids, vitamins, lipids, and other nutrients (101). All larvae are fed for the first three days after hatching, but only those destined to become queens are fed royal jelly thereafter. Recently, Kucharski et al. (78) provided an important insight into how nutrient exposure in early life can induce dramatic differences in the phenotype of the adult. They showed that injecting larvae on day 1 with Dnmt3 siRNA produced 72% queens, while the control siRNA produced 77% workers. Dynactin p62 expression in the corpora allata is up-regulated in larvae destined to become queens. This is associated with induced lower methylation of specific CpG dinucleotides in the dynactin p62 gene. The level of methylation of the dynactin

p62 gene was comparable in hive-reared and Dnmt3 si-RNA-treated bees. Thus, one mechanism by which royal jelly may induce different bee castes from the same genome is through altered Dnmt3 expression leading to changes in DNA methylation of specific genes.

Evidence from Human Studies of Induced Epigenetic Change by Maternal Nutrition

To date, one study has shown hypomethylation of the differentially methylated regions of the imprinted insulin-like growth factor-2 gene in genomic DNA isolated from whole blood from individuals who were in utero during the Dutch Hunger Winter compared to unexposed same-sex siblings (64). The study found that the mean level of methylation of exposed individuals was 52% compared to 49% in unexposed controls, with standard deviations of around 5%. The same group also investigated the effect of periconceptional exposure to famine compared to unexposed same-sex siblings on the methylation status of loci in imprinted (IMP) and nonimprinted (NIMP) genes implicated in growth and metabolic disease (133). Insulin-like growth factor (IMP) was hypomethylated in exposed individuals, whereas interleukin-10 (NIMP), leptin (NIMP), ATP binding cassette A1 (NIMP), guanine nucleotide binding protein (IMP), and maternally expressed 3 (IMP) were hypermethylated. The maximum difference between exposed and unexposed individuals was 6%. Although statistically significant, such small differences are difficult to interpret, particularly since the difference between groups of subjects is similar to the analytical error of the technique used (38). Further studies are clearly needed to substantiate these findings.

Retrotransposon Models of Altered Epigenetic Regulation by Maternal Diet

Three mouse models have been described in which the phenotype is controlled by metastable epialleles, identical alleles that are expressed variably due to epigenetic modifications: agouti viable yellow (A^{vy}) (37), axin fused ($Axin^{Fu}$) (140), and CDK5 activator-binding protein ($Cabp^{IAP}$) (36). In each case, an intracisternal A particle (IAP) retrotransposon is inserted upstream of the transcription start site, which alters the level of transcription by the methylation status of the cryptic promoter. The level of methylation of this promoter is determined during development and is modified by maternal nutrition and other environmental agents including endocrine disrupters (34, 146, 147). For example, in A^{vy} mice, the metastable allele is due to insertion of an IAP retrotransposon upstream of the *Agouti* gene, which encodes a paracrine signaling molecule that produces black eumelanin or yellow pheomelanin. The methylation status of seven CpG dinucleotides in the cryptic promoter at the proximal end of the A^{vy} IAP produces a range of coat colors between yellow (unmethylated) and pseudoagouti (methylated) (147, 155). Feeding pregnant A^{vy} mice diets containing increasing amounts of the methyl donors choline and betaine and the one-carbon metabolism cofactors folic acid and vitamin B12 changes the phenotype from agouti to pseudoagouti, which is consistent with increased methylation of the A^{vy} IAP (155). Comparable effects of increasing maternal folic acid, vitamin B12, choline, and betaine intakes have also been shown in $Axin^{Fu}$ (146). The mechanism by which increasing maternal intakes of nutrients involved in one-carbon metabolism induce such changes in the level of methylation in these models is not known.

IMP: imprinted

NIMP: nonimprinted

A^{vy}: Agouti viable yellow

$Axin^{Fu}$: axin fused

IAP: intracisternal A particle

Effect of Maternal Protein Restriction or Global Nutrient Restriction During Pregnancy on the Epigenome of the Offspring in Rodents

Feeding a PR diet to rats during pregnancy induces hypomethylation of the PPARα and GR promoters and increased expression of the GR and PPARα in the liver of juvenile (92) and day-80 adult (26) offspring. These studies showed for the first time that, in contrast to modifying the maternal intake of nutrients

directly involved in one-carbon metabolism (147), stable changes to the epigenetic regulation of the expression of transcription factors can be induced in the offspring by modest changes to maternal macronutrient intake during pregnancy. The interaction between maternal protein intake and one-carbon metabolism is discussed below. mRNA expression of PPARα and GR, and of their respective target genes, acyl-CoA oxidase and carnitine palmitoyl-transferase-1, and PEPCK was increased in juvenile and adult offspring (26, 92, 95). This is consistent with raised plasma β-hydroxybutyrate and glucose concentrations in the fasting offspring (23). Sequencing analysis of the PPARα promoter showed that four specific CpGs were hypomethylated and that two CpGs located within transcription factor response elements predicted the level of the transcript (93). Thus, the effects of the maternal PR diet on the offspring are targeted to specific CpGs. Taken together, these results are consistent with the view that modest dietary modification induces an altered phenotype through epigenetic changes in specific genes. Methylation of the GR and PPARα promoters was also reduced in the heart (91), and the PPARα promoter was hypomethylated in the whole umbilical cord (21) in the offspring of rats fed a PR diet during pregnancy. These findings are consistent with increased GR mRNA expression in a range of tissues from the offspring of rats fed a PR diet during pregnancy (12). Hypomethylation of the GR promoter has also been found in the offspring of mice fed a PR diet during pregnancy (21), which suggests that the effect of the PR diet may not be specific to one species.

The fundamental role of changes in the epigenetic regulation of transcription factor expression in altering the activity of pathways controlled by their target genes is underlined by the observation that although glucokinase expression was increased in the liver of the PR offspring, this was not accompanied by changes in the methylation status of the glucokinase promoter (16). Since GR activity increases glucokinase expression, greater glucokinase expression in the PR offspring may have been due to increased GR activity as a result of hypomethylation of the GR promoter rather than a direct effect of prenatal undernutrition on glucokinase. Hypomethylation of the angiotensin receptor 1b has also been reported in adrenal glands from the offspring of dams fed a PR diet during pregnancy (17). This adds support to the observations in liver and to the hypothesis that altered epigenetic regulation is central to the induction of an altered phenotype.

Hypomethylation of the GR promoter was associated with an increase in histone modifications that facilitate transcription: acetylation of histones H3 and H4 and methylation of histone H3 at lysine K4, whereas histones that suppress gene expression were reduced or unchanged (95). Although functionally consistent, the mechanistic relationship between GR hypomethylation and the associated histone changes is not known.

The process by which environmental cues induce altered epigenetic regulation in the embryo remains unknown. Studies in liver from juvenile offspring have provided some insights into the underlying mechanisms. Feeding a PR diet to pregnant rats induced lower Dnmt1 expression and reduced binding of Dnmt1 at the GR promoter (95). However, the expression of Dnmt3a, Dnmt3b, and MBD-2 and binding of Dnmt3a at the GR promoter were unaltered (95). This suggests that hypomethylation of the hepatic GR promoter in the offspring, and probably other genes including PPARα, is induced by reduced capacity to maintain patterns of cytosine methylation during mitosis rather than failure of methylation de novo or active demethylation (21, 95). This is consistent with lower MeCP2 binding and increased levels of histone modifications, which facilitate transcription at the GR promoter. Reduced Dnmt1 activity might be expected to result in global demethylation. However, studies in vitro show loss of Dnmt1 induced demethylation of only a subset of genes (118). This indicates that Dnmt1 is targeted to specific genes, which

is consistent with selective hypomethylation in the liver in the PR offspring (92). Dnmt1 activity is also required for progression through mitosis (106), and its expression is substantially reduced in nonproliferating cells (128). Thus, suppression of Dnmt1 activity in the preimplantation period could also account for the reduction in cell number during the early development in this model (79).

In contrast to the effects of maternal PR diet on the epigenetic regulation of hepatic genes in the offspring, reduction of total food intake during pregnancy in rats to 30% of ad libitum induced hypermethylation and lower expression of PPARα and GR in the liver of 170-day-old offspring (52). One possible explanation may lie in the differences in severity of nutritional constraint between these two dietary regimens. If the induction of altered phenotypes is predictive, then it may be anticipated that induced changes in the epigenome would differ according to dietary regimen in order to match the phenotype to the predicted future environment. Thus, the maternal PR diet could be regarded as a moderate nutrient constraint that induces in the offspring increased capacity for using nutrient reserves for energy production. Global undernutrition is a relatively more severe constraint that induces conservation of energy substrates. These interpretations are consistent with the phenotypes induced in the offspring (23, 141).

Neonatal Care and Offspring Stress Response

Weaver and colleagues reported the effects of different levels of nursing of rat offspring in their subsequent behavior and on the regulation of their response to stress (149). They observed that the offspring of dams that had increased tendency to arch their backs during suckling and for grooming their pups had a better response to future stress than did offspring of mothers that showed poorer nursing. Better response to stress was causally associated with hypomethylation of a single CpG dinucleotide within the NGF1-A binding domain in the $GR1_7$ promoter in the hippocampus, which resulted in increased GR mRNA expression. Thus, like the effects of maternal diet on the PPARα promoter, the effects of maternal nursing behavior on the offspring involve highly specific changes in the epigenome. These findings are consistent with the view that an adverse environment in early life induces changes in the offspring that predict future environmental challenge. A recent study has shown that the methylation status of the equivalent CpG dinucleotide in GR promoter in human brain is associated with suicide in individuals who were abused as children (105). These findings have important implications for understanding how the social environment in early life may induce lifelong personality traits and/or facilitate perpetuation of cultural practices. Furthermore, although a lower stress response in humans may have health benefits, induction of more nervous offspring may be beneficial in rodents, where it could be associated with improved avoidance of predators and so confer greater fitness.

Transmission of Induced Changes in DNA Methylation Between Generations

There is substantial evidence for transgeneration epigenetic inheritance in nonmammalian species, and its role in evolutionary biology has been recently reviewed (50, 72). Although epidemiological and experimental studies have shown transmission of induced phenotypes between generations, to date only one study has reported transmission of induced epigenetic marks between generations. GR and PPARα promoters in 80-day-old male grand-offspring of rats exposed to maternal PR diet during gestation were hypomethylated compared to controls, even though their dams received adequate nutrition throughout pregnancy (26). These findings imply that the female line is sufficient for transmission of such epigenetic information between generations, although other studies have shown transmission of phenotypes

induced in the offspring by maternal exposure to dexamethasone in pregnancy through both male and female lines up to the F_2, but not the F_3, generation (35). The tendency toward obesity in A^{vy} mice is exacerbated through successive generations (148). Transmission of the obese phenotype was prevented by supplementation of females with methyl donors and cofactors, although this was not associated with a change in the methylation status of the A^{vy} locus.

The mechanism by which induced epigenetic marks are transmitted to subsequent generations is not known. Since the transmission was only to the F_2 generation, a direct effect of the diet fed to the F_0 dams on germ cells that gave rise to the F_2 offspring cannot be ruled out. Sequential transmission from F_1 to F_2, and possibly beyond, would involve induction in the germ line of altered epigenetic marks, and such changes in DNA methylation would have to be preserved during genome-wide demethylation during fertilization, possibly by a similar mechanism to that which preserves the methylation of imprinted genes (82) and/or by targeted preservation of nucleosome structure as occurs for specific developmental genes during spermatogenesis (60). Alternatively, prenatal nutritional constraint induces physical or physiological changes in the female that in turn restrict the intrauterine environment in which her offspring develop. In this case, transmission of an altered phenotype between generations would involve induction of changes in gene methylation de novo in each generation. If so, the magnitude of the induced effect, epigenetic or phenotypic, would differ between generations. However, this is not supported by the similarity in the reduction in birth size and blood glucose concentration in the F_1 and F_2 generations born to rat dams exposed to dexamethasone in late gestation (35) or the degree of hypomethylation of the hepatic GR and PPARα in the F_1 and F_2 offspring of dams fed a PR diet in pregnancy (26). Furthermore, induced phenotypic traits would not be passed through the male line (35).

THF: tetrahydrofolate

PE N-MET: phosphatidylethanolamine N-methyltransferase

NUTRITIONAL INTERVENTIONS TO PREVENT OR REVERSE INDUCED PHENOTYPES AND EPIGENETIC CHANGES

Identification of the epigenetic mechanisms that underlie induction of increased further disease risk by prenatal nutrition raises the possibility of nutritional interventions to prevent or reverse such processes. Attempts at such interventions have been tested in well-characterized animal models using nutrients involved in one-carbon metabolism. Although these experiments have reached only the proof of principle stage, they have identified possible benefits and pitfalls that are of relevance to preventing human disease.

Prevention of Phenotypes Induced by Maternal Undernutrition

DNA and histone methylation are closely linked to pathways that supply methyl substrates for their respective methyltransferases (99). For DNA methylation, methyl groups are primarily supplied from serine by the action of cytoplasmic serine hydroxymethyltransferase, which transfers CH_3 to tetrahydrofolate (THF) to form 5,10-methylene THF, which is reduced to 5-methyl THF by 5,10-methylenetetrahydrofolate reductase. This methyl group is used to convert homocysteine to methionine by methionine synthase with vitamin B_{12} as cofactor. S-adenosylmethionine is the substrate for Dnmts, which compete for CH_3 with phosphatidylethanolamine N-methyltransferase (PE N-MET) activity. PE N-MET uses three moles of CH_3 to synthesize one mole phosphatidylcholine and in the liver is the major terminal reaction in one-carbon metabolism. Betaine is an alternative substrate for remethylation of homocysteine, which is catalyzed by betaine homocysteine methyltransferase.

Supplementation of the maternal PR diet with 5 g/kg diet glycine (73) or 5 mg/kg diet folic acid (135) has been shown to prevent the

induction of hypertension and endothelial dysfunction in the offspring. Supplementation of the maternal PR diet with folic acid also prevented dyslipidemia in the adult offspring (23). In contrast, supplementation of the control protein diet with 5 mg/kg diet folic acid induced impaired endothelial dysfunction and dyslipidemia in the offspring (23, 135). Increasing the folic acid content of the PR diet prevented hypomethylation of the PPARα and GR promoters in the liver of the offspring (92). However, detailed analysis of the PPARα promoter showed that although increased maternal folic acid intake prevented hypomethylation of the majority of CpG dinucleotides induced by the PR diet alone, two CpGs were hypermethylated (93). Thus, increasing maternal folic acid intake does not simply prevent the effects of the PR diet, but may also induce subtle changes in gene regulation.

The mechanism by which increasing maternal folic acid or glycine intake prevents induction of altered DNA methylation is not clear. Pregnant rats fed a PR diet show increased blood homocysteine concentration in early gestation (114) and a trend towards a higher homocysteine concentration in late gestation (19, 135), although some studies did not find this (85). Thus, it is likely that perturbation of the supply of methyl groups through the folate-dependent pathway is involved. Two possible mechanisms are as follows. First, decreased availability of glycine leads to an altered flux of methyl groups between different metabolic fates, leading to constraint in the remethylation of homocysteine to methionine. Second, increased maternal corticosteroid levels (86), possibly as a result of stress induced by constrained nutrient availability, may reduce folic acid availability (131), again constraining the supply of methyl groups. The latter mechanism could explain how maternal corticosteroid blockade prevents induction of hypertension in the PR offspring (84). Furthermore, Dnmt1 expression is negatively regulated by Hcyst and increased by folic acid (45), although the mechanism is not known. Thus, modulation of Dnmt1 expression by differences in one-carbon metabolism may provide a link between maternal diet and epigenetic regulation of gene expression in the fetus.

Reversal of Phenotypes Induced by Maternal Undernutrition

Interventions that reverse the adverse effects of prenatal nutrition after the neonatal period may be a valuable strategy in reducing the prevalence of noncommunicable disease. One recent study investigated whether supplementing the diet of the offspring with folic acid during their juvenile-pubertal period could reverse changes in phenotype and epigenotype induced by a maternal PR diet (24). The folic acid content of the offspring's diet was increased by the same amount as that added to the maternal PR, which prevented changes in the phenotype and epigenotype induced by the maternal PR diet alone (23, 92). The results showed that in contrast to supplementation of the maternal PR diet with folic acid, supplementation during the juvenile-pubertal period induced impaired lipid homeostasis, including down-regulation of hepatic fatty acid β-oxidation, hepatosteatosis, and increased weight gain, irrespective of the maternal diet. This was associated with altered methylation of specific genes, including hypermethylation of PPARα in the liver of the offspring. These findings suggest that the period between weaning and adulthood in rats represents a period of increased plasticity, possibly reflecting ongoing growth and development. This is consistent with the view that puberty is one of four periods of increased instability of the epigenome: prenatal development, neonatal development, puberty, and aging (33). Although the effects of folic acid supplementation on the offspring were deleterious, these findings do suggest that it may be possible to reverse the adverse effect of prenatal nutrition by nutritional interventions before adulthood.

Together, the findings from studies of folic acid supplementation during gestation or after weaning in rats using the maternal protein-restriction model show that the outcomes of

such interventions are complex and cannot be easily predicted because there are interactions between folic acid and the background diet that are influenced by the timing of the intervention. Thus, although prevention or reversal of the adverse effects of poor prenatal nutrition appears feasible, the design of any supplementation regimen for use in humans would need to include careful consideration of the timing and magnitude of the intervention.

LIFE COURSE AND EVOLUTIONARY IMPLICATIONS OF INDUCED PHENOTYPIC AND EPIGENETIC CHANGE

Developmental plasticity provides a mechanism by which different phenotypes can be expressed from the same genome. Examples often quoted from natural history, such as helmet formation in response to threat of predation in *Daphnia* (81), induction of migratory behavior with associated metabolic and morphological changes in response to increased population density in *Schistocerca gregaria* (113), worker or queen phenotypes in *Apis mellifera* contingent on feeding of royal jelly (101), and variation in coat thickness and color in response to day length in *Microtus pennsylvanicus* (88), are polyphenic. In contrast, phenotypes induced by variations in maternal nutrition in mammals appear pleiotropic and graded, such as the range of coat color induced in the A^{vy} mouse by differences in the dietary methyl donors (155) or the dose-response relationship between maternal protein intake and offspring blood pressure in rats (83). The duration and timing of the nutritional change also induces a range of phenotypes in the offspring. It is possible that influences from other aspects of the intrauterine environment and interactions between these parameters may produce an even greater number of phenotypes. Thus, embryos appear to be highly sensitive to a range of environmental cues. It is not clear why rodents and humans are able to change phenotype in response to modest variations in a range of nutrients. One possibility is that the majority of induced phenotypes are cryptic and are only manifest when an individual is exposed to a particular environmental challenge in later life. If so, then the majority of adaptive responses may be neutral.

It has been suggested that the capacity to generate multiple phenotypes from a single genome may be important in evolution (151). The predictive adaptive response model proposed by Gluckman & Hanson implies that induced phenotypes confer a Darwinian fitness advantage (47). It is reasonably easy to link the environmental cue and the subsequent advantage of the induced phenotype for the polyphenic changes listed above and for induction of increased stress response by poor nursing behavior in rats (149): More nervous offspring may be less susceptible to being stalked by predators. The specific advantage of the phenotypes induced by variations in maternal nutrition, principally altered energy homeostasis, is less clear. To address this, it may be helpful to think of the induced phenotype in terms of reproduction, and hence Darwinian fitness, rather than as a source of disease. One possible role of such induced phenotypes may lie in the energy demands of reproduction. Body size is important for reproductive success in males in a number of animal species; thus, increased tendency to deposit fat in adipose stores may increase reproductive success by raising the status of a male within a hierarchy. Capacity to meet the demands of the developing offspring is important for females. Pregnancy represents a substantial energy demand on the female; thus, alternative reproductive strategies have evolved to facilitate reproductive success when challenged by nutrient restriction (71, 153). These include more efficient generation of energy substrates by the liver (139) or by metabolic adaptations during pregnancy to enhance nutrient supply to the offspring or to withhold nutrients and thus generate poorer-quality or fewer offspring until environmental conditions are more favorable (122, 123). The origins of such reproductive

strategies may lie in adaptive responses during development. Thus, moderate nutrient restriction during development induces in females a greater potential capacity to supply energy substrates to the offspring (23), whereas more severe global prenatal nutrient constraint may be associated with withholding nutrients from the offspring and storing them in the dam to facilitate future reproductive cycles (141). If so, then the adverse consequences for health associated with prenatal nutritional constraint become irrelevant to the biological purpose of these adaptations, since they have little or no effect on reproductive capacity and/or become manifest after the reproductive period or the natural lifespan.

It may be anticipated that after an initial period of plasticity, induced changes in the phenotype and epigenotype of the offspring would be resistant to further change in order to convey the fitness advantage associated with adaptation to the predicted future environment. However, increased exposure to folic acid during the juvenile-pubertal period appeared to supersede epigenetic and phenotypic changes induced before birth (24). Superficially, such resetting of an induced phenotype and epigenotype by a modest nutritional intervention may appear to disprove the hypothesis that such induced changes predict the future environment. However, plasticity before and during puberty may allow further adaptations or correction of an incorrect prenatal prediction before the onset of reproduction. If so, an extended period of plasticity may offer a means of producing phenotypes better suited to the prevailing environment than would be achieved by prenatal prediction alone, provided the offspring receives appropriate cues.

In addition to providing a means of increasing fitness, the expression of altered phenotypes in response to environmental cues and their transmission between generations may also contribute to speciation and phylogeny (151). The epigenetic changes that underlie induced phenotypes may provide a means by which they may become fixed by increasing the susceptibility of the genome to mutation. The mechanisms by which this may occur include the following. Both hyper and hypo DNA methylation induce instability of the genome. Deamination of methyl cytosine to uracil induces a single nucleotide polymorphism by allowing insertion of thymidine instead of guanine in the complementary strand during DNA replication. This is supported by the enrichment of single nucleotide polymorphisms in highly methylated sequences (157). Feeding mice a methyl donor-deficient diet increased the rate of DNA damage in response to irradiation (5). Furthermore, hypomethylation of the genome of the white-cheeked gibbon (*Nomascus leucogenys leucogenys*) is associated with greater chromosomal rearrangement, which may have contributed to the evolution of this species (28). Thus, induced changes in DNA methylation in response to environmental cues during development may contribute to the generation of genetic variation in a manner that may contribute to evolution. It is not clear whether such genetic changes fix induced phenotypes in a population or would tend to produce random changes in the genome.

CONCLUSIONS

Variation in the expression of the genome leads to expression of novel phenotypes that have implications for understanding evolutionary biology and risk of disease. Epigenetic changes, in particular DNA methylation, are central to the generation of novel phenotypes and their stability throughout the life course. Although this area of research is progressing rapidly, key questions remain to be answered. It seems clear that induced epigenetic change is central to variation in phenotype, yet it is likely to be secondary to the pathway by which signals from the future environment are transmitted to the embryo/fetus and subsequently interpreted to give rise to epigenetic modifications. Understanding this process will provide a substantial step forward in biological research, including the early life origins of disease.

SUMMARY POINTS

1. Variation in nutrition during early life induces different phenotypes that are contingent on the timing, duration, and nature of nutritional challenge.
2. Epigenetic changes, in particular DNA methylation, underlie the induction and persistence of altered phenotypes.
3. Nutritional interventions during pregnancy can prevent or alter the phenotype induced by poor maternal nutrition during pregnancy. However, modification of the induced phenotype involves developmental trade-offs.

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LITERATURE CITED

1. Armitage JA, Khan IY, Taylor PD, Nathanielsz PW, Poston L. 2004. Developmental programming of the metabolic syndrome by maternal nutritional imbalance: How strong is the evidence from experimental models in mammals? *J. Physiol.* 561:355–77
2. Armitage JA, Taylor PD, Poston L. 2005. Experimental models of developmental programming: consequences of exposure to an energy rich diet during development. *J. Physiol.* 565:3–8
3. Barker DJ, Osmond C. 1986. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* 1:1077–81
4. Barreto G, Schafer A, Marhold J, Stach D, Swaminathan SK, et al. 2007. Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. *Nature* 445:671–75
5. Batra V, Mishra KP. 2007. Modulation of DNA methyltransferase profile by methyl donor starvation followed by gamma irradiation. *Mol. Cell Biochem.* 294:181–87
6. Bellinger L, Lilley C, Langley-Evans SC. 2004. Prenatal exposure to a maternal low-protein diet programmes a preference for high-fat foods in the young adult rat. *Br. J. Nutr.* 92:513–20
7. Bellinger L, Sculley DV, Langley-Evans SC. 2006. Exposure to undernutrition in fetal life determines fat distribution, locomotor activity and food intake in ageing rats. *Int. J. Obes. (Lond.)* 30:729–38
8. Benvenisty N, Mencher D, Meyuhas O, Razin A, Reshef L. 1985. Sequential changes in DNA methylation patterns of the rat phosphoenolpyruvate carboxykinase gene during development. *Proc. Natl. Acad. Sci. USA* 82:267–71
9. Benyshek DC, Johnston CS, Martin JF. 2006. Glucose metabolism is altered in the adequately-nourished grand-offspring (F-3 generation) of rats malnourished during gestation and perinatal life. *Diabetologia* 49:1117–19
10. Benyshek DC, Martin JF, Johnston CS. 2001. A reconsideration of the origins of the type 2 diabetes epidemic among Native Americans and the implications for intervention policy. *Med. Anthropol.* 20:25–64
11. Bertram C, Khan O, Ohri S, Phillips DI, Matthews SG, Hanson MA. 2008. Transgenerational effects of prenatal nutrient restriction on cardiovascular and hypothalamic-pituitary-adrenal function. *J. Physiol.* 586:2217–29

12. Bertram C, Trowern AR, Copin N, Jackson AA, Whorwood CB. 2001. The maternal diet during pregnancy programs altered expression of the glucocorticoid receptor and type 2 11beta-hydroxysteroid dehydrogenase: potential molecular mechanisms underlying the programming of hypertension in utero. *Endocrinology* 142:2841–53
13. Bertram CE, Hanson MA. 2001. Animal models and programming of the metabolic syndrome. *Br. Med. Bull.* 60:103–21
14. Bhattacharya SK, Ramchandani S, Cervoni N, Szyf M. 1999. A mammalian protein with specific demethylase activity for mCpG DNA. *Nature* 397:579–83
15. Bird A. 2002. DNA methylation patterns and epigenetic memory. *Genes Dev.* 16:6–21
16. Bogdarina I, Murphy HC, Burns SP, Clark AJ. 2004. Investigation of the role of epigenetic modification of the rat glucokinase gene in fetal programming. *Life Sci.* 74:1407–15
17. Bogdarina I, Welham S, King PJ, Burns SP, Clark AJ. 2007. Epigenetic modification of the renin-angiotensin system in the fetal programming of hypertension. *Circ. Res.* 100:520–26
18. Brandeis M, Kafri T, Ariel M, Chaillet JR, McCarrey J, et al. 1993. The ontogeny of allele-specific methylation associated with imprinted genes in the mouse. *EMBO J.* 12:3669–77
19. Brawley L, Torrens C, Anthony FW, Itoh S, Wheeler T, et al. 2004. Glycine rectifies vascular dysfunction induced by dietary protein imbalance during pregnancy. *J. Physiol.* 554:497–504
20. Brennan KA, Gopalakrishnan GS, Kurlak L, Rhind SM, Kyle CE, et al. 2005. Impact of maternal undernutrition and fetal number on glucocorticoid, growth hormone and insulin-like growth factor receptor mRNA abundance in the ovine fetal kidney. *Reproduction* 129:151–59
21. Burdge GC, Hanson MA, Slater-Jefferies JL, Lillycrop KA. 2007. Epigenetic regulation of transcription: a mechanism for inducing variations in phenotype (fetal programming) by differences in nutrition during early life? *Br. J. Nutr.* 97:1036–46
22. Burdge GC, Lillycrop KA, Jackson AA. 2009. Nutrition in early life, and risk of cancer and metabolic disease: alternative endings in an epigenetic tale? *Br. J. Nutr.* 101:619–30
23. Burdge GC, Lillycrop KA, Jackson AA, Gluckman PD, Hanson MA. 2008. The nature of the growth pattern and of the metabolic response to fasting in the rat are dependent upon the dietary protein and folic acid intakes of their pregnant dams and post-weaning fat consumption. *Br. J. Nutr.* 99:540–49
24. Burdge GC, Lillycrop KA, Phillips ES, Slater-Jefferies JL, Jackson AA, Hanson MA. 2009. Folic acid supplementation during the juvenile-pubertal period in rats modifies the phenotype and epigenotype induced by prenatal nutrition. *J. Nutr.* 139:1054–60
25. Burdge GC, Phillips ES, Dunn RL, Jackson AA, Lillycrop KA. 2004. Effect of reduced maternal protein consumption during pregnancy in the rat on plasma lipid concentrations and expression of peroxisomal proliferator–activated receptors in the liver and adipose tissue of the offspring. *Nutr. Res.* 24:639–46
26. Burdge GC, Slater-Jefferies J, Torrens C, Phillips ES, Hanson MA, Lillycrop KA. 2007. Dietary protein restriction of pregnant rats in the F0 generation induces altered methylation of hepatic gene promoters in the adult male offspring in the F1 and F2 generations. *Br. J. Nutr.* 97:435–39
27. Calder PC, Yaqoob P. 2000. The level of protein and type of fat in the diet of pregnant rats both affect lymphocyte function in the offspring. *Nutr. Res.* 20:995–1005
28. Carbone L, Harris RA, Vessere GM, Mootnick AR, Humphray S, et al. 2009. Evolutionary breakpoints in the gibbon suggest association between cytosine methylation and karyotype evolution. *PLoS Genet.* 5:e1000538
29. Catalano PM, Kirwan JP. 2001. Maternal factors that determine neonatal size and body fat. *Curr. Diab. Rep.* 1:71–77
30. Davies MJ. 2006. Evidence for effects of weight on reproduction in women. *Reprod. Biomed. Online* 12:552–61
31. de Assis S, Khan G, Hilakivi-Clarke L. 2006. High birth weight increases mammary tumorigenesis in rats. *Int. J. Cancer* 119:1537–46
32. Delaval K, Feil R. 2004. Epigenetic regulation of mammalian genomic imprinting. *Curr. Opin. Genet. Dev.* 14:188–95
33. Dolinoy DC, Das R, Weidman JR, Jirtle RL. 2007. Metastable epialleles, imprinting, and the fetal origins of adult diseases. *Pediatr. Res.* 61:30–37R

34. Dolinoy DC, Huang D, Jirtle RL. 2007. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. *Proc. Natl. Acad. Sci. USA* 104:13056–61
35. Drake AJ, Walker BR, Seckl JR. 2005. Intergenerational consequences of fetal programming by in utero exposure to glucocorticoids in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288:R34–38
36. Druker R, Bruxner TJ, Lehrbach NJ, Whitelaw E. 2004. Complex patterns of transcription at the insertion site of a retrotransposon in the mouse. *Nucleic Acids Res.* 32:5800–8
37. Duhl DMJ, Vrieling H, Miller KA, Wolff GL, Barsh GS. 1994. Neomorphic agouti mutations in obese yellow mice. *Nat. Genet.* 8:59–65
38. Ehrich M, Nelson MR, Stanssens P, Zabeau M, Liloglou T, et al. 2005. Quantitative high-throughput analysis of DNA methylation patterns by base-specific cleavage and mass spectrometry. *Proc. Natl. Acad. Sci. USA* 102:15785–90
39. Eriksson JG, Lindi V, Uusitupa M, Forsen TJ, Laakso M, et al. 2002. The effects of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma 2 gene on insulin sensitivity and insulin metabolism interact with size at birth. *Diabetes* 51:2321–24
40. Fernandez-Twinn DS, Ekizoglou S, Gusterson BA, Luan J, Ozanne SE. 2007. Compensatory mammary growth following protein restriction during pregnancy and lactation increases early-onset mammary tumor incidence in rats. *Carcinogenesis* 28:545–52
41. Fernandez-Twinn DS, Wayman A, Ekizoglou S, Martin MS, Hales CN, Ozanne SE. 2005. Maternal protein restriction leads to hyperinsulinemia and reduced insulin-signaling protein expression in 21-mo-old female rat offspring. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288:R368–73
42. Fuks F, Burgers WA, Brehm A, Hughes-Davies L, Kouzarides T. 2000. DNA methyltransferase Dnmt1 associates with histone deacetylase activity. *Nat. Genet.* 24:88–91
43. Fuks F, Hurd PJ, Wolf D, Nan X, Bird AP, Kouzarides T. 2003. The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. *J. Biol. Chem.* 278:4035–40
44. Gheorghe CP, Goyal R, Holweger JD, Longo LD. 2009. Placental gene expression responses to maternal protein restriction in the mouse. *Placenta* 30:411–17
45. Ghoshal K, Li X, Datta J, Bai S, Pogribny I, et al. 2006. A folate- and methyl-deficient diet alters the expression of DNA methyltransferases and methyl CpG binding proteins involved in epigenetic gene silencing in livers of F344 rats. *J. Nutr.* 136:1522–27
46. Gidekel S, Bergman Y. 2002. A unique developmental pattern of Oct-3/4 DNA methylation is controlled by a cis-demodification element. *J. Biol. Chem.* 277:34521–30
47. Gluckman PD, Hanson MA. 2004. Living with the past: evolution, development, and patterns of disease. *Science* 305:1733–36
48. Gluckman PD, Hanson MA. 2005. *The Fetal Matrix: Evolution, Developmental and Disease*. London: Cambridge Univ. Press
49. Gluckman PD, Hanson MA. 2006. Changing times: the evolution of puberty. *Mol. Cell. Endocrinol.* 254–255:26–31
50. Gluckman PD, Hanson MA, Beedle AS. 2007. Non-genomic transgenerational inheritance of disease risk. *Bioessays* 29:145–54
51. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. 2008. Effect of in utero and early-life conditions on adult health and disease. *N. Engl. J. Med.* 359:61–73
52. Gluckman PD, Lillycrop KA, Vickers MH, Pleasants AB, Phillips ES, et al. 2007. Metabolic plasticity during mammalian development is directionally dependent on early nutritional status. *Proc. Natl. Acad. Sci. USA* 104:12796–800
53. Gnanalingham MG, Mostyn A, Dandrea J, Yakubu DP, Symonds ME, Stephenson T. 2005. Ontogeny and nutritional programming of uncoupling protein-2 and glucocorticoid receptor mRNA in the ovine lung. *J. Physiol.* 565:159–69
54. Godfrey KM, Barker DJ. 1995. Maternal nutrition in relation to fetal and placental growth. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 61:15–22
55. Godfrey KM, Barker DJ. 2001. Fetal programming and adult health. *Public Health Nutr.* 4:611–24
56. Goto K, Numata M, Komura JI, Ono T, Bestor TH, Kondo H. 1994. Expression of DNA methyltransferase gene in mature and immature neurons as well as proliferating cells in mice. *Differentiation* 56:39–44

57. Grainger RM, Hazard-Leonards RM, Samaha F, Hougan LM, Lesk MR, Thomsen GH. 1983. Is hypomethylation linked to activation of delta-crystallin genes during lens development? *Nature* 306:88–91
58. Guy J, Hendrich B, Holmes M, Martin JE, Bird A. 2001. A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. *Nat. Genet.* 27:322–26
59. Hales CN, Barker DJ. 1992. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 35:595–601
60. Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT, Cairns BR. 2009. Distinctive chromatin in human sperm packages genes for embryo development. *Nature* 460:473–78
61. Harrison M, Langley-Evans SC. 2009. Intergenerational programming of impaired nephrogenesis and hypertension in rats following maternal protein restriction during pregnancy. *Br. J. Nutr.* 101:1020–30
62. Hata K, Okano M, Lei H, Li E. 2002. Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. *Development* 129:1983–93
63. Hawkins P, Steyn C, McGarrigle HH, Saito T, Ozaki T, et al. 2000. Effect of maternal nutrient restriction in early gestation on responses of the hypothalamic-pituitary-adrenal axis to acute isocapnic hypoxaemia in late gestation fetal sheep. *Exp. Physiol.* 85:85–96
64. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, et al. 2008. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc. Natl. Acad. Sci. USA* 105:17046–49
65. Hendrich B, Guy J, Ramsahoye B, Wilson VA, Bird A. 2001. Closely related proteins MBD2 and MBD3 play distinctive but interacting roles in mouse development. *Genes Dev.* 15:710–23
66. Herrick K, Phillips DI, Haselden S, Shiell AW, Campbell-Brown M, Godfrey KM. 2003. Maternal consumption of a high-meat, low-carbohydrate diet in late pregnancy: relation to adult cortisol concentrations in the offspring. *J. Clin. Endocrinol. Metab.* 88:3554–60
67. Hershko AY, Kafri T, Fainsod A, Razin A. 2003. Methylation of HoxA5 and HoxB5 and its relevance to expression during mouse development. *Gene* 302:65–72
68. Hofman PL, Regan F, Jackson WE, Jefferies C, Knight DB, et al. 2004. Premature birth and later insulin resistance. *N. Engl. J. Med.* 351:2179–86
69. Hovi P, Andersson S, Eriksson JG, Jarvenpaa AL, Strang-Karlsson S, et al. 2007. Glucose regulation in young adults with very low birth weight. *N. Engl. J. Med.* 356:2053–63
70. Howell CY, Bestor TH, Ding F, Latham KE, Mertineit C, et al. 2001. Genomic imprinting disrupted by a maternal effect mutation in the Dnmt1 gene. *Cell* 104:829–38
71. Hoyenga KB, Hoyenga KT. 1982. Gender and energy balance: sex differences in adaptations for feast and famine. *Physiol. Behav.* 28:545–63
72. Jablonka E, Raz G. 2009. Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Q. Rev. Biol.* 84:131–76
73. Jackson AA, Dunn RL, Marchand MC, Langley-Evans SC. 2002. Increased systolic blood pressure in rats induced by a maternal low-protein diet is reversed by dietary supplementation with glycine. *Clin. Sci. (Lond.)* 103:633–39
74. Jaenisch R, Bird A. 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat. Genet.* 33(Suppl.):245–54
75. Jost JP. 1993. Nuclear extracts of chicken embryos promote an active demethylation of DNA by excision repair of 5-methyldeoxycytidine. *Proc. Natl. Acad. Sci. USA* 90:4684–88
76. Kind KL, Clifton PM, Katsman AI, Tsiounis M, Robinson JS, Owens JA. 1999. Restricted fetal growth and the response to dietary cholesterol in the guinea pig. *Am. J. Physiol.* 277:R1675–82
77. Ko YG, Nishino K, Hattori N, Arai Y, Tanaka S, Shiota K. 2005. Stage-by-stage change in DNA methylation status of Dnmt1 locus during mouse early development. *J. Biol. Chem.* 280:9627–34
78. Kucharski R, Maleszka J, Foret S, Maleszka R. 2008. Nutritional control of reproductive status in honeybees via DNA methylation. *Science* 319:1827–30
79. Kwong WY, Wild AE, Roberts P, Willis AC, Fleming TP. 2000. Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. *Development* 127:4195–202
80. Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T. 2001. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* 410:116–20

81. Laforsch C, Tollrian R. 2004. Embryological aspects of inducible morphological defenses in *Daphnia*. *J. Morphol.* 262:701–7
82. Lane N, Dean W, Erhardt S, Hajkova P, Surani A, et al. 2003. Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. *Genesis* 35:88–93
83. Langley SC, Jackson AA. 1994. Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. *Clin. Sci. (Lond.)* 86:217–22
84. Langley-Evans SC. 1997. Hypertension induced by foetal exposure to a maternal low-protein diet, in the rat, is prevented by pharmacological blockade of maternal glucocorticoid synthesis. *J. Hypertens.* 15:537–44
85. Langley-Evans SC, Lilley C, McMullen S. 2006. Maternal protein restriction and fetal growth: lack of evidence of a role for homocysteine in fetal programming. *Br. J. Nutr.* 96:578–86
86. Langley-Evans SC, Phillips GJ, Benediktsson R, Gardner DS, Edwards CR, et al. 1996. Protein intake in pregnancy, placental glucocorticoid metabolism and the programming of hypertension in the rat. *Placenta* 17:169–72
87. Langley-Evans SC, Sculley DV. 2005. Programming of hepatic antioxidant capacity and oxidative injury in the ageing rat. *Mech. Ageing Dev.* 126:804–12
88. Lee TM, Zucker I. 1988. Vole infant development is influenced perinatally by maternal photoperiodic history. *Am. J. Physiol.* 255:R831–38
89. Li E. 2002. Chromatin modification and epigenetic reprogramming in mammalian development. *Nat. Rev. Genet.* 3:662–73
90. Li E, Bestor TH, Jaenisch R. 1992. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* 69:915–26
91. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC. 2006. Dietary protein restriction in the pregnant rat induces altered epigenetic regulation of the glucocorticoid receptor and peroxisomal proliferator-activated receptor alpha in the heart of the offspring which is prevented by folic acid. *Proc. Nutr. Soc.* 65:65A
92. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC. 2005. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J. Nutr.* 135:1382–86
93. Lillycrop KA, Phillips ES, Torrens C, Hanson MA, Jackson AA, Burdge GC. 2008. Feeding pregnant rats a protein-restricted diet persistently alters the methylation of specific cytosines in the hepatic PPARalpha promoter of the offspring. *Br. J. Nutr.* 100:278–82
94. Lillycrop KA, Rodford J, Garratt ES, Slater-Jefferies JL, Godfrey KM, et al. 2010. Maternal diet during pregnancy induces specific changes in the hepatic transcriptome of the adult male offspring. *Br. J. Nutr.* doi: 10.1017/S0007114509993795
95. Lillycrop KA, Slater-Jefferies JL, Hanson MA, Godfrey KM, Jackson AA, Burdge GC. 2007. 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.* 97:1064–73
96. Litt MD, Simpson M, Gaszner M, Allis CD, Felsenfeld G. 2001. Correlation between histone lysine methylation and developmental changes at the chicken beta-globin locus. *Science* 293:2453–55
97. Lucarelli M, Fuso A, Strom R, Scarpa S. 2001. The dynamics of myogenin site-specific demethylation is strongly correlated with its expression and with muscle differentiation. *J. Biol. Chem.* 276:7500–6
98. Lucas A, Baker BA, Desai M, Hales CN. 1996. Nutrition in pregnant or lactating rats programs lipid metabolism in the offspring. *Br. J. Nutr.* 76:605–12
99. Lucock M. 2000. Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. *Mol. Genet. Metab.* 71:121–38
100. Maher B. 2008. Personal genomes: the case of the missing heritability. *Nature* 456:18–21
101. Maleszka R. 2008. Epigenetic integration of environmental and genomic signals in honey bees. *Epigenetics* 3:188–92
102. Maloney CA, Gosby AK, Phuyal JL, Denyer GS, Bryson JM, Caterson ID. 2003. Site-specific changes in the expression of fat-partitioning genes in weanling rats exposed to a low-protein diet in utero. *Obes. Res.* 11:461–68

103. Martin JF, Johnston CS, Han CT, Benyshek DC. 2000. Nutritional origins of insulin resistance: a rat model for diabetes-prone human populations. *J. Nutr.* 130:741–44
104. Mayer W, Niveleau A, Walter J, Fundele R, Haaf T. 2000. Demethylation of the zygotic paternal genome. *Nature* 403:501–2
105. McGowan PO, Sasaki A, Huang TC, Unterberger A, Suderman M, et al. 2008. Promoter-wide hypermethylation of the ribosomal RNA gene promoter in the suicide brain. *PLoS ONE* 3:e2085
106. Milutinovic S, Zhuang Q, Niveleau A, Szyf M. 2003. Epigenomic stress response. Knockdown of DNA methyltransferase 1 triggers an intra-S-phase arrest of DNA replication and induction of stress response genes. *J. Biol. Chem.* 278:14985–95
107. Morison IM, Paton CJ, Cleverley SD. 2001. The imprinted gene and parent-of-origin effect database. *Nucleic Acids Res.* 29:275–76
108. Nakayama J, Rice JC, Strahl BD, Allis CD, Grewal SI. 2001. Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science* 292:110–13
109. Okano M, Bell DW, Haber DA, Li E. 1999. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 99:247–57
110. Osmond C, Barker DJ, Winter PD, Fall CH, Simmonds SJ. 1993. Early growth and death from cardiovascular disease in women. *BMJ* 307:1519–24
111. Oswald J, Engemann S, Lane N, Mayer W, Olek A, et al. 2000. Active demethylation of the paternal genome in the mouse zygote. *Curr. Biol.* 10:475–78
112. Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, et al. 2006. Sex-specific, male-line transgenerational responses in humans. *Eur. J. Hum. Genet.* 14:159–66
113. Pener MP, Yerushalmi Y. 1998. The physiology of locust phase polymorphism: an update. *J. Insect Physiol.* 44:365–77
114. Petrie L, Duthie SJ, Rees WD, McConnell JM. 2002. Serum concentrations of homocysteine are elevated during early pregnancy in rodent models of fetal programming. *Br. J. Nutr.* 88:471–77
115. Reik W, Maher ER. 1997. Imprinting in clusters: lessons from Beckwith-Wiedemann syndrome. *Trends Genet.* 13:330–34
116. Reik W, Walter J. 2001. Genomic imprinting: parental influence on the genome. *Nat. Rev. Genet.* 2:21–32
117. Relethford JH. 2008. Genetic evidence and the modern human origins debate. *Heredity* 100:555–63
118. Rhee I, Jair KW, Yen RWC, Lengauer C, Herman JG, et al. 2000. CpG methylation is maintained in human cancer cells lacking DNMT1. *Nature* 404:1003–7
119. Roseboom T, de RS, Painter R. 2006. The Dutch famine and its long-term consequences for adult health. *Early Hum. Dev.* 82:485–91
120. Rountree MR, Bachman KE, Baylin SB. 2000. DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. *Nat. Genet.* 25:269–77
121. Sanders VM. 2006. Epigenetic regulation of Th1 and Th2 cell development. *Brain Behav. Immun.* 20:317–24
122. Schneider JE. 2004. Energy balance and reproduction. *Physiol. Behav.* 81:289–317
123. Shanley DP, Kirkwood TB. 2000. Calorie restriction and aging: a life-history analysis. *Evolution* 54:740–50
124. Shiell AW, Campbell-Brown M, Haselden S, Robinson S, Godfrey KM, Barker DJ. 2001. High-meat, low-carbohydrate diet in pregnancy: relation to adult blood pressure in the offspring. *Hypertension* 38:1282–88
125. Skinner MK. 2008. What is an epigenetic transgenerational phenotype? F3 or F2. *Reprod. Toxicol.* 25:2–6
126. Stein AD, Lumey LH. 2000. The relationship between maternal and offspring birth weights after maternal prenatal famine exposure: the Dutch Famine Birth Cohort Study. *Hum. Biol.* 72:641–54
127. Strahl BD, Ohba R, Cook RG, Allis CD. 1999. Methylation of histone H3 at lysine 4 is highly conserved and correlates with transcriptionally active nuclei in *Tetrahymena*. *Proc. Natl. Acad. Sci. USA* 96:14967–72
128. Suetake I, Shi LH, Watanabe D, Nakamura M, Tajima S. 2001. Proliferation stage-dependent expression of DNA methyltransferase (Dnmt1) in mouse small intestine. *Cell Struct. Funct.* 26:79–86
129. Szyf M. 2007. The dynamic epigenome and its implications in toxicology. *Toxicol. Sci.* 100:7–23
130. Temple IK. 2007. Imprinting in human disease with special reference to transient neonatal diabetes and Beckwith-Wiedemann syndrome. *Endocr. Dev.* 12:113–23

131. Terzolo M, Allasino B, Bosio S, Brusa E, Daffara F, et al. 2004. Hyperhomocysteinemia in patients with Cushing's syndrome. *J. Clin. Endocrinol. Metab.* 89:3745–51
132. Timmermans S, Jaddoe VW, Hofman A, Steegers-Theunissen RP, Steegers EA. 2009. Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. *Br. J. Nutr.* 102:777–85
133. Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, et al. 2009. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum. Mol. Genet.* 18:4046-53
134. Tollefsbol TO, Andrews LG. 2001. Mechanisms for telomerase gene control in aging cells and tumorigenesis. *Med. Hypotheses* 56:630–37
135. Torrens C, Brawley L, Anthony FW, Dance CS, Dunn R, et al. 2006. Folate supplementation during pregnancy improves offspring cardiovascular dysfunction induced by protein restriction. *Hypertension* 47:982–87
136. Torrens C, Poston L, Hanson MA. 2008. Transmission of raised blood pressure and endothelial dysfunction to the F2 generation induced by maternal protein restriction in the F0, in the absence of dietary challenge in the F1 generation. *Br. J. Nutr.* 100:760–66
137. Turner BM. 2000. Histone acetylation and an epigenetic code. *Bioessays* 22:836–45
138. Tycko B, Morison IM. 2002. Physiological functions of imprinted genes. *J. Cell Physiol.* 192:245–58
139. Valle A, Guevara R, Garcia-Palmer FJ, Roca P, Oliver J. 2007. Sexual dimorphism in liver mitochondrial oxidative capacity is conserved under caloric restriction conditions. *Am. J. Physiol. Cell. Physiol.* 293:C1302–8
140. Vasicek TJ, Zeng L, Guan XJ, Zhang T, Costantini F, Tilghman SM. 1997. Two dominant mutations in the mouse Fused gene are the result of transposon insertions. *Genetics* 147:777–86
141. Vickers MH, Breier BH, Cutfield WS, Hofman PL, Gluckman PD. 2000. Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. *Am. J. Physiol. Endocrinol. Metab.* 279:E83–87
142. Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, et al. 2006. The Polycomb group protein EZH2 directly controls DNA methylation. *Nature* 439:871–74
143. Waddington CH. 1942. Canalization of development and the inheritance of acquired characters. *Nature* 150:563–65
144. Waddington CH. 1959. Canalization of development and genetic assimilation of acquired characters. *Nature* 183:1654–55
145. Wang SC, Oelze B, Schumacher A. 2008. Age-specific epigenetic drift in late-onset Alzheimer's disease. *PLoS ONE* 3:e2698
146. Waterland RA, Dolinoy DC, Lin JR, Smith CA, Shi X, Tahiliani KG. 2006. Maternal methyl supplements increase offspring DNA methylation at axin fused. *Genesis* 44:401–6
147. Waterland RA, Jirtle RL. 2003. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol. Cell Biol.* 23:5293–300
148. Waterland RA, Travisano M, Tahiliani KG, Rached MT, Mirza S. 2008. Methyl donor supplementation prevents transgenerational amplification of obesity. *Int. J. Obes. (Lond.)* 32:1373–79
149. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, et al. 2004. Epigenetic programming by maternal behavior. *Nat. Neurosci.* 7:847–54
150. Weinkove C, Weinkove EA, Pimstone BL. 1976. Glucose tolerance and insulin release in malnourished rats. *Clin. Sci. Mol. Med.* 50:153–63
151. West-Eberhard MJ. 1986. Alternative adaptations, speciation, and phylogeny (a review). *Proc. Natl. Acad. Sci. USA* 83:1388–92
152. Whorwood CB, Firth KM, Budge H, Symonds ME. 2001. Maternal undernutrition during early to midgestation programs tissue-specific alterations in the expression of the glucocorticoid receptor, 11beta-hydroxysteroid dehydrogenase isoforms, and type 1 angiotensin II receptor in neonatal sheep. *Endocrinology* 142:2854–64
153. Widdowson EM. 1976. The response of the sexes to nutritional stress. *Proc. Nutr. Soc.* 35:175–80
154. Winick M, Noble A. 1966. Cellular response in rats during malnutrition at various ages. *J. Nutr.* 89:300–6
155. Wolff GL, Kodell RL, Moore SR, Cooney CA. 1998. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *FASEB J.* 12:949–57

156. Woodall SM, Johnston BM, Breier BH, Gluckman PD. 1996. Chronic maternal undernutrition in the rat leads to delayed postnatal growth and elevated blood pressure of offspring. *Pediatr. Res.* 40:438–43
157. Xie H, Wang M, Bonaldo MD, Smith C, Rajaram V, et al. 2009. High-throughput sequence-based epigenomic analysis of Alu repeats in human cerebellum. *Nucleic Acids Res.* 37:4331–40
158. Yoder JA, Soman NS, Verdine GL, Bestor TH. 1997. DNA (cytosine-5)-methyltransferases in mouse cells and tissues. Studies with a mechanism-based probe. *J. Mol. Biol.* 270:385–95
159. Zambrano E, Martinez-Samayoa PM, Bautista CJ, Deas M, Guillen L, et al. 2005. Sex differences in transgenerational alterations of growth and metabolism in progeny (F-2) of female offspring (F-1) of rats fed a low protein diet during pregnancy and lactation. *J. Physiol. (Lond.)* 566:225–36
160. Zegerman P, Canas B, Pappin D, Kouzarides T. 2002. Histone H3 lysine 4 methylation disrupts binding of nucleosome remodeling and deacetylase (NuRD) repressor complex. *J. Biol. Chem.* 277:11621–24
161. Zhu B, Zheng Y, Angliker H, Schwarz S, Thiry S, et al. 2000. 5-Methylcytosine DNA glycosylase activity is also present in the human MBD4 (G/T mismatch glycosylase) and in a related avian sequence. *Nucleic Acids Res.* 28:4157–65

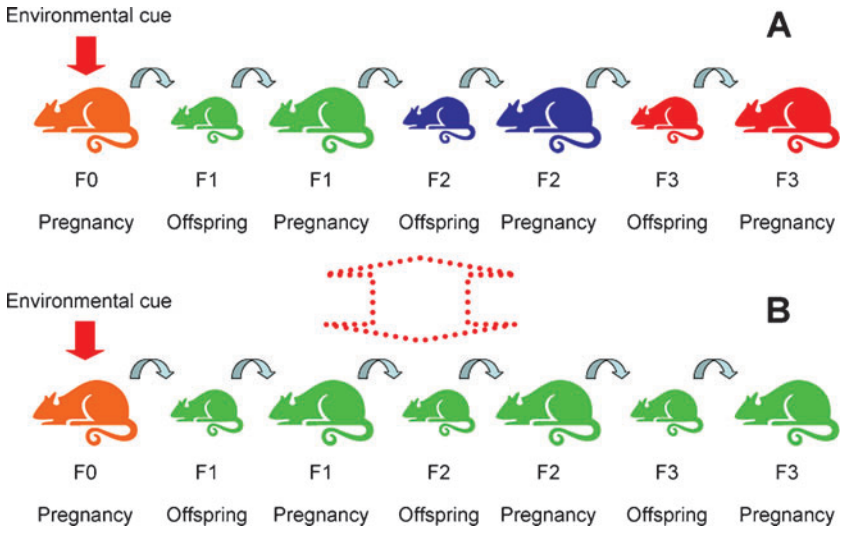

Figure 1

Possible mechanisms for transmission of induced phenotypes between generations through the female line. F0 Dams are exposed during pregnancy to an environmental cue, such as a change in food availability or stress, that induces changes in the phenotype and epigenotype of the F1 female offspring. In scenario *A*, the altered phenotype induced in F1 offspring leads to changes in their physiological adaptations to pregnancy that, in turn, induce a phenotype in F2 offspring that differs from that of the F1 offspring. Thus, a series of phenotypes is generated over successive generations, each of which differs from the previous generation (indicated by the changing colors of each generation). In this model, the underlying epigenotype is not transmitted between generations. In scenario *B*, the epigenotype induced in the F1 offspring is transmitted between generations and so produces a stable phenotype. This process is implied by the findings described in (26, 136).

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Errata

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