CELLSCIENCE
Commentary
Cell Science Reviews Vol 6 No 1
ISSN 1742-8130

Linking nutrient metabolism to epigenetics

Jianjun Luo [1] & Min-Hao Kuo [1,2]

[1] Dept. of Biochemistry & Molecular Biology, & [2] Programs in Genetics & in Cell & Molecular Biology, Michigan State University, East Lansing, MI 48824, USA.

Received 21st July

Epigenetic regulation of gene activity is of immense importance in maintaining normal and dynamic transcription profiles of practically all cells in our body. In the meantime, cells metabolize nutrients to sustain through different tasks and challenges of life. Both metabolism and gene activity respond to changes in environment. By assessing the relative abundance of certain key compounds central to cellular energy flux, cells adjust transcription of selective genes by modulating epigenetic parameters including DNA and histone modifications. This commentary summarizes the epigenetic impacts of fluctuations of acetyl coenzyme A, S-adenosyl methionine, and nicotinamide adenine dinucleotide.

Changes in chromatin structure and dynamics by histone post-translational modifications, chromatin remodeling, and DNA methylation, are correlatively associated with transcriptional regulation. The reversible acetylation of histones by histone acetyltransferases (HATs) and deacetylases (HDACs) regulates the accessibility of DNA and recruits select proteins for regulating transcription. Histone methylation, catalyzed by histone methyltransferases (HMTs) and cleared by a variety of demethylases (Culhane and Cole, 2007; Klose and Zhang, 2007), acts primarily as docking sites for different proteins whose recruitment regulates the biochemical and structural characters of the underlying chromatin loci. DNA methylation exerts mighty effects, both locally and spanning an expansive chromatin locus, on gene activity. Importantly, all of these chromatin modulators rely on essential cofactors that themselves are linked closely to nutrient status. These cofactors include acetyl coenzyme A (Ac-coA), S-adenosyl methionine (SAM or AdoMet), and nicotinamide adenine dinucleotide (NAD). It seems obvious that nutrient status that affects the availability of these cofactors will also cast significant impacts on chromatin dynamics and gene activity. Several recent reports lend conclusive support for this notion, and forecast a new wave of exciting research toward this direction.

Acetyl coenzyme A (Ac-coA) and acetylation

Acetyl coA is the acetate donor for acetylation reactions. In mammalian cells, Ac-coA can be

synthesized via two routes. Ac-coA synthetase, like many fungal cells, condenses acetate and coenzyme A into Ac-coA. In the second pathway, the ATP-citrate lyase (ACL) uses energy from ATP hydrolysis to convert citrate, a TCA cycle product/intermediate, and coenzyme A into Ac-coA and oxaloacetate. Cytoplasmic Ac-coA is a precursor for lipid synthesis, whereas the nuclear Ac-coA contributes to acetylation of histones and other nuclear factors. This compartmentalization of Ac-coA metabolism correlates with the local need of this coenzyme. Wellen *et al*. (2009) recently demonstrated that histone acetylation in several human cell lines relies primarily on the activity of the ACL, hence linking intracellular energy status (i.e. TCA cycle and glycolysis) to gene activity. Global acetylation of histones and expression of a selective subset of genes are downregulated if the ACL activity is perturbed. By feeding ACL-deficient cells with acetate, the aforementioned phenotypes are suppressed, indicating that the acetate-utilizing acetyl coA synthetase contributes insignificantly to nuclear Ac-coA pool under a normal situation, but can supplement the needed Ac-coA when cells are shifted from glucose- to acetate-driven metabolism. The critical implication of this study is that, because the Ac-coA for epigenetic control arises from the TCA cycle, energy flux, and hence the metabolic status, of cells is tied to gene activities.

An earlier study by J. Boeke and colleagues using the budding yeast as the model shows that one of two Ac-coA synthetases in yeast, Acs2p, also regulates the global acetylation of histones, and that synthetic lethality results from deleting a key HAT, Gcn5p, in an *acs2*$^{-}$ conditional mutant (Takahashi *et al*., 2006). These results resonate well with the Wellen *et al*. report in that global and gene-specific control of transcription can be intertwined with the metabolic status of cells. Importantly, given the requirement of several high-energy compounds (*e.g.* Ac-coA, SAM) and coenzyme (*e.g.* NAD) for chromatin modifications, it is of immense interest to examine whether the abundance of these pluripotent compounds also contribute to transcriptional regulation via epigenetic mechanisms.

SAM and SAH (AdoMet and AdoHcy)

The majority of biological methylation reactions are catalyzed by methyltransferases that transfer the methyl group from S-adenosyl methionine (SAM, SAMe, or AdoMet) to the acceptor molecules including DNA, RNA, and selective arginine or lysine residues in proteins. Methylation of DNA and histones has tremendous impacts on local and global gene activities (Cedar and Bergman, 2009; Vaillant and Paszkowski, 2007). Synthesis of SAM involves the methionine adenosyltransferases (MATs), ATP, and methionine. After giving away the high-energy methyl group in a transmethylation reaction, SAM becomes S-adenosyl homocysteine (SAH or AdoHcy) that is a potent inhibitor of all methyltransferases (Lu and Mato 2008; Luka *et al*., 2009). Hence, cells produce the SAH hydrolase (SAHH) that efficiently breaks down SAH into adenosine and homocysteine. Homocysteine can further act as a precursor for methionine (and thus is recycled back to SAM synthesis) and cysteine synthesis. SAHH is conserved and essential for viability. A genetic disorder due to the deficiency of SAHH activity has been demonstrated (Baric *et al*., 2004). Several antiparasite and antiviral drugs targeting SAHH have been described (Cai *et al*., 2007).

Epigenetic regulation involving SAM includes methylation of DNA, histones, and several non-histone transcriptional regulators, such as the tumor suppressor protein p53 and the general transcriptional factor TAF10 (Huang and Berger, 2008). DNA methylation represses transcription

and is essential for the maintenance of chromatin structure and genomic stability. Alterations of the promoter methylation have been associated with changes of transcription profiles of cancers. Consistently, transgenic mice with one of the two methionine adenosyltransferase genes (MAT1A) deleted revealed stunning development of hepatocellular cancer (HCC) by 18 months of age (Lu and Mato, 2007). Deleting MAT1A leads to drastic decrease of the liver SAM level and increase of serum methionine concentration. However, it should be noted that, epigenetically, it is unclear whether the cancerous development was ascribed to misregulation of DNA or histone methylation, or both. In this regard, it is interesting to note that the budding yeast *Saccharomyces cerevisiae* does not have DNA methylation, and hence may be a good model to delineate the effect of deleting the methionine adenosyltransferase genes (*SAM1* and *SAM2*) on protein methylation and gene expression.

In addition to the biosynthesis by MATs, the abundance of SAM is determined by a variety of other factors, including its breakdown in transmethylation and other reactions, and the accumulation/removal of SAH. The SAM/SAH ratio, or methylation potential, is critically dependent on the action of SAHH. Intriguingly, the affinity of SAHH for adenosine is affected by the relative amount of NAD and NADH, and that NAD is an essential coenzyme for the SAHH action (Grillo and Colombatto, 2008). These observations are reminiscent of the NAD-dependent class III HDACs (sirtuins; see below). It is thus tempting to speculate that a crosstalk between the NAD/NADH and SAM/SAH pathways may coordinate expression of certain genes in response to changes in cellular nutrient status. Consistent with this speculation are the reports that the life span of budding yeast *Saccharomyces cerevisiae* is increased upon deleting a SAM synthetase gene, *SAM1*, but decreased in *SIR2* (a sirtuin family) knockout cells (Boselli *et al*., 2009; Smith *et al*., 2008).

Furthermore, methylation of homocysteine re-generates methionine. The methyl donor in this case is either betaine, which is derived from choline (an abundant phospholipids constituent), or 5-methyl-tetrahydrofolate. Thus, the SAM/SAH ratio may also be tied to metabolism of phospholipids (see, for example, Tehlivets *et al*., 2004) and folic acid. How the status of SAM/SAH, phospholipids, and folate may affect the life span, and whether such an effect contributes to epigenetic control are interesting questions, and they may turn out to be highly relevant to human health.

NAD

NAD (nicotinamide adenine dinucleotide, or NAD^+) is a key compound that captures electrons in the form of hydride ($H:^-$) during oxidative catabolism, *e.g.* glycolysis and TCA cycle, and is critical for several reactions that directly or indirectly modulate chromatin dynamics. As stated above, the availability of nucleocytosolic Ac-coA directly impacts the process of histone acetylation in both yeast and mammalian cells (Takahashi *et al*., 2006; Wellen *et al*., 2009). Interestingly, the yeast Ac-coA synthetase Acs2p, and its human homologue AceCS1, are regulated in a nutrient responsive manner, as they assume full activity after they are deacetylated by sirtuins (Hallows *et al*., 2006; Starai *et al*., 2002). Contrary to many reactions in which NAD is the essential coenzyme that only changes its redox state, in sirtuin-mediated deacetylation reactions, NAD is hydrolyzed into nicotinamide and, as the acceptor of the liberated acetate function, O-acetyl-ribose. The former is a potent inhibitor of sirtuin HDAC activity, whereas the

latter is a novel signaling molecule related to calcium homeostasis and other pathways (Sauve *et al*., 2006). Because of the obligatory need of NAD for catalysis and the susceptibility for nicotinamide inhibition, the activity of sirtuins is controlled by the intracellular ratio of NAD: NADH (as well as their derivatives; Fulco *et al*., 2003; Imai *et al*., 2000). In each round of glycolysis and TCA cycle in which cells extract energy from glucose and pyruvate breakdown, multiple molecules of NAD are reduced into NADH, hence decreases the NAD/NADH ratio and downregulates the overall sirtuin activity. Conceptually, this reduction of sirtuin function may then be compensated for by keeping the Ac-coA synthetase at bay, due to its inactivation by acetylation.

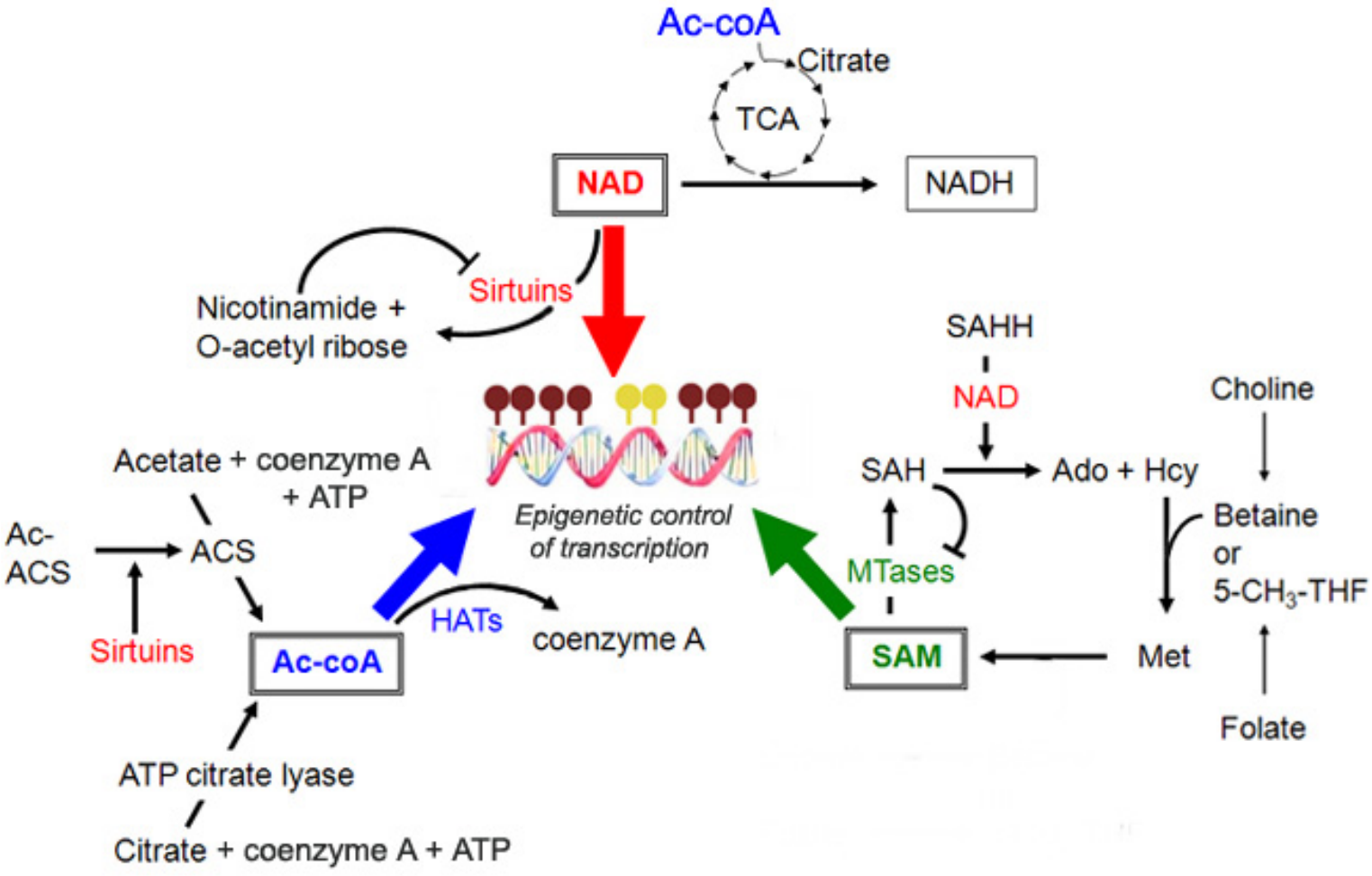

Figure 1. NAD, acetyl coA and SAM are elemental for epigenetic control of transcription including methylation of DNA ('lollypops') and post-translational modifications of histones and non-histone chromatin factors (not shown). NAD contributes to transcriptional control mainly via the activity of the protein deacetylase sirtuin, which uses NAD as one of the substrates. Sirtuins is also important for maintaining the activity of the acetyl coA synthetase by removing the inhibitory acetylation. Ac-coA provides the acetyl group for histone and protein acetyltransferases. Ac-coA is synthesized by acetyl coA synthetase and ATP-citrate lyase that use acetate and citrate as the precursor, respectively. Citrate is an intermediate/product of the TCA cycle. SAM is the methyl donor for DNA, RNA, histones, and non-histone proteins methylation. SAH generated in each round of methylation reaction is a potent inhibitor of methyltransferases, and has to be cleared by SAH hydrolase. NAD is an essential coenzyme for SAHH. Synthesis of methionine from homocysteine is achieved through extracting the methyl group from betaine, derived from choline, or 5-methyl-THF, a derivative of folic acid. Metabolism of phospholipids and folic acid may thus indirectly contribute to epigenetic regulation. Likewise, the abundance of

NAD and citrate is linked to the cellular energy flux, e.g. the TCA cycle. Changes in the expression of certain genes may therefore be influenced significantly. Abbreviations used in this figure are: Ac-coA, acetyl coenzyme A; ACS, acetyl coA synthetase; Ac-ACS, acetylated ACS; Ado, adenosine; HATs, histone acetyltransferases; Hcy, homocysteine; Met, methionine; MTases, methyltransferases; NAD, nicotinamide adenine dinucleotide; SAH, S-adenosyl homocysteine; TCA, tricarboxylic cycle; THF, tetrahydrofolate.

Fluctuation of the NAD abundance modulates the activity of sirtuin HDACs that act on acetylated histones. Histone deacetylation not only represses transcription, but also inhibits recombination. One of the functions of a yeast sirtuin Sir2p is to suppress the formation of rDNA extrachromosomal circles (ERCs) that have been postulated to be related to cellular senescence (Kaeberlein *et al*., 1999). Thus, the metabolism and availability of NAD may impact the genome and cellular physiology in multiple ways, including global and local changes in chromatin structures and transcriptional activities.

In conclusion, epigenetic control of gene activity is of immense importance in human health. Many activities controlling chromatin dynamics require compounds that shuttle between different cellular functions and pathways. Gene regulation is thus linked to the metabolic status of cells. In the era of ‘omics’ and systems biology in which researchers focus their attention on coordinating different disciplines and approaches, the same integrative efforts can and should be applied to epigenetics and metabolism. Studies of Ac-coA, NAD, and SAM mentioned above have paved the foundation for further exciting research.

Acknowledgements

Due to the page limitation, we apologize to our colleagues for being unable to include all relevant references. JL is currently supported by a doctoral thesis completion fellowship from the College of Natural Science, Michigan State University.

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