Nutrition and Developmental Biology—Implications for Public Health

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Recent advances in understanding genome-nutrient and nutrient-network interactions, and the modifying effects of genetic variation on their function, have strengthened interests in acute and long-lasting diet/nutrition influences on health. Relationships between early and mid-gestational and perinatal conditions (including those related to maternal nutrition) and outcomes, and later-onset chronic diseases have received particular attention. Controlled animal experiments support views that responses with long-lasting effects to nutritional milieus are enabled by epigenetic and other metabolic adjustments during critical windows. Thus, underlying mechanisms are beginning to be understood. For example, chromatin remodeling during development can alter gene expression levels, fix or determine future set points critical to intra- and inter-organ communication networks, alter morphogenesis, initiate remodeling events, etc., all with lifelong consequences. These also may affect DNA mutation rates and thereby influence adult cancer and other risks. There is increasing evidence that nutrient-based strategies will be of value to the prevention or delay of onset of chronic diseases and that these strategies may require initiation during embryonic or fetal stages of development to achieve maximal benefit.

Key words: developmental biology, gene-nutrient interactions, public health

INTRODUCTION

Mammalian development proceeds because of orchestrated parallel, overlapping, and/or sequential programs that are coordinated through interactive gene, signaling, and metabolic networks. Network outputs direct responses that are critical for development. These include cell migration, anatomical and metabolic differentiation, apoptosis, and maintenance of stem cell populations. The availability of the human genome sequence and sequences of model organisms provides the equivalent of comprehensive blueprints and parts lists that describe dynamic networks and the bases for understanding their responses to external and internal perturbations. These sequences also enable an appreciation of the plasticity of genome-nutrient interactions.

Nutrients, like pharmaceuticals, are powerful modifiers of network function and stability. Salient examples of this are provided by the knowledge that attention to maternal and perinatal nutrition improves birth outcomes, including cognitive development (e.g., ensuring iodine sufficiency), lifelong chronic disease resistance (e.g., preventing small-for-gestational age births), and increased longevity (e.g., optimizing dietary fat consumption). It also is apparent that inappropriate uses of nutrition to maximize reproductive outcomes will present new potential risks. Exploiting roles such as these for benefit, however, is restricted severely by the limited understanding of nutrient-related network characteristics and functions, including their robustness, regulation, and interaction. This is a prerequisite for the rational design of strategies to manipulate cell functions and/or cell fates for benefit. Incomplete knowledge of network function also restricts abilities to predict quantitative or qualitative relationships between specific health outcomes and diverse patterns and levels of nutrient intakes in genetically diverse individuals and populations.

Network Characteristics

Metabolic, transcriptional, and cell signaling networks can be classified as hardwired, environmentally responsive, and/or environmentally adaptive. Hardwired networks function blindly, with no mechanism to sense environmental changes. Other networks have dynamic features, and as such they are environmentally respon-
sive and therefore require periodic nutritional, metabolic, or hormonal inputs that serve as signals for sustaining homeostasis. Nutrients can direct “yes-no” or non-continuous types of responses (also referred to as “checkpoints”), or they can be key components of feedback loops that permit or prevent system outputs or cellular responses to the index input(s). Thus, response magnitudes can be dose sensitive or exhibit threshold characteristics, and typically are transient. Still other networks are adaptive and potentially learning in character; that is, they sense their environments and reorganize, either transiently and/or permanently, to adjust flux or signal strength, thus sustaining optimal function within environmental contexts.

Additionally, cellular networks likely vary in their “robustness,” a term that describes a system’s insensitivity to internal or external fluctuations and is an inherent property of networks. Robustness can be achieved through network wiring or architecture and/or through network dynamics (i.e., interactions among a defined set of network components). Network wiring that includes structural redundancy or degeneracy provides multiple or alternative pathways, and such architectures avoid the need for dynamic responses to achieve robustness. Fully redundant pathways that exist without any independent function are predicted to be evolutionarily unstable unless the redundancy is required to maintain minimal flux through the network. The same may apply to degenerative networks.

Plasticity of the Genome-Nutrient Interaction: Lessons from Yeast

Gene deletion studies indicate that 80% of yeast genes are nonessential for survival under laboratory conditions, observations that seem inconsistent with the conservation of gene sequences and protein function through evolution. A recent examination of yeast metabolic networks using an in silico model revealed that culture conditions, especially the use of nutrient-rich culture media, can compensate for the disruption of 37% to 68% of the organism’s genes. Only 18% of dispensable genes are compensated for by gene duplicates, whereas other types of network buffering or robustness accounted for 4% to 17% of apparently “dispensable” genes. In microbial systems, the maintenance of enzymatic flux under highly diverse environmental conditions appears to be a primary selective pressure that maintains gene sequence, with starvation being among the most common environmental stresses.

The relative essentiality of specific genes determined by culture conditions represents one important dimension of robustness. In the absence of rich culture conditions, robustness is conserved throughout evolution because of genomic changes that alter transcript dosage and/or through conservation of redundancy or degeneracy that are essential for survival. Laboratory conditions can be designed to effectively relax selective pressures by supplying end products through external sources. Thus, high-nutrient broths can relax dispensable gene selection, which favors resilience under less-permissive conditions. These and other studies of yeast biological networks reveal several key paradigms that must be validated in mammalian systems. If valid, they are of exceptional salience to human modernity because of the unprecedented degree to which we can manipulate our nutritional environments. These paradigms include: 1) nutrient-rich, controlled environments can functionally compensate for a substantial proportion of genes; 2) network dynamics, reorganization, and survival are attained primarily through environmentally driven changes in transcript dosage (gene duplication and adaptive alterations in gene expression) and metabolite regulatory mechanisms (feedback loops), many of which are highly conserved; and 3) redundancy and degeneracy contribute to network robustness, but the primary contributions come from environmental and internal sensing mechanisms that enable compensation through network dynamics.

NUTRIENT-GENOME INTERACTIONS IN HUMAN DEVELOPMENT

Genomic Evolution: Diet as a Selective Pressure on Genome Primary Sequence

Nuclear-encoded developmental programs evolve in the context of their environments, and only genomes that adapt to the environment survive and expand in populations. The human genome is optimized for survival in the maternal environment and for postnatal reproductive success. Genotypes that confer advantage during one life stage do not necessarily confer advantage in subsequent life stages when selective pressures change dramatically (e.g., during the transition from the maternal to the external environment or from reproductive to non-reproductive life stages).

Gestational Pressures On Genome Survival—Interactions Among Genetics And Maternal Nutrition

Genotypes that cannot support basic physiological processes in the embryonic and fetal stages usually are eliminated. This is achieved in primates by spontaneous abortions. Humans may be unique relative to other mammalian species in their high rates of gestational fetal loss; the high rates of spontaneous abortion may be a unique selective pressure that accelerates the expansion
of polymorphic alleles within human populations. Approximately 75% of human conceptions are lost “spontaneously” before term; 80% of spontaneous abortions occur within the first trimester.\textsuperscript{12-14} Half of spontaneous abortions occur before the first 3 weeks of gestation, and generally are unnoticed; many embryos fail to implant.\textsuperscript{15} Spontaneous abortion may have evolved to limit defective offspring in human populations, but no molecular mechanisms of spontaneous abortion have been elucidated.\textsuperscript{12}

Fertility declines and risks of spontaneous abortion increase in women over the age of 30 years.\textsuperscript{16,17} Although specific etiologies of spontaneous abortion are usually unknown, many spontaneously aborted fetuses have structural and/or genetic anomalies.\textsuperscript{12,18} The etiologies of most spontaneous abortions are likely multifactorial. Potential inducers of spontaneous abortion include maternal immune responses; fetal genotypes; maternal and/or fetal endocrine, nutritional, or hormonal imbalances; maternal and/or fetal infection; and endometriosis (Table 1).\textsuperscript{16} Although few specific environmental risk factors are identified, low maternal folate status, diabetes (type 1), and elevated homocysteine (which most often results secondarily to primary or conditioned folate deficiency) are associated with spontaneous abortion.\textsuperscript{19,20-25} For example, variant alleles of \textit{MTHFR} (A222V)\textsuperscript{21,22,26} and \textit{TC} (776G)\textsuperscript{22} impair the metabolism of folic acid and homocysteine and are independent risk factors for spontaneous abortion. Their effect on spontaneous abortion risk is cumulative and may have direct effects on folate “requirements.”\textsuperscript{2,24,27-29}

Table 1. Maternal Risk-Genotypes and Reproductive Outcomes

<table>
<thead>
<tr>
<th>Gene Variant</th>
<th>Pathway</th>
<th>Fetal Risk</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{MTHFR} V222A</td>
<td>One-carbon metabolism</td>
<td>SA NTD Down’s syndrome Adult cardiovascular disease</td>
<td>Bailey and Gregory, 1999\textsuperscript{108}</td>
</tr>
<tr>
<td>\textit{MTFD1}</td>
<td>One-carbon metabolism</td>
<td>NTD</td>
<td>Brody et al., 2002\textsuperscript{27}</td>
</tr>
<tr>
<td>\textit{TC} (transcobalamin)</td>
<td>Vitamin B\textsubscript{12}/one-carbon metabolism</td>
<td>SA NTD</td>
<td>Zetterberg, 2004\textsuperscript{20}</td>
</tr>
<tr>
<td>\textit{IL6} (−174 G → C)</td>
<td>Cytokine</td>
<td>SA</td>
<td>von Linsingen et al., 2005\textsuperscript{109}</td>
</tr>
<tr>
<td>\textit{IFN-gamma 874 A → T}</td>
<td>Cytokine</td>
<td>SA</td>
<td>Prigoshin et al., 2004\textsuperscript{110} and Daher et al., 2003\textsuperscript{111}</td>
</tr>
<tr>
<td>\textit{IL1RN}*1</td>
<td>Cytokine</td>
<td>SA</td>
<td>Perni et al., 2004\textsuperscript{112}</td>
</tr>
<tr>
<td>\textit{IL1RN}*2</td>
<td>Cytokine</td>
<td>Preterm birth</td>
<td>Perni et al., 2004\textsuperscript{112}</td>
</tr>
<tr>
<td>\textit{CYP17A2}</td>
<td>Steroid biosynthesis</td>
<td>SA</td>
<td>Sata et al., 2003\textsuperscript{113}</td>
</tr>
<tr>
<td>\textit{CYP1A1}*2A</td>
<td>Phase 1 detoxification</td>
<td>SA</td>
<td>Suryanarayana et al., 2004\textsuperscript{114}</td>
</tr>
<tr>
<td>\textit{PR}*2</td>
<td>Progesterone receptor</td>
<td>SA</td>
<td>Schweikert et al., 2004\textsuperscript{115}</td>
</tr>
<tr>
<td>\textit{GSTM1}</td>
<td>Phase 2 detoxification</td>
<td>SA</td>
<td>Sata et al., 2003\textsuperscript{116}</td>
</tr>
<tr>
<td>\textit{Prothrombin G20210A}</td>
<td>Clotting</td>
<td>SA</td>
<td>Finan et al., 2002\textsuperscript{117}</td>
</tr>
<tr>
<td>\textit{Factor V G1691A}</td>
<td>Clotting</td>
<td>SA</td>
<td>Finan et al., 2002\textsuperscript{117}</td>
</tr>
<tr>
<td>\textit{Nos3B}</td>
<td>Vascular function</td>
<td>SA</td>
<td>Tempfer et al., 2001\textsuperscript{118}</td>
</tr>
<tr>
<td>\textit{PGM1}*2</td>
<td>Phosphoglucomutase</td>
<td>SA</td>
<td>Gloria-Bottini et al., 2001\textsuperscript{119}</td>
</tr>
</tbody>
</table>

\textit{NTD} = Neural tube defect; \textit{SA} = spontaneous abortion.
Evolutionary Pressures on Genome Integrity: Survival to Reproduce

Human genetic variation is, in part, the result of ongoing environmental interactions. Rapid environmental shifts, however, can compromise the function of environmentally adaptive genes, and result in their presentation as disease alleles. In specific situations, such reductions in gene function can enhance evolutionary fitness. 

A222V is a polymorphism in the MTHFR gene that confers risk for iron overload disease also displays evidence for selection; the recent expansion of this polymorphism may have conferred advantages in iron-poor regions or resistance to microbial infection. SNPs that prevent efficient ethanol metabolism also display genomic signatures of selection.

The compatibility of a genome with the postnatal external environment does not always predict success in the maternal environment. Although the MTHFR A222V genotype is a risk factor for spontaneous abortion and developmental anomalies, it is highly protective against colon and other cancers in folate-replete adults. The MTHFR A222V variant protein has reduced affinity for riboflavin cofactors and is thermolabile, resulting in reduced cellular MTHFR activity; its stability is increased when folate is bound. Although the biochemical roles of this polymorphism in the etiologies of NTDs and cancer are unknown, the effects of this SNP on the folate network appear to confer advantage against adult colon cancer, but also confer risk for in utero death. The HFE hemochromatosis allele (C282Y), on the other hand, confers risk for iron overload in adults, but is not a risk factor for fetal loss.

Genomic Adaptations to Gestational and Other Environments

The “thrifty gene” hypothesis was introduced over 40 years ago to explain the type 2 diabetes epidemic occurring in non-Western cultures that adopted Western diets and lifestyles. The hypothesis is based on historical records of recurrent famines, and postulates that sparse environments selected for genomes that promoted and enabled efficient conversion of food into energy and fat disposition and efficient adaptations to fasting. Genomes that evolved in more ample food environments putatively resulted in genomes less likely to result in metabolic disease under conditions of plenty. To date, no conclusive “thrifty gene” or selected allelic variants have been verified to support this hypothesis. However, there is increasing evidence that nutritional exposures during critical windows early in life alter genomic responses to environmental exposures, including diet, by reorganizing cellular networks with lifelong consequences. Often referred to as “epigenetic programming,” “metabolic imprinting,” or “early-life programming,” network reorganization may have evolved as a mechanism for fetal survival in nutrient-poor conditions.

Waterland and Garza framed specific criteria for metabolic imprinting as an “adaptive” phenomenon, rather than a toxicological response, that resulted in “permanent” changes during the affected individual’s lifetime: 1) a susceptibility limited to a critical ontogenic window in development, 2) a persistent effect lasting through adulthood, 3) a specific and measurable outcome, and 4) a dose-response or threshold relation between a specific exposure and outcome. Mechanisms for metabolic imprinting are still largely unresolved and appear to be much more complex than those associated with toxic or deficiency states. Whereas many teratogens are foreign agents that disrupt cellular networks, metabolic imprinting was described as a conserved adaptive response that optimizes network function in one life stage through genomic mechanisms resulting in permanent functional characteristics. Such changes, however, may prevent or limit the range of other adaptive mechanisms that are protective in subsequent life stages when environments change (e.g., in a transition from “want” to “surplus”). Thus, once the “imprint” is established, a system’s buffering capacity is limited. The “novelty” of sustained surplus in an evolutionary sense may explain the limited response capability apparently at the core of the present obesity/type 2 diabetes epidemic.

Changes in transcript dosage are a hallmark of network dynamics. Epigenetic events, including metabolic imprinting, programming and/or re-programming of networks, effect either a change in mean responses or phenotypes or variance of the distribution of responses or phenotypes. The learning phase requires sensing of the environment; the network adaptation requires a stable or meta-stable genomic signature that does not alter primary sequence and survives DNA replication and potentially is heritable. Alterations in DNA and histone methylation remain the most likely epigenetic signatures because of their potential connections to metabolic networks, chromatin structure, and transcriptional networks.
Furthermore, DNA and histone methylations are meta-stable, heritable, and alter genome expression and stability. Methylation is a higher-order genomic signal that can override transient metabolic or hormonal signals such as the regulation of transcriptional networks through nuclear receptors (e.g., vitamin A, vitamin D, and steroid hormones).

Example 1: “Canalization”—Epigenetic Masking of Genetic Variation?

Perhaps the first mechanistic insight into metabolic imprinting was described by Conrad Waddington when he coined the term “canalization.” Originally described as the concealment of phenotypic variation in the absence of stress, it was described more recently as phenotype buffering that preserves developmental pathways against the penetrance of genetic polymorphisms and/or environmental challenges. 

Canalization evolves through stabilizing natural selection for robust developmental processes and optimal phenotypes, otherwise referred to as the reduction in the variation of a trait to achieve similar phenotypic outcomes. The prevalence, limits, and functional capacity of canalization are unknown, and the underlying mechanisms that conceal phenotypic variation, enable environmental tolerance, and/or result in their expression are under intense investigation. Canalization appears to enable networks to achieve intermediate optimal equilibrium states. It is an inherent property of, and most effective in, highly connected or degenerate networks that also can be an inherent property of, and most effective in, highly connected or degenerate networks. 

Hsp90 was the first reported canalizer gene candidate. As a chromatin-associated heat-shock protein, Hsp90 interacts with proteins involved in chromatin organization and signal transduction, and stabilizes its interactive proteins by inducing their conformation between functional and inactive forms. Its deletion in Drosophila and Arabidopsis dramatically unmasks pre-existing phenotypic variation that can undergo subsequent selection and expansion in their respective inbred populations. The revealed phenotypic variation is dependent on an underlying genetic capability that pre-existed in recombinant inbred lines. Unmasked traits can be outcrossed with high penetrance to unselected lines, but only in Hsp90 functionally deficient strains of Drosophila. In this manner, Hsp90 expression alters the mean, threshold, and/or variation of potential phenotypes. These and other experimental observations support a role for Hsp90 as a genetic buffer and canalizer. However, other evidence indicates that Hsp90 primarily evolved by enabling environmental adaptations, and its genetic buffering capacity is a by-product of selection through environment. Hsp90 may function by enabling epigenetic changes that buffer against environmental perturbations. Hsp90 effects are, in turn, modifiable by epigenetic mechanisms; for example, histone deacetylase inhibitors reduce the penetrance of Hsp90 deletion on sensitized backgrounds.

Example 2: Folate-Mediated One-Carbon Metabolism—Convergence of Genetic and Epigenetic Pathways through Metabolic Competition

Folate is a B vitamin and metabolic cofactor that carries and activates one-carbon units for the de novo synthesis of purine nucleotides and thymidylate (dTMP), and for the remethylation of homocysteine to methionine, a network known as folate-mediated one-carbon metabolism (Figure 1A). Methionine can then be adenylylated to form S-adenosylmethionine (AdoMet), which is a cofactor for numerous cellular methylation reactions, including histone and DNA methylation. Impairments in this metabolic network by nutritional deficiencies or highly penetrant SNPs increase the risk for pathologies including cancer and cardiovascular disease, and developmental anomalies such as spontaneous abortion and NTDs. Folate supplementation can reduce the risk for these disorders; maximal benefit is achieved in genetically susceptible individuals/populations.

Methionine and dTMP synthesis are the most vulnerable pathways within the network; their impairments compromise the fidelity of DNA synthesis and cellular methylation reactions. AdoMet-dependent methyltransferases, including histone and DNA methyltransferases, are subject to product inhibition by S-adenosylhomocysteine (AdoHcy), which accumulates during folate deficiency (Figure 1B). Therefore, methyltransferases sense the efficiency of the folate metabolic network because their activity is determined by the cellular AdoMet/AdoHcy ratio, otherwise known as the “methylation potential” of the cell. Global genomic methylcytosine content is highly sensitive to the AdoMet/AdoHcy ratio, which can affect both gene expression and DNA stability and mutation rates.

The mechanisms underlying folate-associated pathologies, including NTDs, spontaneous abortions, and cancer, are unknown and assumed to be the result of insufficient flux through the dTMP and/or AdoMet synthesis pathways. Therefore, the etiology involves impairments in genome synthesis (mitotic) rates, genome stability, and/or methylation-sensitive gene expression.

Numerous studies have indicated that folate-dependent dTMP synthesis, catalyzed by thymidylate synthase, and 5-methylTHF synthesis (leading to AdoMet synthesis), catalyzed by methylenetetrahydrofolate reductase (MTHFR), are competitive pathways within the network. They compete for a limiting pool of the cofactor methylenetetrahydrofolate (methyleneTHF) (Figure 1B).
bolic competition is unbalanced by the previously described \textit{MTHFR} A222V polymorphism. This functional SNP reduces \textit{MTHFR} activity and has two effects on the network: 1) it impairs the remethylation of homocysteine to methionine, thereby reducing global DNA methylation and thus also influencing gene expression,\textsuperscript{69,70} and 2) it increases the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP).\textsuperscript{71} This change in the network is associated with an increased risk for spontaneous abortion and NTDs,\textsuperscript{47} but a decreased risk for adult colon cancer,\textsuperscript{47} illustrating that optimal network function or outputs differ between the fetal and adult environments. Identifying the precise mechanism for folate-related pathologies in experimental systems is challenging, because any factor, genetic or environmental, that influences the metabolic competition for methyleneTHF simultaneously will alter the efficiency of both dTMP and AdoMet synthesis (Figure 1B).

Alterations in one-carbon metabolism, and the AdoMet cycle in particular, can have dramatic effects on genome methylation. Both genome-wide and allele-specific DNA methylation are influenced by folate metabolism.\textsuperscript{72,73} DNA hypomethylation induced by folate deficiency has three primary effects on the mammalian genome: 1) it can alter transcription of genes regulated by promoter methylation, including tumor suppressor genes,\textsuperscript{73,74} 2) it influences genome mutation rates, and 3) it enables interchromosomal recombination events through common retroviral repeat sequences whose activity normally is silenced by methylation.\textsuperscript{75} Hyperhomocysteinemia is a clinical state that results from the inability to effectively metabolize homocysteine, and results in the accumulation of cellular AdoHyc. Patients exhibit DNA hypomethylation and a homocysteine-dependent shift from mono-allelic to bi-allelic expression of both sex-linked and imprinted genes, including \textit{H19}. 

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{A, The folate-mediated one-carbon metabolic network in the cytoplasm. Folate-mediated one-carbon metabolism occurs in the mitochondria and cytoplasm. Mitochondrial one-carbon metabolism generates formate from serine. Formate traverses the mitochondrial membrane, where it is condensed with tetrahydrofolate (THF) and incorporated into the purines dTMP and methionine. B, cSHMT is shown to mediate the competition for methyleneTHF by the dTMP and methionine synthesis pathways. \textit{MTHFS}, methenyltetrahydrofolate synthetase; \textit{cSHMT}, cytoplasmic serine hydroxymethyltransferase; \textit{mSHMT}, mitochondrial serine hydroxymethyltransferase; \textit{TS}, thymidylate synthase; \textit{MTHFR}, methylenetetrahydrofolate reductase; \textit{AdoMet}, S-adenosylmethionine; \textit{AdoHyc}, S-adenosylhomocysteine.}
\end{figure}
Folate supplementation in these patients restores homocysteine levels to baseline, reverses global DNA hypomethylation, and restores mono-allelic expression of imprinted genes. Hyperhomocysteinemic patients with bi-allelic expression of H19 displayed negligible expression of the tightly linked IGF2 gene, but expression of IGF2 increases significantly following folate therapy. Interestingly, SNPs in the H19 gene are associated with cord blood IGF-II levels and birth size.

Folate-mediated alterations in genome methylation can be set irreversibly, or “imprinted,” during early development. In the viable yellow agouti (Avy) mouse model, maternal diet determines the coat color of offspring. This mouse strain contains a transposon that integrates into a 5’ exon of the agouti gene, which attracts DNA methylation to that locus. In this model, maternal one-carbon status determines the density of cytosine methylation at the agouti locus and hence the level of agouti gene transcription. The methylation patterns and subsequent effects on coat color and, presumably, associated metabolic characteristics, are maintained throughout the lifetime of experimental animals. However, study of the Axin-fused mouse model failed to show an effect of maternal folate and one-carbon status on promoter and transposon methylation, indicating that not all methylated genomic loci sense metabolic alterations in the one-carbon metabolic network. The identification of transcriptional units that are influenced by alterations in the AdoMet/AdoHyc ratio through DNA or histone methylation, and the critical developmental windows that enable their nutritional imprinting, are essential to elucidating the mechanisms of folate-related pathologies such as developmental anomalies.

**Cytoplasmic Serine Hydroxymethyltransferase as a Nutritional Sensor, Metabolic Switch, and Potential Epigenetic Programmer**

The one-carbon metabolic network is unique in its ability to transform directly metabolic signals into epigenetic signatures that enable adaptation. There is increasing evidence that the one-carbon network serves as a conduit and sensor for many nutrients and metabolic states; identification of the network’s range of sensing capability is a focus of active investigation. One enzyme within the network, cytoplasmic serine hydroxymethyltransferase (cSHMT), has been identified as a nutrient sensor that buffers the metabolic competition between nucleotide biosynthesis and cellular methylation, and thereby influences the rate and fidelity of DNA synthesis and alters the epigenetic state of chromatin by regulating cellular methylation. When expressed, cSHMT has two independent effects on the network: it increases the flux of one-carbons through the dTMP synthesis pathway and it suppresses the AdoMet synthesis pathway by sequs-

**IMPROVING REPRODUCTIVE OUTCOMES THOUGH NUTRITION**

Public health interventions, including micronutrient food fortifications, often have been successful in preventing adverse outcomes associated with maternal nutrient deficiencies or excesses. Successful initiatives include the fortification of grain products with folate to prevent NTDs, the fortification of salt with iodine to prevent cretinism, discouraging alcohol consumption during pregnancy to prevent fetal alcohol syndrome, and the fortification of various vehicles to prevent iron deficiency. These successes encourage further improvements of maternal diets and infant formulas to optimize short- and long-term outcomes and presage the likely acceleration of biomedical approaches for optimizing functional abilities rather than the more limited goals of preventing disease and improving therapeutic approaches. For example, there is evidence that docosahexaenoic acid (DHA) or choline supplementation during prenatal and/or early postnatal life improves central nervous system function and cognitive performance throughout life. Other new challenges include the determination of human embryonic nutritional requirements and toxicities as assisted reproductive technologies be-
come used more widely. Continued progress in these areas requires the identification and understanding of nutritionally modifiable developmental networks that are key to successful adaptations in successive life stages, their associated critical windows, and validation of the efficacy and effectiveness of nutritional interventions. Two examples of challenges that require a better understanding of fetal and embryonic nutrition are described below.

Embryonic Nutrition and Optimal Culture Medium

Some, but not all, studies indicate elevated spontaneous abortion rates in human in vitro fertilization pregnancies compared with natural conception, findings that may be due to harvest and early manipulations of eggs and embryos. Other studies have found that human in vitro fertilization procedures result in higher than expected incidences of intrauterine growth restriction. The composition of embryo culture medium in ovine and other mammalian species affects the expression and methylation status of imprinted genes, including H19, Igf2, and Igf2r, resulting in large offspring syndrome. There appear to be many critical windows associated with the establishment of environmentally sensitive methylation patterns from early embryogenesis through the suckling period that appear to be network and/or allele specific. Some of these networks may be sensitive to one-carbon metabolism. The potential impact of genomic methylation is evident in the viable yellow agouti (A/y) mouse; others may respond to the allelic- or locus- specific targeting of methylase/demethylase activity, as is seen with glucocorticoid receptor function. Differences in network function, the timing of critical windows associated with epigenetic modifications that exist among species, and the realization that early metabolic events result in late-onset adult diseases through epigenetic and other mechanisms illuminate major challenges that are made immediate by the increased demand for assisted reproduction.

Maternal Nutrition and Embryo Rescue

The concept of embryo rescue, or "good diet hides genetic mutations," is supported by numerous examples of nutritional rescue or compensation (viability or phenotype) of gene disruptions through diet in mice and yeast. The shared risk for both developmental anomalies and spontaneous abortions conferred by MTHFR and TC polymorphic allels, and the knowledge that folic acid lowers the risk of developmental anomalies associated with MTHFR SNPs, has led to the suggestion that women predisposed to hyperhomocystinemia might benefit from folate and vitamin B supplementation to reduce their risk of spontaneous abortion. The first study to validate the concept of embryo rescue through nutrition in humans came from southern Spain. This study, and a subsequent follow-up study, indicated that maternal folate supplementation increases the prevalence of the MTHFR A222V allele by rescuing fetuses at risk of spontaneous abortion in this population. This observation has yet to be confirmed in other populations. The long-term consequences of fortification and supplementation interventions that result in maternal nutrient intakes above what can be achieved with healthy, unfortified food-based diets, and their potential rescue of fetuses at risk of spontaneous abortion, may present public health and ethical challenges to communities and the health professionals who serve them.

CONCLUSION

The successes of genomic sequencing efforts have expanded our understanding of the functions associated with individual genes. These successes facilitate similar gains in understanding mechanisms responsive to more complex genetic interactions that are driven by endogenous and/or exogenous influences. Epidemiological, whole-animal, and tissue- and cell-culture studies demonstrate nutrition’s pivotal role as an exogenous determinant of health. Clearly, the responsiveness of epigenetic and other mechanisms to nutritional milieu has short- and long-lasting consequences to current and possibly future generations. The expanded understanding of the diet/health/genomic relationships made possible by these advances support the view that this “responsiveness” can be used to “engineer” healthy and unhealthy phenotypes. How best to attain the former is not completely discernable in large part because of the limited knowledge of the role of most nutrients in genomic regulation in early development and subsequent life stages. Our collective ability to manipulate the composition of global and local food supplies gives great saliency to this and related issues. The wisdom of the adage “you are what you eat” may be much more profound than previously appreciated.

REFERENCES

4. Morohashi M, Winn AE, Borisuk MT, Bolouri H,


