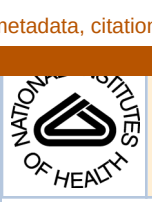

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Metaboloepigenetics: Interrelationships between energy metabolism and epigenetic control of gene expression

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Abstract

Diet and energy metabolism affect gene expression, which influences human health and disease. Here, we discuss the role of epigenetics as a mechanistic link between energy metabolism and control of gene expression. A number of key energy metabolites including SAM, acetyl-CoA, NAD^+, and ATP serve as essential co-factors for many, perhaps most, epigenetic enzymes that regulate DNA methylation, posttranslational histone modifications, and nucleosome position. The relative abundance of these energy metabolites allows a cell to sense its energetic state. And as co-factors, energy metabolites act as rheostats to modulate the activity of epigenetic enzymes and upregulate/downregulate transcription as appropriate to maintain homeostasis.

Keywords

Energy Metabolism; Epigenetics; SAM; CpG Methylation; Acetyl-CoA; Histone Modifications; NAD^+

It is generally accepted that diet and energy metabolism affect gene expression, which, in turn, influences human health and disease. Although our knowledge of how this occurs is still rather limited, there is strong evidence to support the role of epigenetic mechanisms. This is not surprising because many gene-environment interactions converge at the level of the epigenome to regulate gene expression profiles and phenotypic outcomes (Figure 1). And in this context, bioactive food components and nutrients are environmental factors that we are exposed to on a regular basis. In this review, we discuss “metaboloepigenetic” mechanisms that link energy metabolism and epigenetic control of gene expression.

Epigenetics refers to how transcription, DNA replication, and other aspects of genome function are regulated in a manner that is independent of DNA sequence. Individuals who are genetically identical but have different traits such as certain cloned animals (including Cc the cat and her calico coat-color patterns due to X chromosome inactivation) and monozygotic twins (who can be afflicted by different diseases) exemplify the concept of epigenetics. Epigenetics also explains why every cell in our body has the same genome sequence (with the exception of lymphocytes due to VDJ recombination), yet each cell type (e.g., neurons, adipocytes, fibroblasts, intestinal epithelial cells) exhibits distinct expression profiles and morphological/functional characteristics.

Mechanistically, epigenetics involves changes in either DNA methylation [which occurs almost exclusively at CG dinucleotides (or CpG) in mammals] or how the DNA is packaged as chromatin (Figure 2). At the most fundamental level, chromatin consists of 147-bp

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segments of DNA wrapped around histone octamers to form nucleosomes. Separated by short DNA linkers, nucleosomes are ubiquitous (>10^7 per nucleus) and have both structural and regulatory roles in genome function. Chromatin-modifying factors can alter the position/density of nucleosomes on the chromosome as well as catalyze posttranslational modifications of histones or the incorporation of histone variants within nucleosomes (Figure 2). DNA methylation and nucleosomes are usually an impediment to transcription, whereas histone modifications and variants are more nuanced or context dependent and can have opposing effects. For example, histone 3 lysine 4 trimethylation (H3K4me3) usually stimulates transcription while the same modification at certain adjacent lysine residues such as H3K9me3 or H3K27me3 has an inhibitory effect. This adds complexity to what is referred to as the histone code, which is written by enzymes that add posttranslational modifications and read by proteins that bind to modified residues as docking sites. Proteins with bromodomains bind to acetylated lysines (Figure 2), while proteins with chromodomains, tudor domains, and PHD fingers bind to various lysine or arginine residues when they are methylated. The histone code can also be erased, as most reactions are reversible. For example, histone acetyltransferases (HATs) are counteracted by histone deacetylases (HDACs), while histone methyltransferases are counteracted by LSD1 or jumonji C (JmjC) domain demethylases (Figure 2). Both small and large non-coding RNAs also participate in epigenetic mechanisms by guiding chromatin-modifying factors to specific sites in the genome. For instance, the long ncRNA XIST physically associates with and coats one X chromosome in female cells. XIST recruits enzymes that posttranslationally modify histones [e.g. HDACs to deacetylate and a Polycomb repressive complex (PRC2) with catalytic subunit EZH2 to induce H3K27me3] followed by the incorporation of the histone variant macroH2A and CpG methylation to stably inactivate one of two X chromosomes to achieve dosage compensation relative to XY male cells (Figure 2).

1C Metabolism, SAM, & DNA Methylation

Many epigenetic enzymes utilize energy metabolites as essential co-factors (Table 1). For example, DNA methyltransferases utilize S-adenosylmethionine (SAM), which is a product of 1-carbon (1C) cycle metabolism that involves zinc, methionine, and vitamin B family members including folate, choline, and vitamin B12 (Figure 3A). The effects of these micronutrients can be easily visualized in viable-yellow agouti mice, which are genetically identical (when they are maintained on an inbred genetic background) but range in color from yellow through increasing amounts of agouti (brown) mottling to completely agouti (Figure 3B), depending on the extent of DNA methylation near the transcriptional start site (Figure 3C). When viable-yellow females are provided a diet supplemented with zinc, methionine, and a 3-fold increase in the aforementioned vitamin B family members while they are pregnant, the developing progeny accumulate increased levels of CpG methylation within the agouti gene (Figure 3C) (Cooney et al., 2002; Waterland and Jirtle, 2003). This is evident based on their coat color after birth where the distribution is shifted away from yellow and lightly mottled (the hypomethylated state) toward heavily mottled and agouti (the hypermethylated state) (Figure 3D). This result demonstrates two very important concepts: 1, Maternal diet epigenetically regulates gene expression in a mother's offspring *in utero* , and these changes can persist and change the offspring's physical characteristics after their birth and for their entire life. This epigenetic effect can even be transmitted to the next generation because maternal methyl-donor supplementation induces CpG methylation in the developing germ cells of the fetus as well as in their skin and hair follicle cells (Morgan et al., 1999). 2, Diet-induced co-factors modulate the activity of epigenetic enzymes and can be rate limiting *in vivo*. This suggests that nutrient availability and co-factor abundance act as a rheostat to increase/decrease enzymatic activity more effectively than changing actual enzyme levels. Another advantage of this mechanism is that it can be subject to feedforward and feedback regulation as is the case in DNA methylation reactions

where SAM is converted to S-adenosylhomocysteine (SAH), which can build up to levels capable of inhibiting DNA methyltransferase activity.

The effect of 1C metabolism and SAM in the viable-yellow agouti mouse model is not limited to coat color but also influences the incidence of obesity and cancer in these mice. Similar effects of methyl-donor supplementation have been observed in other mice such as axin-fused mutants, which exhibit a variable kink-tail phenotype, and are probably not uncommon but are difficult to detect for genes that do not confer observable phenotypes. There is also evidence that 1C metabolism is important for human health. It is known from epidemiological studies that adequate folate intake during pregnancy [approximately twice the recommended daily allowance (RDA) of 400 μg] prevents neural tube defects such as spina bifida in the fetus (1991; Czeizel and Dudas, 1992). In fact, the importance of these findings from a public health perspective led to flour being fortified with folate because the neural tube closes during the first trimester before many women know they are pregnant and have had an opportunity to modify their diets. Increased folate consumption has also been implicated in colorectal cancer prevention, but this is controversial and some evidence suggests that folate may actually increase the progression of existing adenomas (Kim, 2007). High levels of alcohol also affect 1C metabolism and are known to cause genome-wide changes in CpG methylation in mouse embryos that are associated with neural tube defects and other teratogenic effects (Liu et al., 2009). Therefore, fetal alcohol syndrome has an epigenetic component.

The importance of maternal diet is also supported by cohort studies of >2,000 people that evaluated the health status of ~50 year olds that were conceived during the Dutch famine of 1944-1945 (Painter et al., 2005). In Nazi-occupied Netherlands, calorie consumption dropped to 400-800 per day, and children that were conceived during this period became adults with a higher incidence of schizophrenia and heart disease than their siblings. Although it is unclear whether this higher incidence was mediated through epigenetic alterations, it is clear that dynamic changes in DNA methylation occur during the earliest stages of development and these studies identified a correlation between disease state and altered CpG methylation in certain genes [including a decrease in the imprinted gene *IGF2* (insulin-like growth factor 2)] (Painter et al., 2005). These studies also suggest increased incidence of other health ailments due to the effects of the famine during later stages of pregnancy.

The 1C mechanism is not completely understood because SAM is an essential co-factor not only for DNA methyltransferases but also for histone methyltransferases [also known as protein arginine methyltransferases (PRMTs) or protein lysine methyltransferases (PKMTs) because non-histone proteins are also modified]. In fact, maternal choline deficiency, which is also associated with neural tube defects and perturbed neurogenesis in the fetus, results in diminished H3K9 methylation as well as CpG methylation (Mehedint et al.). SAM is also the methyl donor for the methylation of RNA and phospholipid substrates. Regardless of the precise mechanism, the transgenerational effects of 1C metabolism and SAM probably involve DNA methylation and should be considered when thinking about the etiology of complex diseases. Although the increased incidence of autism and schizophrenia spectrum disorders in recent years reflects a change in how they are diagnosed (i.e., an ascertainment issue), an actual increase contributed by maternal diet or fetal/neonatal exposure to certain agents (other than the MMR vaccine) cannot be ruled out (Rutter, 2005). For example, bisphenol A (BPA) is a chemical used to manufacture polycarbonate plastic that has been implicated in autism. When viable-yellow agouti mice are exposed to BPA during fetal or neonatal development, their coat-color distribution is shifted toward yellow, opposite of methyl-donor supplementation, due to decreased CpG methylation (Dolinoy et al., 2007). Although it is not clear whether this effect is linked to the role of BPA as an endocrine

disruptor, maternal supplementation of methyl donors (including folate) or the phytoestrogen genistein (an isofalvone derived from soy beans that is abundant in soy-based foods) negates the effect of BPA in viable-yellow mice (Dolinoy et al., 2007).

Effect of Diet and Metabolites on the Emerging Mechanism of DNA Demethylation

The effect of diet and energy metabolism on DNA methylation has been evolutionary conserved. A particularly good example is a honeybee colony where the vast majority of females are infertile worker bees with usually just one fertile queen per hive. The queen is genetically identical to the worker bees but destined to become a queen as a larva when she is fed relatively high levels of a substance produced by nurse bees called royal jelly. Although royal jelly is enriched in various B vitamins, the mechanism apparently involves decreased DNA methylation rather than an increase based on RNAi experiments. When a DNA methyltransferase (DNMT3A) was knocked down in honeybee larvae, 72% of the females developed into queens without being fed royal jelly (Kucharski et al., 2008). This finding suggests that royal jelly decreases DNA methylation in order to endow queens with their ability to reproduce via fully developed ovaries and other profound physical and behavioral changes including increased body size and longevity.

Several bioactive components of royal jelly have recently been identified (Kamakura; Robinson; Spannhoff et al.), and the study described above suggests that at least one should decrease DNA methylation by either inhibiting DNA methyltransferase activity or increasing demethylase activity. Although much is known about the former, where well-characterized *de novo* methyltransferases (DNMT3A and DNMT3B) are followed by another methyltransferase (DNMT1) that maintains CpG methylation during DNA replication in a semi-conservative manner, little is known about the latter. Over the years, reports of bona fide DNA demethylases have not been confirmed, and recently much attention has focused on a less direct route to active demethylation involving a DNA repair mechanism DNA demethylation.

Three TET family enzymes convert 5-methylcytosine to 5-hyroxymethylcytosine and can further oxidize this base to 5-formylcytosine and 5-carboxylcytosine (He et al.; Ito et al.). Although these 5-methylcytosine derivatives may have their own epigenetic properties (they do prevent bisulfite mutagenesis and therefore complicate our understanding of CpG methylation profiles), they appear to be intermediates in the process of demethylation as they can be removed by base excision repair (utilizing glycosylases similar to how uracil is replaced by thymine) and replaced by unmethylated cytosines (Bhutani et al.; Nabel and Kohli). In support of this idea, a similar mechanism occurs in plants *Arabidopsis* utilizing the DEMETER 5-methylcytosine-specific glycoslylases/lyases.

The TET enzymes are dioxygenases dependent on iron (Fe^{2+}) and α-ketoglutarate (α-KG) as co-substrates. α-KG plays an important role in oxidative metabolism as an intermediate in the tricarboxylic acid (TCA) cycle and is derived from glutamine, which is an abundant amino acid and has important functions in cellular energetics (Figure 4). Although it is currently unclear whether α-KG levels are ever rate limiting for TET activity, other metabolites that competitively inhibit α-KG, such as 2-hydroxyglutarate, fumarate, and succinate (the latter two are also TCA cycle intermediates), accumulate in certain tumors [so-called oncometabolites, which arise because of altered tumor cell metabolism, and in this case are a consequence of isocitrate dehydrogenase 1 (*IDH*1) mutations] (Xu et al.). The importance of TET enzymes is underscored by RNAi knockdown experiments demonstrating that TETs are involved in ES cell pluripotency, and it is likely that TETs have many additional functions yet to be identified.

DNA demethylation can also occur passively, in the absence of any demethylase, but only in cells that are proliferating. In this case, if DNMT1 is either expressed at low levels or if most of it is excluded from the nucleus, then CpG methylation cannot be maintained in a semi-conservative manner and it is reduced by 50% after each S phase. Interestingly, this might also be regulated by energetics because AKT, a kinase that integrates growth factor signaling and energy homeostasis that is frequently upregulated in cancer, phosphorylates and stabilizes DNMT1 (Esteve et al.).

Acetyl-CoA and Histone Acetylation

Metaboloepigenetics is also exemplified by comparing histone acetylation in tissue-culture cells that are grown under normal conditions versus serum starvation or glucose deprivation. Serum starvation or glucose deprivation causes a marked decrease in global levels of histone acetylation under these tightly controlled conditions (Figure 5A). Recent work suggests that the effect of serum starvation is dependent on glucose and has delineated the signaling pathway that is responsible as shown in Figure 5B (Wellen et al., 2009). Glucose is metabolized by glycolytic enzymes to pyruvate, which is converted by pyruvate dehydrogenase (PDH) to acetyl-CoA inside of mitochondria where it condenses with oxaloacetate to yield citrate in the first step of the TCA cycle. Citrate can be transferred to the cytosol via the citrate shuttle, particularly when nutrients meet or surpass the energy needs of the cell. The enzyme ATP-citrate lyase (ACL) utilizes the energy of ATP hydrolysis to convert citrate to acetyl-CoA and oxaloacetate in the cytosol as an initial step in *de novo* lipid biogenesis. This step is followed by reactions catalyzed by acetyl-CoA carboxylase and fatty acid synthase to synthesize phospholipids and triglcyerides. The former is crucial for doubling the biomass of the plasma membrane every time a cell divides, and the latter is utilized for energy storage purposes, especially in adipocytes and hepatocytes. It has been shown that ACL is expressed at relatively high levels in the nucleus and that ACL is important for maintaining global histone acetylation levels (Wellen et al., 2009). Mechanistically, ACL-generated acetyl-CoA is an essential co-factor for histone acetyltransferases (HATs) by serving as the acetyl group donor. Not surprisingly, this pathway is subject to feedforward activation as glucose and insulin signaling upregulate ACL activity via phosphorylation by AKT (Figure 5B).

The importance of cytosolic/nuclear acetyl-CoA is underscored by it being produced not only by ACL but also by two acetyl-CoA synthetase enzymes that condense acetate and coenzyme A. Knocking down one of these enzymes in addition to ACL results in very low levels of global histone acetylation (Wellen et al., 2009). Because acetyl-CoA is a crucial metabolite in several energy metabolism pathways (Table 1), it is tempting to speculate that acetyl-CoA links energy status and gene expression to regulate cell-cycle progression. When cells are serum starved or lack enough energy to complete the next round of cell division, they do not pass a G_1 checkpoint called the restriction point and become quiescent because it would be deleterious to begin DNA replication without being able to complete it and the ensuing mitosis. This checkpoint protects against genomic instability and cancer. Re-addition of serum or nutrients allows cells to move beyond this restriction point by exiting G_0 and moving into the G_1/S phase transition. RB phosphorylation is involved in this process, but many other factors must also contribute. Acetyl-CoA is a good candidate because it is influenced by energy balance, is necessary to build a new cell as the substrate for plasma membrane biogenesis, and regulates gene expression as a HAT co-factor. In support of this idea, when carbon sources are added back to nutrient-deprived yeast that are quiescent, acetyl-CoA levels increase and induce histone acetylation (via the GCN5 HAT) and transcription of growth-related genes (Cai et al.). It is also likely that acetyl-CoA-driven HAT activity triggers the G_1/S phase transition by facilitating DNA replication because origins of replication are enriched in histone acetylation (Goren et al., 2008; Unnikrishnan et

al.). Acetyl-CoA could also contribute to this process by increasing the acetylation of non-histone proteins [HATs are also known as lysine acetyl transferases (KATs) for this reason] such as metabolic enzymes and cell-cycle regulators (Burnett et al.; Goren et al., 2008; Grummt and Ladurner, 2008; Guarente; Hallows et al., 2006).

NAD^+ and Class III HDACs (Sirtuins)

HDACs are divided into several classes [and are also known as lysine deacetylases (KDACs) because they deacetylate non-histone substrates]. HDACs 1-10 belong to class I or II and are dependent on Zn^{2+} (so is HDAC 11 in class IV). The other seven members, known as Sirtuins 1-7, belong to class III and require nicotinamide adenine dinucleotide (NAD^+) as an essential co-factor. NAD^+ accepts electrons from other molecules as it is reduced to NADH. This change in redox status is particularly important in the mitochondria where it links the TCA cycle (where NAD^+ is reduced to NADH, Figure 4) to the electron transport chain (where NADH is the initial electron donor) for ATP production. A relatively high NADH/NAD^+ ratio is therefore a measure of a favorable energetic condition that is "sensed" by Sirtuins to downregulate their HDAC activity. For this reason, when glucose is abundant, SIRT1 activity is relatively low, whereas periods of prolonged fasting and energy limitation upregulate SIRT1 HDAC activity (Grummt and Ladurner, 2008; Guarente). This suggests that glucose strongly induces global histone acetylation, as shown in Figure 5A, because of dual mechanisms. By contributing carbon to the TCA cycle, glucose not only increases acetyl-CoA and HAT activity (via the citrate shuttle and ACL as discussed above) but also decreases NAD^+ (relative to NADH) to attenuate class III HDAC activity (Figure 5B). There is also crosstalk between the two pathways as Sirtuins can regulate acetyl-CoA synthetases (Hallows et al., 2006). The second HDAC inhibition mechanism has been shown to link the energetic state of the cell with its ability to grow: low glucose triggers NAD^+-induced SIRT1, which deacetylates histones (with a reported preference for H3K9ac and H4K16ac) at ribosomal DNA (rDNA) repeats and downregulates rDNA transcription, ribosome biogenesis, and consequently protein synthesis (Grummt and Ladurner, 2008).

Calorie restriction (down to ~60% of normal) is known to increase lifespan (up to ~150% of normal) in organisms ranging from yeast to humans, and this has been attributed to increased SIRT1 activity. Although the involvement of SIRT1 in the lifespan of *C. elegans* and *Drosophila* was recently refuted by rigorously controlled genetic experiments (Burnett et al.), SIRT1 is still believed to protect against metabolic and age-related diseases in mammals. Although caloric restriction increases longevity in wild-type mice, it does not do so in SIRT1-deficient mice (Herranz and Serrano). This suggests that the effect of caloric restriction on longevity is SIRT1 dependent, but it should be noted that SIRT1 null mutants have a short lifespan to begin with (and most actually die in the neonatal period). Transgenic mice that overexpress SIRT1 are protected from glucose intolerance and hyperinsulinemia in response to a high-fat diet although longevity is unaffected (Herranz and Serrano). Similarly, resveratrol, which is a naturally occurring phenol in the skin of red grapes (but present at low levels in red wine) and other fruits, activates SIRT1 and can improve insulin sensitivity and extend lifespan of mice provided a high-fat diet. However, resveratrol probably activates SIRT1 indirectly via adenosine monophosphate kinase (AMPK) activation, and the relative importance of SIRT1 compared to other targets is not known. Taken together, these findings support the idea that SIRT1 confers metabolic flexibility to protect against energetic stress. Furthermore, they suggest that calorie intake is linked to good health and possibly lifespan by epigenetics. However, this link is probably not exclusive to histone acetylation because SIRT1 also deacetylates non-histone proteins including p53, FOXO transcription factors, PPARγ, and LXR. But SIRT1 is nuclear, whereas other SIRTs are present in additional subcellular compartments and SIRT4 and SIRT5 are primarily or exclusively mitochondrial.

The redox state of NAD also affects the ability of SIRT1 to regulate our circadian rhythms, which influence energy metabolism by controlling daily fluctuations in body temperature, feeding behavior, locomotor activity, and hormone secretion. SIRT1 physically binds to the clock transcription factor, which is a key component of the regulatory network, and SIRT1 HDAC activity counterbalances clock's HAT activity (Bellet and Sassone-Corsi). Although SIRT1 and clock protein levels do not fluctuate on a daily basis, NAD^+ biosynthesis does display circadian oscillations that match SIRT1 HDAC activity [via NAMPT as the rate-limiting step of the salvage pathway (Nakahata et al., 2009; Ramsey et al., 2009)]. This results in an oscillating pattern of histone acetylation and transcription for the period (*PER*) and cryptochrome (*CRY*) genes and other targets [microarray studies estimate that 10-15% of transcripts show circadian oscillations (Bellet and Sassone-Corsi)] (Figure 6). NAD^+ can promote deacetylation not only by promoting SIRT1 activity but also by inhibiting the binding of clock to DNA (whereas NADH is reported to increase binding of clock to DNA) (Figure 6). As part of a negative feedback loop, the PER and CRY proteins dimerize and translocate to the nucleus to inhibit clock, and this is apparently dependent on redox state because CRY binds FAD. The NAD^+ salvage pathway must maintain the circadian network as part of a feedback loop rather than kick starting it because it is under circadian control itself. Not surprisingly, such a complicated process has additional inputs. AMPK, which is activated by low energy levels (via LKB1 phosphorylation in response to a low ATP/AMP ratio), shows rhythmic nuclear localization and activity and phosphorylates/destabilizes CRY1 (Lamia et al., 2009). A JmjC domain demethylase (JARID1a), HDAC3, and cAMP are also involved. Circadian rhythms are of biomedical importance because perturbations are associated with insomnia, behavioral disorders (depression and possibly bipolar disorder), obesity, and metabolic syndrome (which includes increased risk of diabetes and heart disease).

Diet can influence total levels of nicotinamide adenine dinucleotide because *de novo* synthesis utilizes the amino acids tryptophan and aspartic acid as well as the vitamin niacin as building blocks. Poly-ADP-ribose polymerases (PARPs) utilize NAD irrespective of redox state as an enzymatic substrate. By cleaving a bond between the ADP-ribose moiety and the nicotinamide, PARP1 (the best characterized of ~18 PARPs and accounts for the majority of PARP activity) forms a branched polymer of ADP-ribose (PAR) at histones and other proteins, and this is important for both transcription and the repair of DNA damage.

Other Metabolic Co-Factors of Epigenetic Enzymes

Energy metabolites serve as co-factors for other epigenetic enzymes including histone methyltransferases (HMTs) and histone demethylases (HDMs) as well as chromatin-remodeling complexes (Table 1). HMTs can be subdivided into PRMTs and PKMTs that methylate either arginine or lysine residues, respectively. The 9 known PRMTs are promiscuous compared to the ~50 PKMTs, which exhibit target specificity and have been characterized in greater detail. For example, EZH2 methylates H3K9 while G9a and SUV39H1/2 methylate H3K9 at different loci. With the exception of DOT1L, all of the PKMTs contain a catalytic SET domain. Similar to DNA methyltransferases, all of the HMTs (both PRMTs and PKMTs) are dependent on SAM as a methyl donor. These enzymes are counteracted by HDMs (Figure 2). PADI4 deiminates methylated arginines but is not a bona fide HDM because it yields citrulline instead of unmethylated arginine. Citrulline is noteworthy, however, because it is a key intermediate in the urea cycle. The JmjC domain (JMJD) family consists of ~30 members within 7 subfamilies in mammals that show target specificity for methylated lysines (and at least one member, JMJD6, demethylates methylates arginines). Similar to the TET DNA "demethylases", the JmjC domain HDMs use Fe^{2+} and α-KG as co-factors to stabilize the enzyme/substrate complex. LSD1 is a structurally distinct demethylase that acts on H3K4me1/2 and H3K9me1/2 but

cannot demethylate trimethyl marks because it requires a protonated hydrogen to yield an imine intermediate. LSD1 activity is regulated by redox state similar to Sirtuins. Rather than being regulated by NAD^+, however, LSD1 uses flavin adenine dinucleotide (FAD), which is composed of riboflavin (vitamin B_2) and ADP. LSD1 activity is stimulated when FAD is in its oxidized form but not when it is reduced to $FADH_2$ such as occurs in the TCA cycle (Figure 4).

SWI/SNF and several other families of chromatin-remodeling complexes are recruited by sequence-specific transcription factors to promoters of target genes where they use ATP as a co-factor to regulate transcription. Each of these complexes has a catalytic subunit with DNA-dependent ATPase activity, and the energy of ATP hydrolysis is utilized to move nucleosomes to alternate positions to either “open” or “close” chromatin and activate or silence transcription. It is not known whether ATP levels in the nucleus are ever rate limiting for the activity of chromatin-remodeling complexes. However, low ATP levels are known to activate AMP kinase (AMPK), which is an energy sensor that phosphorylates many substrates including H2B Serine 36, which serves as an active mark for transcription and is thought to help mediate adaptation to bioenergetic stress (Bungard et al.).

Dietary HAT and HDAC Inhibitors

Certain foods and herbs include naturally occurring HAT or HDAC inhibitors (Table 2), which have an even more direct impact on epigenetics. Biochemical analysis of plant-derived extracts, particularly those used as traditional medicines in China and India, has led to the identification of a number of bioactive molecules (Table 2) (Dashwood et al., 2006; Kim et al.). Because these are small molecules, they are not destroyed by the acidic environment of the stomach and are readily absorbed. In addition to generally inhibiting HATs or HDACs, these molecules alter histone acetylation of specific target genes leading to altered expression. Targets include cell-cycle regulators such as p21, components of the NF-κB inflammation pathway, and pro-apoptotic factors such as BAX and BAK. In some cases, these dietary agents can also alter the acetylation of important non-histone proteins including p53. Some of these HAT and HDAC inhibitors are known to have beneficial properties consistent with the function of their downstream targets such as inhibiting cell proliferation, diminishing inflammation as well as reactive oxygen species, and promoting apoptosis.

These naturally occurring HAT and HDAC inhibitors are not as potent as synthetic inhibitors (such as trichostatin A or SAHA, which are being used in clinical trials to treat hematopoietic malignancies). However, depending on our diet, we can be chronically exposed to high levels of these agents. For example, butyrate is present at >5 mM in the lumen of our colon. Butyrate is so abundant that it, rather than glucose, is the preferred energy source of colonocytes, and it plays a key role in maintaining energy homeostasis to prevent autophagy as well as functioning as an epigenetic HDAC inhibitor (Donohoe et al.).

Butyrate is also noteworthy because it is produced in the colon by bacterial fermentation of dietary fiber. However, it is not alone as we also have microbes with a curcumin converting enzyme that is dependent on NADPH (Hassaninasab et al.). Microbial cells in our body outnumber all of our somatic and germ cells by 10X. Most of these microbes are bacteria in our GI tract (Figure 7), and their collective genomes, which is referred to as the microbiome, harbors ~100X more genes than the human genome (Walker et al., 2006). The Human Microbiome Project, which is using high-throughput sequencing to catalog all of these microbes, is receiving much attention because they have been implicated in obesity, inflammatory bowel disease, and metabolic syndrome of the host (Tilg and Kaser, 2011). They are also known to have widespread effects on the production of metabolites in our

bodies including those involved in choline metabolism (and therefore possibly CpG methylation) (Dumas et al., 2006; Li et al., 2008; Wikoff et al., 2009).

As mentioned above, honeybee larvae that are fed royal jelly become queen bees rather than worker bees, and genetics is not a factor. One recent study showed that a major constituent (~5%) of royal jelly is a short-chain fatty acid that is structurally similar to butyrate (Spannhoff et al.). This fatty acid, 10-hydroxy-2-decenoic acid, is also a potent HDAC inhibitor. Although it does not inhibit global levels of DNA methylation, this is not necessarily incompatible with the DNMT3 RNAi experiments. It is possible that it increases histone acetylation directly and decreases DNA methylation indirectly (the two are often coupled) at specific loci involved in caste switching during larval development.

Future Directions

It is likely that future studies will discover many more connections between energy metabolism and epigenetics. For example, a very recent study showed that glucose induces histone GlcNAcylation (H2B-S112-GlcNAC) via the hexosamine biosynthesis pathway and histone monoubiquitination (H2B-K120ub), which is an active mark unrelated to proteosome-mediated degradation (Fujiki et al.). As our knowledge of metaboloepigenetics grows, it will become an increasingly important factor in understanding human health and disease prevention. For example, genome-wide association studies (GWAS) have identified many genetic variants, but collectively they account for <20% of the phenotypic variance of most complex diseases including asthma, type 2 diabetes, and heart disease (Manolio et al., 2009). Although some of the missing heritability from these GWAS will be revealed by whole-genome sequencing efforts, we feel that there will still be a large gap. And this will be most evident for complex diseases that have a significant contribution from the environment in general (such as asthma) and the growing obesity epidemic in particular (such as type 2 diabetes and heart disease). Fortunately, the high-throughput sequencing technologies that are being used to find rare genetic variants to improve GWAS are also being used to compile epigenomic profiles from cases and controls (*via* ChIP-seq and related techniques such as MeDIP-seq or bisulfite-seq/BS-seq and FAIRE-seq) (Zhou et al., 2011). Although these latter studies have become a point of emphasis because of the NIH Epigenomics Roadmap Initiative and should be very informative, some human epigenomic studies will face a major technical challenge. Unlike genetic studies, where DNA can be prepared from any tissue, usually one that is easy to obtain such as blood, the epigenome varies in a cell-type dependent manner. So understanding the epigenomic basis of autism, for example, will require access to human brain tissue from cases and controls. Not only can it be difficult to procure this tissue in a timely manner postmortem (and the inability to biopsy makes it difficult to study disease progression), but it can be difficult to decide which region(s) of the brain to study and the degree of cellular purity that is acceptable. Inflammation and fibrosis are common in many pathological conditions, and this cellular heterogeneity can complicate comparisons between cases and controls. Obtaining precisely matched samples is not necessarily trivial if flow sorting or laser capture microdissection are not possible.

A strong precedent for the epigenomic basis of human disease is the role of DNA methylation in cancer. Unlike the brain/autism example, tumors are surgically removed from cancer patients and available for study. And the mutations and “epimutations” that drive tumorigenesis are selected for and therefore highly represented in tissue from the cases but not the controls. In fact, this led to the realization that the “second hit” in many tumor suppressor genes, which results in loss of heterozygosity (LOH), is not a somatic mutation but promoter CpG hypermethylation that silences the wild-type allele (Esteller, 2008). This is not surprising because our DNA sequence is static and does not change (with the

exception of rare mutations) as it is transmitted from one generation to the next, whereas CpG methylation is dynamic and reversible. Because of genomic imprinting, CpG methylation profiles are erased and re-established in the germline before being transmitted to the next generation. As a consequence of this malleability, CpG methylation profiles are not maintained in somatic tissues with as high a degree of fidelity as DNA sequence. This is apparent not only from tumor studies but is also based on the divergence of CpG profiles in tissues from identical twins as they age. The reversible nature of other epigenomic marks (Figure 2), in both germ cells [mediated, in part, by piwi ncRNAs (piRNAs)] and somatic cells, suggests that epimutations may account for much of the missing heritability of GWAS. Consistent with this idea, only a small percentage of SNPs are non-synonymous variants located in coding exons, while a high percentage of SNPs are found in putative regulatory elements located many kilobases (kb) proximal or distal to the nearest transcription unit (Chorley et al., 2008; Wang et al., 2005). Some of these SNPs have been shown to affect the binding of transcription factors that recruit chromatinmodifying factors and alter epigenomic marks such as DNase I hypersensitive sites (McDaniell et al.).

Metabolomics/metabonomics using NMR and mass spectrometry platforms is a promising approach to studying human disease because metabolites are a readout of gene-environment interactions and may therefore be better biomarkers than SNPs or epigenomic marks (Nicholson and Lindon, 2008). In addition, metabolites can be analyzed from easily obtained biofluids such as serum and urine. Integrating genomic, epigenomic, and metagenomic datasets should become increasingly commonplace, and cancer should be particularly promising for two reasons. First, it is amenable to epigenomic analyses as discussed above. Second, due to the Warburg effect, tumors take up large amounts of glucose and undergo aerobic glycolysis rather than oxidative metabolism (i.e., TCA cycle and electron transport chain in the mitochondria) (Vander Heiden et al., 2009). This presumably accounts for high levels of oncometabolites in tumors but not normal tissues and will likely affect how glucose, fatty acids, and other nutrients influence histone acetylation and other epigenomic marks.

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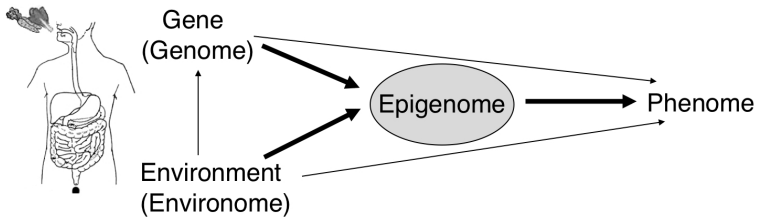

Figure 1. A simple model of metaboloepigenetics

The importance of food is not restricted to its nutritional content (e.g., calories and essential vitamins/minerals), but it is also important because bioactive food components and energy metabolites can function as environmental factors. And many gene-environment interactions converge at the level of the epigenome, which regulates gene expression profiles and determines phenotypic outcomes, referred to collectively as the phenome, at both the cellular and organismal levels. This is particularly true for certain energy metabolites and bioactive food components that activate or inhibit chromatinmodifying factors.

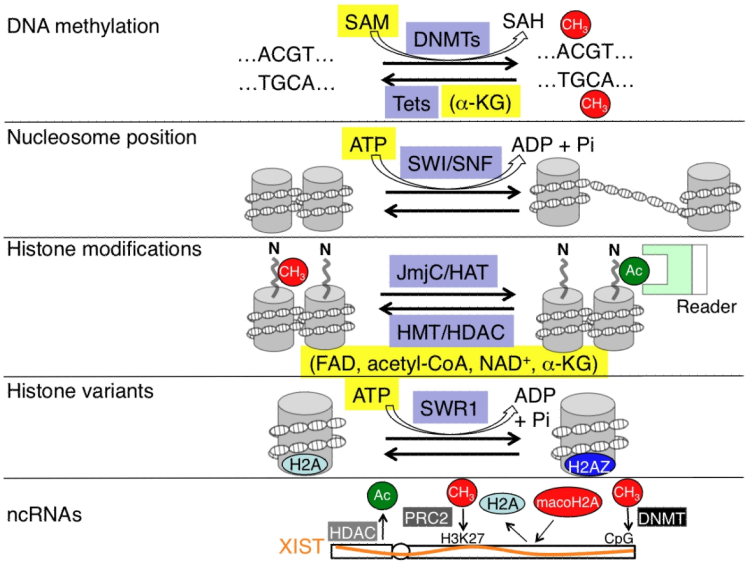

Figure 2. Epigenetic marks are catalyzed by chromatin-modifying factors

General types of epigenetic/epigenomic marks are listed at the left and a schematic of each reversible reaction is shown to the right. In the top 4 panels, enzymes are highlighted in blue and co-factors are highlighted in yellow. Nucleosome position and incorporation of histone variants are also reversible as indicated. In the bottom panel, XIST recruits enzymes and epigenomic marks in a sequential manner (from left to right) with stimulatory and inhibitory marks shown in green and red, respectively. See text for additional details.

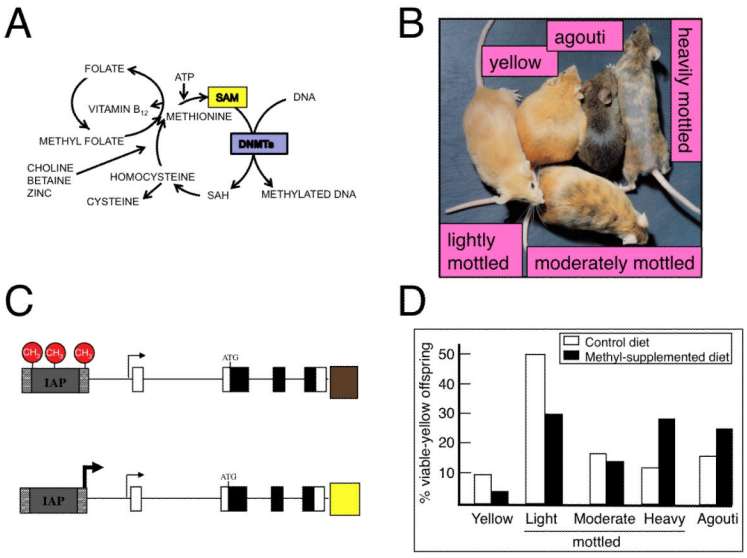

Figure 3. 1C-cycle metabolism and SAM regulate DNA methylation

(A) Schematic of 1C cycle and synthesis of SAM. **(B)** Photograph of 5 genetically-identical agouti viable-yellow mice with different coat-color phenotypes ranging from yellow through 3 degrees of mottling to agouti. **(C)** Schematic of agouti-viable yellow “epialleles”. Agouti exons are shown as boxes with the filled portions corresponding to coding sequence. An IAP retrovirus-like element is immediately upstream of the agouti gene. In the top panel, CpG methylation (red circles labeled CH_3) of the IAP renders it functionally inert. The endogenous promoter of the agouti gene (arrow) drives modest gene activity resulting in a brown (agouti) coat-color appearance in these mice. In the bottom panel, the IAP is not methylated and a strong, cryptic promoter (thicker arrow) drives high-level gene activity resulting in yellow pigmentation. **(D)** Methyl-donor supplementation provided to pregnant viable-yellow mice changes the coat-color distribution of their progeny after birth. Adapted from references 1 and 2.

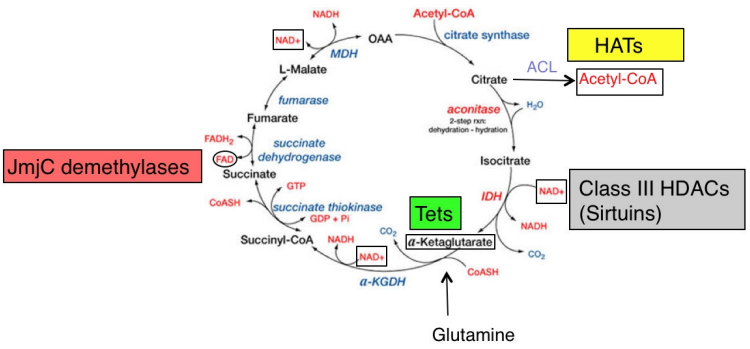

Figure 4. TCA cycle intermediates serve as co-factors for epigenetic enzymes

Shown is a schematic of the TCA cycle. Several intermediates that are boxed or encircled (acetyl-Co-A, NAD^+, α-ketoglutarate, and FAD) serve as essential co-factors for epigenetic enzymes (highlighted by colors).

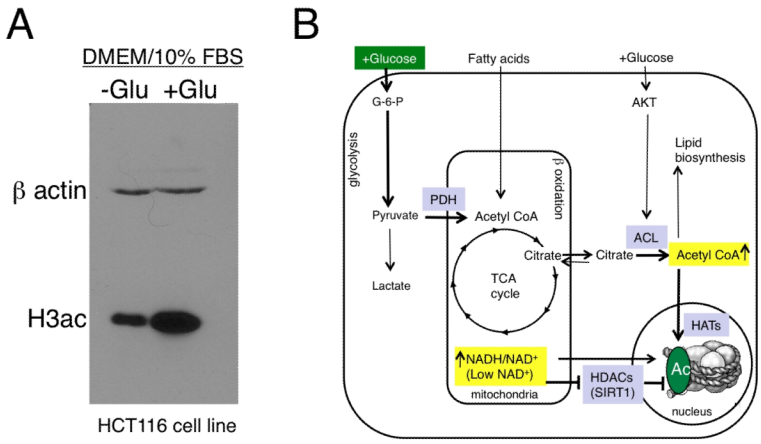

Figure 5. Glucose regulates histone acetylation

(A) Western blot showing pan-histone 3 acetylation (H3ac) levels in HCT116 cells grown in DMEM formulated without glucose (−Glu, left lane) or formulated with 25mM glucose (+Glu, right lane). β actin serves as the loading control. **(B)** A model of how glucose increases histone acetylation. First, it contributes carbons that can be incorporated into acetyl-CoA *via* the following pathway (denoted by thick arrows): glycolysis, PDH (pyruvate dehydrogenase), the TCA cycle and citrate shuttle, and ACL. Second, increased TCA-cycle activity increases the NADH/NAD^+ ratio to inhibit SIRT1, which inhibits histone acetylation. Enzymes and metabolites that are most relevant are highlighted in blue and yellow, respectively.

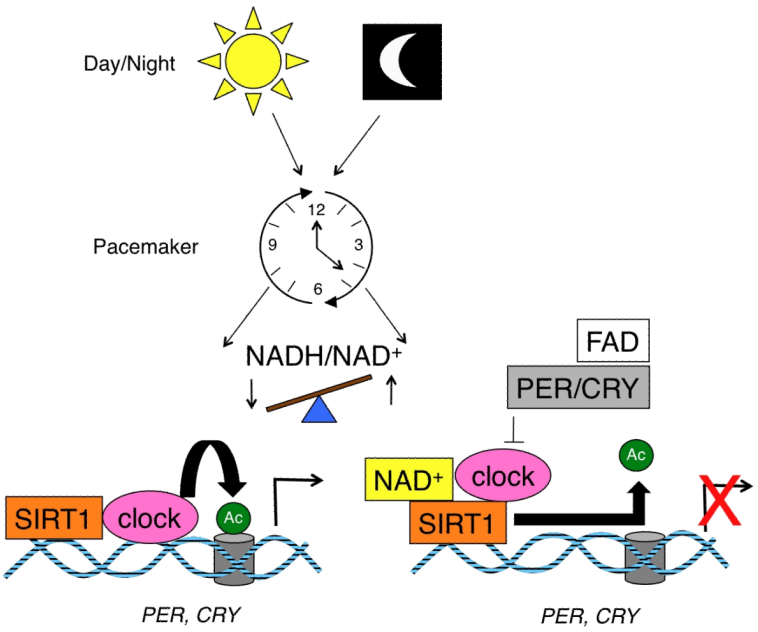

Figure 6. The redox state of NAD regulates SIRT1 and circadian oscillations

The day-night cycle sets an internal pacemaker that regulates circadian rhythms. Daily fluctuations in the NADH/NAD^+ ratio contribute by regulating SIRT1 and its ability to counteract clock HAT activity. This helps determine histone acetylation and transcriptional activity of downstream target genes such as *PER* and CRY. See text for more details.

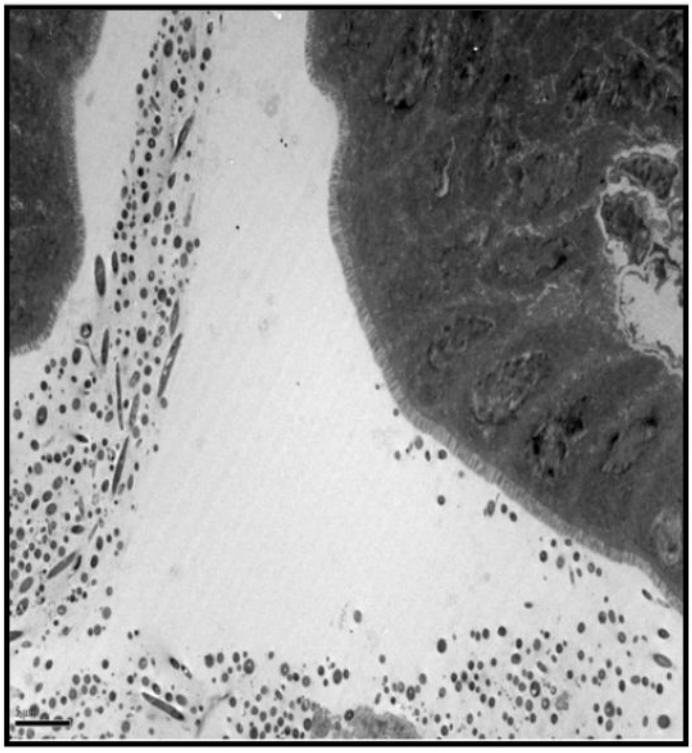

Figure 7. There is a preponderance of microbial cells in the lumen of the GI tract

Shown is a transmission electron micrograph of the proximal mouse colon. This image exemplifies the number and diversity of bacteria present in the mammalian GI tract. Bacterial metabolites such as butyrate can influence host epigeneitcs.

TABLE 1

Metabolites that function as co-factors for epigenetic enzymes

Metabolic process	Co-factor	Epigenetic enzyme
1C metabolism (folate, choline, etc.)	SAM	DNA methyltransfeases (DNMTs) Histone methyltransferases (HMTs)
β oxidation, PDH, TCA, lipid biosynthesis	acetyl-CoA	Histone acetyltransfeases (HATs)
TCA, redox	NAD^+ FAD	Class III histone deacetylases (HDACs)-Sirtuins LSD1 histone demethylases
TCA, glutamine catabolism	α-ketoglutarate	Tet family of DNA “demethylases” JmjC family of histone demethylases
Urea cycle	Citrulline *	PADI4 histone “demethylase”
Electron transport	ATP	Chromatin-remodeling complexes

* Not a co-factor but product of PADI4 deimination of methylated arginines

TABLE 2

Dietary HAT and HDAC Inhibitors

Dietary Agent	Source	Chemical Structure	Enzymes Inhibited
Anacardic acid	shell of cashew nut	several salicylic acids with alkyl chains	HATs (p300)
Garcinol	rind from kokum fruit	polyisoprenylated bezophenone	HATs (p300)
Curcumin	roots of tumeric herb	polyphenol	HATs (p300/CBP)
Plumbagin	roots of scarlet leadwort plant	hydroxynaphthoquinone	HATs
Lunasin	soy, wheat, barley	43 residue polypeptide	HATs (GSN5, PCAF)
Sulforaphane	cruciferous	isothiocyanate	HDACs (HDAC6)
Allyl Mercaptan	garlic	organosulfur compound	HDACs (HDAC8)
Diallyl Disulfide	garlic	organosulfur compound	HDACs
Butyrate	microbial fermentation of fiber	short-chain fatty acid	HDACs
MCP30	seeds of bitter melon	30 kDa protein	HDACs (HDAC1)

Adapted from Kim et al. 2010