REVIEW

Methyl Metabolism and the Clock: An Ancient Story With New Perspectives

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Abstract   Methylation, that is, the transfer or synthesis of a –CH$_3$ group onto a target molecule, is a pervasive biochemical modification found in organisms from bacteria to humans. In mammals, a complex metabolic pathway powered by the essential nutrients vitamin B9 and B12, methionine and choline, synthesizes *S*-adenosylmethionine, the methyl donor in the methylation of nucleic acids, proteins, fatty acids, and small molecules by over 200 substrate-specific methyltransferases described so far in humans. Methylations not only play a key role in scenarios for the origin and evolution of life, but they remain essential for the development and physiology of organisms alive today, and methylation deficiencies contribute to the etiology of many pathologies. The methylation of histones and DNA is important for circadian rhythms in many organisms, and global inhibition of methyl metabolism similarly affects biological rhythms in prokaryotes and eukaryotes. These observations, together with various pieces of evidence scattered in the literature on circadian gene expression and metabolism, indicate a close mutual interdependence between biological rhythms and methyl metabolism that may originate from prebiotic chemistry. This perspective first proposes an abiogenetic scenario for rhythmic methylations and then outlines mammalian methyl metabolism, before reanalyzing previously published data to draw a tentative map of its profound connections with the circadian clock.

Keywords   methylation, circadian, evolution, folic acid, adenosylmethionine, SAM, prebiotic

EVOLUTION

In the Beginning There Was. . . Chemistry

The characteristics of life, that is, responsiveness to the environment, growth, reproduction, adaptation, and homeostasis, are all found at the level of one single cell. Cellular metabolism likely originates from chemical reactions occurring on the prebiotic earth, when eobionts were evolving in an environment of countless chemical possibilities. Indeed, when bioenergetic processes are compared between extant organisms at the cellular level, the most diverse of these processes are seen among prokaryotes (Kluyver and van Niel, 1956).

Amino Acids and Central Carbon Metabolism

One of the most famous experiments seeking to explain the origin of organic molecules now essential for life is the 1953 Miller experiment (Miller, 1953) and its later iteration (Van Trump and Miller, 1972). In a simulated primitive atmosphere, spark discharges were shown to catalyze the synthesis of the amino acids glycine, alanine, aspartic acid and methionine. Re-analysis of Miller's archived samples with state-of-the-art analytical techniques revealed that more than 20 amino acids were abiotically synthesized (Johnson et al., 2008; Parker et al., 2011a, 2011b, 2011c). More recently, others have confirmed synthesis of

amino acids in prebiotic conditions (Mompean et al., 2019; Wollrab et al., 2016; Cooper et al., 2017).

A Step Toward Biological Methylation: Sulfur-Containing Amino Acids

Among the amino acids synthesized abiotically by a spark discharge in a reducing atmosphere containing H_2S are the sulfur-containing amino acids methionine, homocysteine, and most likely cysteine (Parker et al., 2011c). The synthesis of methionine under an alternative sulfur-containing simulated prebiotic condition was independently reported (Steinman et al., 1968). In an evolutionary context these are especially important, because among all elements used to build amino acids (*i.e.* O, N, C, H, S) sulfur, being the most electronegative, gives amino acids different properties and makes the methyl of methionine more readily attacked by a nucleophilic molecule, essentially resulting in its methylation, with the conversion of methionine to homocysteine. Thus, methionine and homocysteine, which constitute half of the methyl cycle, may have been present on the prebiotic earth. Moreover, methionine has been shown to spontaneously react with ATP or adenosine to yield S-adenosylmethionine (SAM), the methyl donor co-substrate used by methyltransferases today (Laurino and Tawfik, 2017). Since (deoxy)ribonucleotides and (deoxy)ribonucleosides, including adenosine, can also be synthesized abiotically under conditions likely to have been prevalent on the prebiotic earth (Xu et al., 2017, 2018, 2019, 2020; Patel et al., 2015; Powner et al., 2009; Xu et al., 2021), methylation reactions using SAM may thus have occurred before the development of life. But spontaneous methylation reactions may have occurred even before methionine or SAM.

Daily Methylation Rhythms May Have Occurred on the Prebiotic Earth

Abiotic methylation of simple amines such as ethanolamine and glycine, both products of the Miller experiment (Parker et al., 2011c), can occur in prebiotic conditions with formaldehyde (Waddell et al., 2000), a legitimate prebiotic alkylating agent readily synthesized by photolysis of water vapor with carbon monoxide (Bar-Nun and Hartman, 1978). The fact that formaldehyde can be synthesized by *photolysis* of water vapor with carbon monoxide (Bar-Nun and Hartman, 1978) is meaningful since it indicates that prebiotic methylation reactions may have occurred more frequently during the day.

Formaldehyde is highly reactive, and has been hypothesized to be the only prebiotic 1-carbon molecule able to generate more complex organic molecule, including amino acids and sugars (Weber, 2002). The main source of formaldehyde was likely gas phase photochemical synthesis in the atmosphere followed by transport into bodies of water by rain (Pinto et al., 1980; Cleaves, 2008). In aqueous solution, subsequent photochemical transformations of formaldehyde occur: oligomerization, and reactions with amines, sulfur species and HCN (Cleaves, 2008). By converting formaldehyde into non-volatile species, these reactions increase the concentration of formaldehyde. Other geochemical mechanisms, including eutectic freezing, may further increase the local concentration of formaldehyde, enabling more demanding reactions (Cleaves, 2008). Together this suggests that, during the day the amount of formaldehyde and dependent methylations would progressively increase. At night, the reactivity of formaldehyde, together with the absence of sunlight, would lead to its progressive decline.

In time, increased methylation reactions during the day may have been a way by which eobionts "sensed" daylight, and later developed strategies to avoid or expose themselves to this increased "methylation potential" according to their chemical needs. Interestingly, formaldehyde remains to this day an intermediate in some methyltransferase reactions (Meller et al., 1975; Stover and Schirch, 1990).

It is thus likely methylations occurred in the prebiotic day and imprinted their influence on abiogenesis, and cellular metabolism may have co-opted methylations as an endogenous regulatory mechanism. Now, most methyltransferases use SAM as a co-substrate, transferring the methyl moiety of SAM to their specific target substrate: nucleic acids, proteins, membrane components, hormones, xenobiotics and small molecules. It is not surprising, therefore, that SAM is the second-most used enzyme substrate (Cantoni, 1975), after ATP. If methylation reactions were more frequent during the day on the prebiotic earth, influencing the development of life, an intimate link between circadian rhythms (biological rhythms ticking with a period of a day) and methyl metabolism should be found in organisms alive today. Keeping this in mind, we first provide an overview of the methyl metabolism and its regulation in mammals.

METHYL METABOLISM TODAY

Vitamin B9: Substrate For *de Novo* Methyl Synthesis

The readers are invited to consult excellent reviews on methyl and folate metabolism (Tibbetts and Appling, 2010; Ducker and Rabinowitz, 2017), as only

a brief overview necessary to understand the next section will be given here. The methyl of methionine mainly originates from a folate cofactor (vitamin B9) at the end of a chain of reduction (the folate cycle, Figure 1). In the folate cycle, a formate ($HCOO^-$) of mitochondrial origin is added to tetrahydrofolate in the cytoplasm, forming 10-formyltetrahydrofolate (10-CHO-THF), further reduced to 5,10-methenyltetrahydrofolate (CH^+-THF) then 5,10-methylenetetrahydrofolate (CH_2-THF) by the same trifunctional enzyme *methylenetetrahydrofolate dehydrogenase, cyclohydrolase, and formyltetrahydrofolate synthetase 1* (MTHFD1). Another enzyme, *methylenetetrahydrofolate reductase* (MTHFR), irreversibly synthesizes 5-methyltetrahydrofolate (CH_3-THF) from CH_2-THF, and *methionine synthase* (MTR) uses CH_3-THF as a cosubstrate to methylate homocysteine to methionine in a reaction that also requires its cofactor cobalamin (vitamin B12). Methionine and ATP are then converted to SAM by *methionine adenosyltransferases*, with MAT1A being expressed mainly in the liver, while MAT2A and MAT2B are ubiquitous (Shimizu-Saito et al., 1997; Ramani and Lu, 2017) (Figure 1). Collectively, folate and methyl metabolism are conventionally grouped together under the term "C1 metabolism."

Folate metabolism is a cycle because the 1-carbon of formate leaving the mitochondria originates from serine entering the mitochondrial folate arc that is a reversal of the reactions explained above (Figure 1). The reason why cytoplasmic folate metabolism requires mitochondrial formate originates in the differential electrochemical potential between mitochondrial NADH and cytosolic NADPH, with the high cytosolic $NADPH/NADP^+$ ratio favoring the flux from formate to serine (Yang and MacKenzie, 1993). Serine is not the only amino acid used as a source of 1-carbon units, however. The glycine cleavage system (GCS) catabolizes excess glycine and converts its α-carbon into CH_2-THF, and the histidine degradation pathway leads to CH^+-THF synthesis (Beaudet and Mackenzie, 1976; Kohls et al., 2000) (Figure 1).

Folates are also used as direct cofactors for several very ancient enzymes catalyzing 1-carbon transfers in the metabolism of nucleotides: TYMS catalyzes the methylation of dUMP to dTMP using CH_2-THF (Mishanina et al., 2012); GART and ATIC use 10-CHO-THF to progressively assemble the purine base of inosine monophosphate (Nelson et al., 2017). Moreover, in bacteria and mitochondria the initiation of translation is coupled to the folate cycle via *methionyl-tRNA formyltransferase* (MTFMT), synthesizing the formyl-methionine-tRNA that is essential for the initiation of proteins (Minton et al., 2018; Morscher et al., 2018). These enzymatic reactions are all shown in Figure 1.

Transport and Sequestration of Folates

Entry of folate into the cell is controlled by the folate transporter SLC19A1, and into the mitochondrial matrix by SLC25A32. Another transporter, SlC46A1, transport folates across membranes in acidic environment. Finally, a reduced folate receptor, *Folr*, with two tissue- and developmental stage-specific isoforms in mice and three in humans, transport CH_3-THF into the cell (Clare et al., 2019; Tibbetts and Appling, 2010) (Figure 1). Dietary folic acid, or dihydrofolate (DHF) generated by the reaction catalyzed by TYMS, need to be reduced to THF by *dihydrofolate reductase* (DHFR) to re-enter the folate cycle. Finally, to improve folate retention in the cell and its use as a cofactor, a poly-γ-glutamate tail is added to the to the *p*-aminobenzoic acid moiety of THF, a reaction catalyzed by *folylpolyglutamate synthetase* (FPGS) (Tibbetts and Appling, 2010) (Figure 1).

Salvage of Methyl Groups From Betaine

During vertebrate evolution, the methyl cycle in adult animals became not completely reliant on folates. An enzyme called *Betaine-homocysteine S-methyltransferase* (BHMT) can re-methylate homocysteine using one of betaine's three methyl groups (betaine is also known as *N*, *N*, -trimethylglycine), but only in specific tissues such as the liver and kidneys in human (Sunden et al., 1997). Unlike the *de novo* methyl of CH_3-THF, however, the three methyl groups of endogenous betaine originate from the sequential SAM-dependent methylation of phosphatidylethanolamine to phosphatidylcholine by the SAM-dependent *phosphatidylethanolamine N-methyltransferase* (PEMT), followed by the conversion to choline, then to betaine by several successive enzymatic activities (Sherriff et al., 2016) (Figure 1). The re-methylation of homocysteine to methionine using betaine is thus the salvage of methyl groups from phospholipids. Choline is an essential nutrient in mouse, human and many other vertebrates because it is a source of betaine as well as phosphatidylcholine.

SAM-Binding MTases

As we have seen above, several enzymes use folates as 1-carbon donors resulting in the methylation of their substrate. However, virtually all methyltransferases use SAM as a cofactor. SAM-binding proteins are usually classified into 9 superfamilies based on domain structures (Martin and McMillan, 2002; Schubert et al., 2003; Petrossian and Clarke, 2009a, 2009b). In 2011, based on primary sequences, predicted secondary structures and solved crystal
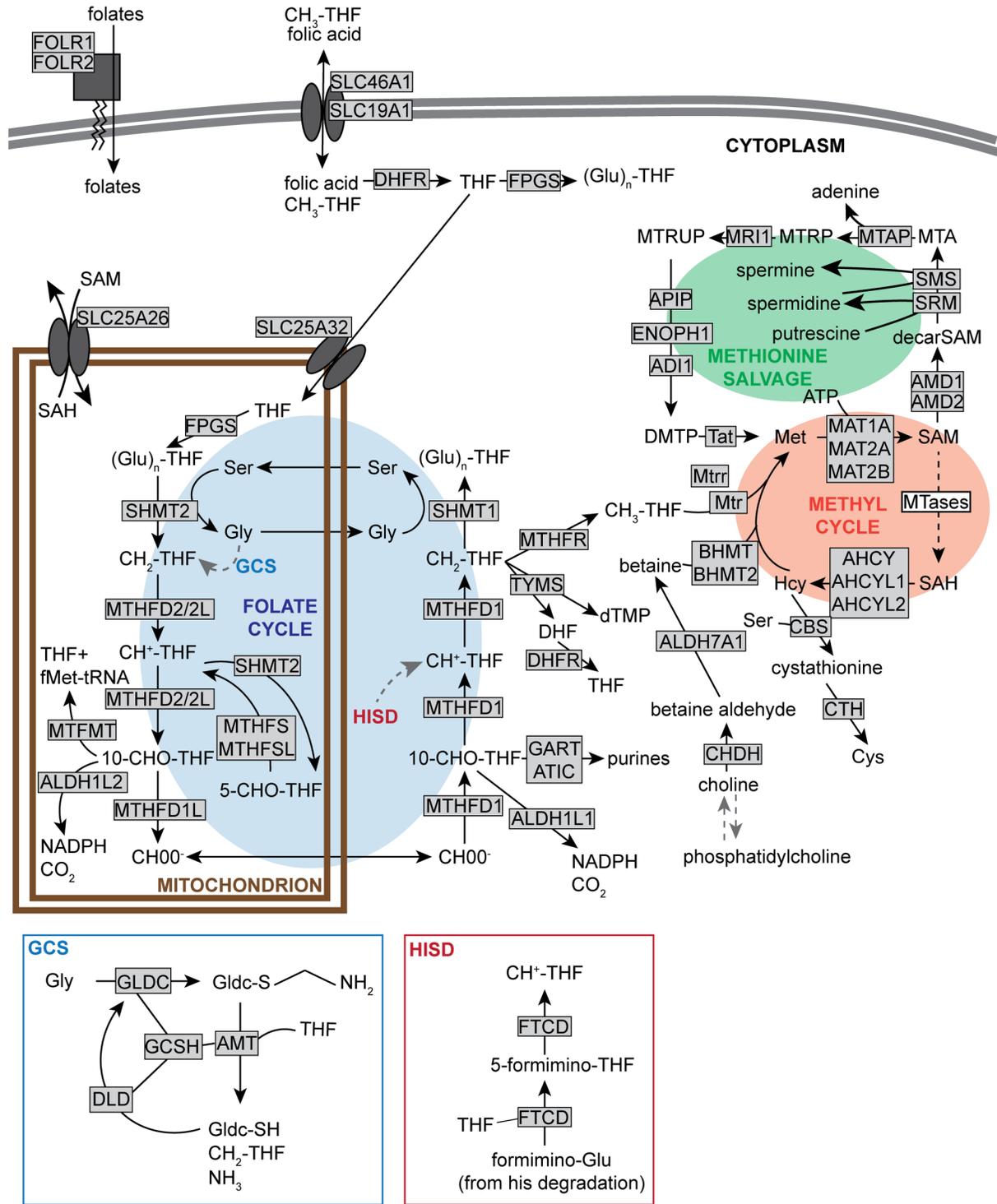
Figure 1. of 1-carbon (folate and methyl) metabolism. Enzymes are shown in grayed rectangle, with the exception of SAM-dependent methyltransferases, collectively represented as "MTases" in a white rectangle. Enzymes using or generating folates and/or methyl groups, as well as enzymes directly dependent on the metabolites of the methyl cycle are represented. GCS is the glycine cleavage system; through its sulfur (S), GLDC forms a bound intermediate with glycine, further cleaved into CH_2-THF and NH_3 by the activity of the other enzymes in the complex, with regenerated GLDC(-SH). HISD is the histidine degradation pathway, ultimately leading to the degradation of formimino-glutamate (Glu) to CH^+-THF mediated by FTCD. The information about enzymes mediating each step was obtained from the KEGG. Based on published reviews and pathways from public databases (Tibbetts and Appling, 2010; Clare et al., 2019; Kanehisa, 2019; Kanehisa et al., 2019; Kanehisa and Goto, 2000). Abbreviations: SAM = S-adenosylmethionine; GCS = glycine cleavage system; GLDC = Glycine decarboxylase; CH_2-THF = 5,10-methylenetetrahydrofolate; GLDC = Glycine decarboxylase;

(continued)

Figure 1.   (continued)

CH⁺-THF = 5,10-methenyltetrahydrofolate; FTCD = Formimidoyltransferase cyclodeaminase; KEGG = Kyoto Encyclopedia of Genes and Genomes; DHFR = Dihydrofolate reductase; FPGS = folylpolyglutamate synthetase; (Glu)n-THF = polyglutamylated-THF; MTRUP = S-Methyl-5-thio-D-ribulose 1-phosphate; MTRP = S-Methyl-5-thio-D-ribose 1-phosphate; Mtap = S-methyl-5'-thioadenosine phosphorylase; MTA = methylthioadenosine; Apip = Apoptotic protease activating factor 1 (APAF1)-interacting protein; Enoph1 = Enolase-phosphatase 1; Sms = Spermine synthase; Srm = Spermidine synthase; fMet-tRNA = N-formylmethionine-tRNA; MTFMT = methionyl-tRNA formyltransferase; 10-CHO-THF = 10-formyltetrahydrofolate; MTHFR = Methylenetetrahydrofolate reductase; TYMS = Thymidylate synthase; DHF = dihydrofolate; GART = phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase; ATIC = 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase; MTR = Methionine synthase; BHMT = Betaine-homocysteine S-methyltransferase; ALDH7A1 = aldehyde dehydrogenase 7 family member A1; Cth = Cystathionine gamma-lyase; CHDH = choline dehydrogenase; Gly = glycine; GCSH = glycine cleavage system protein H; AMT = aminomethyltransferase; DLD = dihydrolipoamide dehydrogenase; SH = thiol; HISD = histidine degradation pathway; THF = tetrahydrofolate; SAH = S-adenosylhomocysteine; MTHFS = Methenyltetrahydrofolate Synthetase; MTHFSL = 5-formyltetrahydrofolate cyclo-ligase; 5-CHO-THF = 5-formyl-THF; dTMP = deoxythymidine monophosphate; DFHR = dehydroflate reductase; ATP = adenosine triphosphate; CBS = Cystathionine beta synthase.

structures of known and putative *S*-adenosylmethionine-dependent methyltransferases, human MTases were assigned to the 9 superfamilies of MTases (Petrossian and Clarke, 2011). It is estimated there are between 200 and 300 methyltransferases in the human genome (Petrossian and Clarke, 2011).

Pathologies and Treatments Related to 1C Metabolism

1C metabolism is at the origin of many pathologies, and comprehensively covering this subject is out of the scope of this perspective. The reader is invited to consult selected previous works (Ducker and Rabinowitz, 2017; Carmel and Jacobsen, 2001; Lippi and Plebani, 2012; Longnecker, 2002; Poirier et al., 2003).

Due to its role in *de novo* dTMP and purines synthesis, the folate cycle is essential for rapidly proliferating cells and tissues, for which dietary folate is critical. The main dietary form of folate is normally polyglutamylated CH₃-THF, in high amounts in green leafy vegetables (from Latin, *folium* means leaf). Natural folates are, however, highly unstable during food storage and preparation, resulting in folate deficiency to be relatively common. Folate deficiency was recognized as a frequent cause of anemia in children and adults in the early 1940s (Hoffbrand and Weir, 2001), and of neural tube defects in newborns in 1965 (Crider et al., 2011), prompting many governments to recommend fortification of flours and cereals with folic acid, a stable form of folate, in the late 1990s. Folate supplementation was also found to reduce the risk of congenital heart defects (Bailey and Berry, 2005).

Many inborn errors of 1C metabolism have been identified in humans, including lethal AHCY mutations (Vugrek et al., 2009), MTR deficiencies causing a wide array of hematological and neurological deficits (Watkins and Rosenblatt, 1989), MTHFR and CBS polymorphisms causing hyperhomocysteinemia and homocystinuria associated with nervous, connective

tissues and vascular damages (Schwahn and Rozen, 2001; Carmel and Jacobsen, 2001; Kraus et al., 1999; Kozich and Kraus, 1992), an MTHFD1 polymorphism (Brody et al., 2002) and MTHFD1 L variants associated with neural tube defects (Parle-McDermott et al., 2009) and late-onset Alzheimer's disease (Naj et al., 2010). Very recently, an MTHFR polymorphism has been associated with chronic insomnia that could be resolved by targeting MTHFR's loss of function (Kapoor et al., 2021).

Considering the importance of 1C metabolism for proliferation, it is not a surprise that it is a major target for chemotherapies: methotrexate, permetrexed and their analogues are antifolates, inhibiting DHFR and other folate-dependent enzymes (Gonen and Assaraf, 2012); methotrexate is also recommended as the standard treatment for arthritis (Cronstein and Aune, 2020). Surprisingly, SAM has also been shown to block the growth of cancer cells (Ilisso et al., 2018; Mosca et al., 2020; Wang et al., 2017), and has been inconclusively proposed as a treatment for depression (Mischoulon et al., 2015), arthritis (Najm et al., 2004), and liver diseases (Guo et al., 2015).

Known 1C pathologies are related to proliferative tissues because their symptoms are the most obvious, but work in mice have shown that 1C metabolism deficiencies in adult animals have consequences in non-proliferative tissues. For example, knock-out of MTHFR, BHMT, CBS, or PEMT in mice all cause liver steatosis (Maclean et al., 2010; Chen et al., 2001; Schwahn et al., 2003; Teng et al., 2011; Zhu et al., 2003), which resonates with increased homocysteine often found in patients with non-alcoholic fatty liver disease (Dai et al., 2016).

While there is no question 1C metabolism underlies many aspects of biology, this very essentiality—knock-out of MTHFD genes, AHCY, MTR, MAT2A are all lethal, and that of MTHFR is semi-lethal depending on the genetic background (Miller et al., 1994; Ducker and Rabinowitz, 2017; Swanson et al., 2001; Chen et al., 2001; Lawrance et al., 2011; Dickinson et al., 2017)—has

hampered a clear understanding of its regulations and ramifications. Future investigations with modern genetic and metabolic tools should clarify the physiological functions of key enzymes of 1C metabolism and how they are regulated in adults.

CIRCADIAN INFLUENCE ON METHYL METABOLISM

### Links Between the Methyl Cycle and the Circadian Clock

Now that we have an overview of how C1 metabolism is organized, we can start looking into how it is linked with the clock. The rhythmic transcriptional programs underlying circadian rhythms in organisms from cyanobacteria to humans have been shown to be dependent on methylation (Fustin et al., 2020). Disruption of the methyl cycle with a pharmacological inhibitor of *Adenosylhomocysteinase* (AHCY, Figure 1) to induce accumulation of SAH, which can displace SAM from SAM-binding methyltransferases and act as a competitive inhibitor (Carmel and Jacobsen, 2001), led to the lengthening of the circadian period in all eukaryotic cells tested (Fustin et al., 2020). This shows that there is a link between circadian rhythms and methyl metabolism conserved during evolution. Available evidence also indicate that this link is bidirectional, *i.e.* that methyl metabolism as a whole is under the control of the circadian clock. Rhythms in histone (Koike et al., 2012), DNA (Oh et al., 2018) and mRNA (Zhong et al., 2018) methylation have been observed, and there has been suggestions that the methyl cycle and its outputs (glutathione and polyamine synthesis), may be under clock control, notably from the observations that polyamines, SAM and SAH display circadian oscillations in the liver (Eckel-Mahan et al., 2012; Atwood et al., 2011; Panda, 2016; Krishnaiah et al., 2017). Moreover, several MTases have been linked to circadian rhythms: the class V-like, SET domain-containing histone lysine MTases EZH2 (Etchegaray et al., 2006), KMT2A (Katada and Sassone-Corsi, 2010), KMT2C (Valekunja et al., 2013) and KMT1/SUV39H1 (Duong and Weitz, 2014), the class I-like mRNA N^6-adenosine MTAse METTL3 and cap N^7-guanine MTase RNMT (Fustin et al., 2013). More recently, AHCY was reported to directly interact with BMAL1 to support the histone methylation that accompanies rhythmic gene expression (Greco et al., 2020).

To provide a comprehensive review of the circadian influence on C1 metabolism at the transcriptional level, we appended the latest Gene Ontology (GO) source files and their annotations from geneontology.org with a new "*C1 metabolism*" (GO:9999999)

biological process node containing all proteins shown in Figure 1. The modified GO source files are provided as supplemental information. Next, we re-analyzed the Gene Expression Omnibus dataset GSE54652, a high quality microarray-based transcriptomics atlas from various mouse tissues sampled at 2 h intervals for 2 days (Zhang et al., 2014), with the newest version of MetaCycle, an R package frequently used for detecting rhythmic signals from large scale time-series data (Wu et al., 2016). Genes whose expression was significantly rhythmic (Benjamini-Hochberg-adjusted p value < 0.05) in each tissue were identified, and GO analysis was performed using the appended GO source files on the Galaxy server (usegalaxy.org) using the GOEnrichment tool (Afgan et al., 2018). One GO analysis was performed on rhythmic genes for each tissue, and additional analyses were performed with genes that were rhythmic in at least a third of the tissues, and with all rhythmic genes in any tissues. To filter the most significant hits, only the biological processes enriched at least 2-fold and with a q value < 0.05 were considered. The MetaCycle and GOEnrichment outputs are provided in Supplementary Tables S1 and S2, respectively.

Several observations stood out. First, *C1 metabolism* was the second most significant hit in the liver, immediately following the "circadian rhythms," clearly showing the profoundly circadian organization of C1 metabolism in this tissue, where 31 out of 50 genes were significantly rhythmic (Figure 2a). Further indicating the liver as a major regulator of methyl metabolism, more than half of the *C1 metabolism* enzymes analyzed here were enriched in the liver (in terms of expression averaged across 48 h, Figure 2b). Second, *C1 metabolism* was significant in the hypothalamus, where the mammalian master clock resides (Moore and Eichler, 1972; Stephan and Zucker, 1972). Two related GO terms, *transsulfuration* and *sulfur amino acid biosynthetic process*, were also significantly enriched in this tissue. Third, *C1 metabolism* remained among significant hits when GO analysis was performed on genes rhythmic in at least 4 of the 12 tissues. And last but not least, when all rhythmic genes from any tissues were grouped for GO analysis, the most significant GO term was *C1 metabolism*, because out of the 50 genes included in *C1 metabolism*, 43 were found to be rhythmic in at least 1 tissue.

To further probe the link between 1C metabolism and circadian rhythms, we next looked at the high temporal resolution metabolome profiling published by Krishnaiah and co-authors (Krishnaiah et al., 2017), and performed overrepresentation analysis using Metaboanalyst 5.0 (Pang et al., 2021; Xia et al., 2009), using rhythmic liver metabolites (BH.Q. < 0.01 in Dataset S2 from
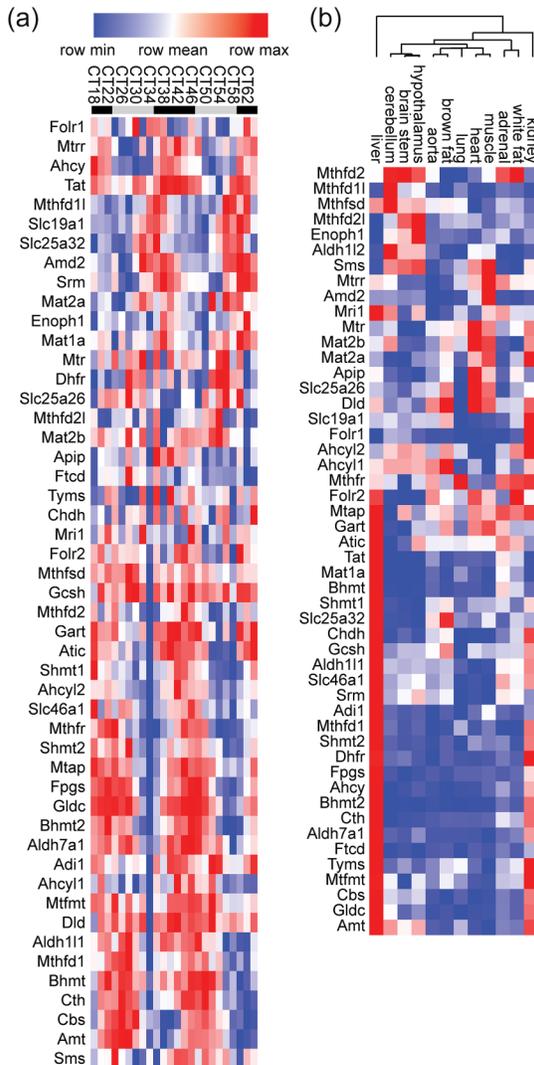
Figure 2. Orchestration of hepatic folate/methyl metabolism by the circadian clock. (a) Heatmap representation of expression values from GSE54652 of all genes shown in Figure 1, in the liver. Rows were organized by Pearson correlation-clustering using GenePattern (Reich et al., 2006; de Hoon et al., 2004; Eisen et al., 1998), leading to the grouping of genes with similar expression patterns. The legend at the top shows Circadian Time (CT), 0 (24, 48) being the start of the rest phase of the mouse. Sampling frequency was every 2 h, but for readability labels only show every 4 h. Mice were kept in constant darkness throughout the experiment; the start of the active phase in these conditions being determined by the endogenous circadian clock. Gray bars represent the inactive phase ("subjective" day), black bars the active phase ("subjective" night). Notice the difference in phase between genes at the top of the heatmap and toward the lower half. **(b)** Pearson correlation-clustering of mean gene expression in GSE54652 across 48 h of the same genes as in (a) in all tissues investigated. Notice the high expression of most genes in the liver, followed by the kidneys. The tree at the top shows the results of the Pearson correlation-clustering that was also performed on the columns. Notice the significant separation between expression patterns in brain tissues and peripheral tissues. See also supplemental information for raw expression values in all tissues.

Krishnaiah et al.) as test dataset and all detected metabolites from the same table as reference (using The Small Molecule Pathway Database as a library (Jewison et al., 2014)]. The results, presented in Figure 3, lead to the same conclusion as above: the 3 most significantly enriched metabolic pathways were methylhistidine metabolism, methionine metabolism and glycine and serine metabolism, all overrepresented notably due to the fact that both SAM and SAH, at the crossroad of these pathways, are rhythmic, together with other C1-derived metabolites including methylthioadenosine (MTA), glycine, histidine and cystathionine (Figure 3).

Together these observations highlight the strong link between the circadian clock and C1 metabolism in mammals. The molecular mechanisms underlying this influence, and the physiological function of such a temporal organization, remain to be identified.
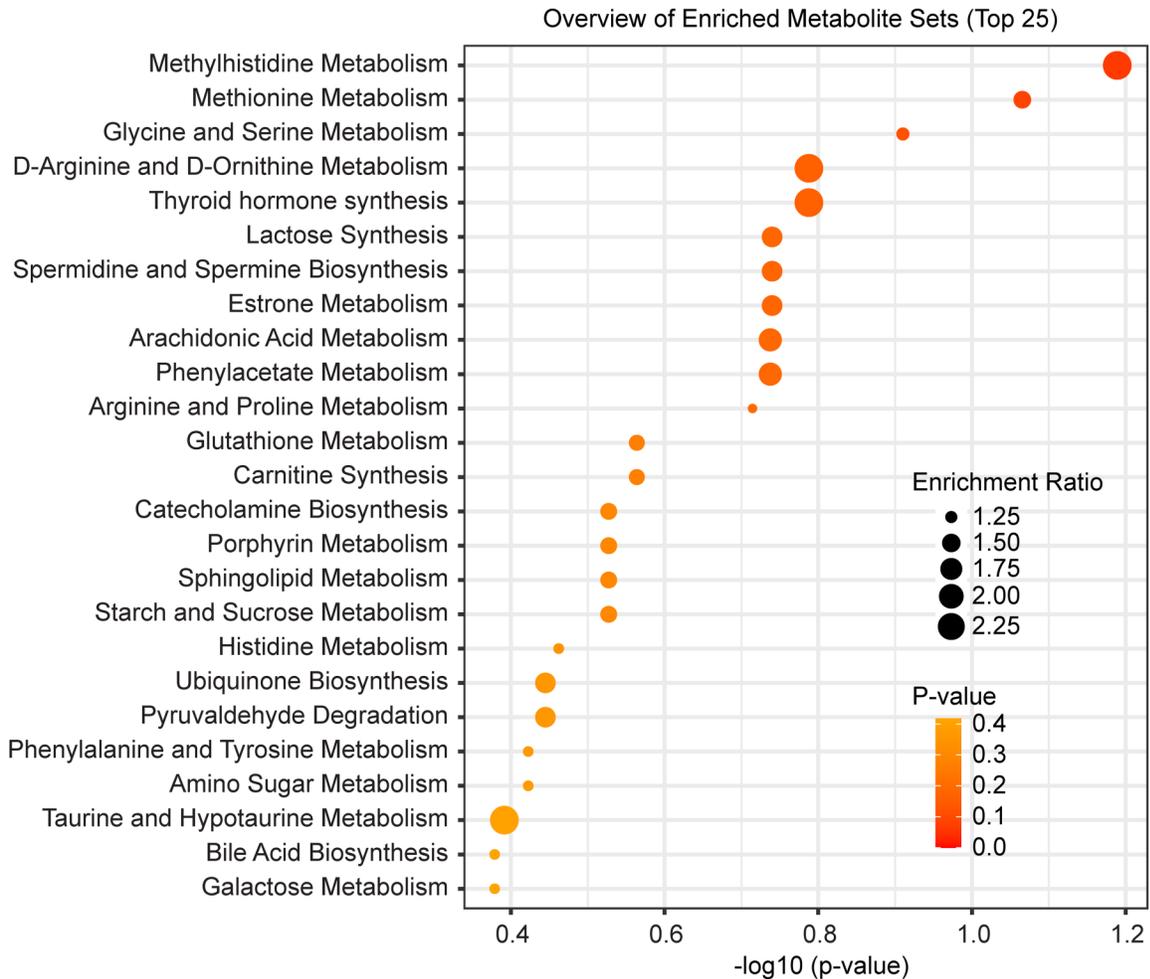
SELECTED FURTHER QUESTIONS

What Is the Physiological Meaning of Temporal Organization?

Among the few genes consistently rhythmic were *Mthfd1l* and *Mthfr*, their antiphasic expression (Figure 4) in many tissues especially interesting considering their key functions: MTHFD1L provides formate to the cytoplasmic folate arc, and MTHFR is the critical link between cytoplasmic folate metabolism and the methyl cycle (Figure 1). This antiphasic expression prompts many questions. Is formate mainly released into the cytoplasm during the night (active phase in mice), activating cytoplasmic folate metabolism, when *Mthfd1l* expression is high? And is CH_3-THF mainly produced during the day, when *Mthfr* expression is high? Why should it be so? Interestingly, hepatic SAM is highest during the subjective day, at CT4, further suggesting a link between the day and methylation (Krishnaiah et al., 2017). What would happen if the link between the circadian clock and folate/methyl metabolism is severed, for example by removing circadian rhythms of *Mthfr* or *Mthfd1l* expression?

A Master for All, or a Division of Labor?

Based on average expression levels across 24 h of genes involved in methyl/folate pathways (Figure 4), there is a considerable level of tissue specificity, with the liver clearly a master regulator in mouse. *Ahcy* and *Mat1a* are enriched in the liver, while their respective homologues *Ahcyl1, Ahcyl2* and *Mat2a* are expressed ubiquitously at comparable levels. What is

Figure 3.   Orchestration of hepatic folate/methyl metabolism by the circadian clock. Results of overrepresentation analysis by Metabo-analyst 5.0 using previously published data (Krishnaiah et al., 2017). The most significant hits are all related to 1C metabolism. At the bottom are listed all metabolites belonging to each of these three pathways present in the dataset, the rhythmic ones written in red. NAD = Nicotinamide adenine dinucleotide; FAD= Flavin adenine dinucleotide; NADH = Reduced nicotinamide adenine dinucleotide; ADP = adenosine diphosphate.

the contribution of each of these genes to systemic folate and methyl metabolism, and what is their involvement in the development of the numerous pathologies related to methylation deficiencies, including cancer, hepatitis, fibrotic and vascular diseases and neurological disorders?
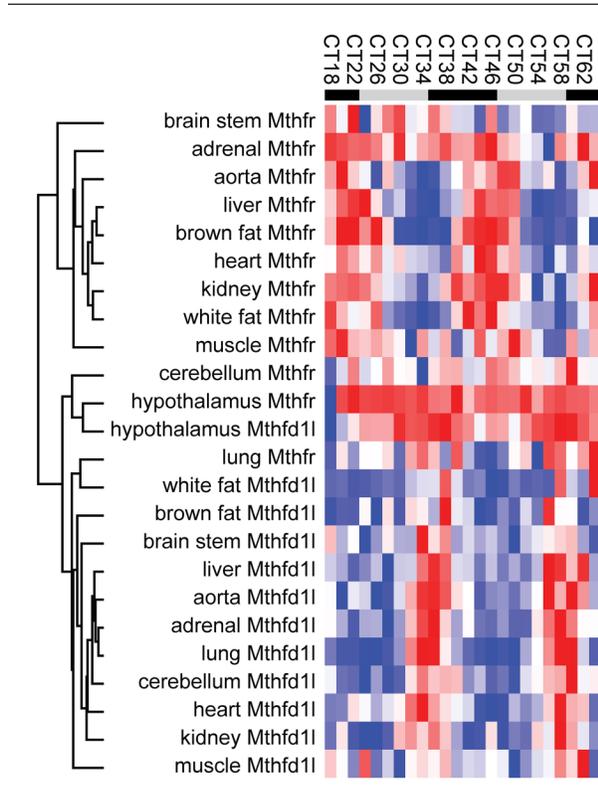
Figure 4. Expression of *Mthfd1l* and *Mthfr* in most tissues. Expression values for *Mthfd1l* and *Mthfr* in GSE54652 were clustered by Pearson correlation, leading to the clear separation of *Mthfd1l* and *Mthfr* into two groups with very few exceptions. Notice higher expression of both genes in the hypothalamus, at the center of the heatmap. Sampling frequency was every 2 h, but for readability labels only show every 4 h.

How Is Methyl Metabolism Regulated in Other Species?

The focus of this review was mammalian methyl metabolism, but what about other species? The circadian clock in invertebrates, plants, unicellular green algae and cyanobacteria is also sensitive to methylation inhibition (Fustin et al., 2020), but is the folate/methyl metabolism in these organisms also orchestrated by the circadian clock? Moreover, is the folate/methyl metabolism in unicellular green algae and cyanobacteria associated not only with the circadian clock but also with the light/dark cycle? Indeed, it would be predicted from the discussion about the prebiotic origin of methylation above that higher methylation in single-celled organisms directly responsive to light should be associated with the light phase. A hint that this may be the case, at least for mRNA methylation, has recently been shown in *Arabidopsis* (Wang et al., 2021). Understanding methyl metabolism in plants and algae may be relevant for biomass production.

CONCLUSIONS

Light-induced prebiotic methylations may have influenced the origin and evolution of life to become the complex array of regulatory mechanisms it is today, and its hypothesized association with daylight may have been an early determinant in the evolution of circadian rhythms. In extant organisms, evidence indicate an intimate relationship between the circadian clock and methyl metabolism. Yet, because of the complexity of the folate/methyl metabolism and the sheer variety of methyltransferases, the regulation of methyl metabolism, and its impact on physiology and behavior, remain poorly understood. Future investigations into the relationship between methyl metabolism and circadian rhythms using state-of-the-art genetic editing and metabolic tracing should provide further insights. Given the clinical importance of folate and methionine metabolism, a more thorough understanding of these pathways is needed.

ABBREVIATIONS

ALDH1L1 and ALDH1L2 = 10-formyltetrahydrofolate dehydrogenases; ALDH7A1 = aldehyde dehydrogenase 7 family member A1; (Glu)n-THF = polyglutamylated-THF; Adi1 = Acireductone dioxygenase 1; AMT = aminomethyltransferase; Apip = Apoptotic protease activating factor 1 (APAF1)-interacting protein; ATIC = 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase; Cth = Cystathionine gamma-lyase; decarSAM = decarboxylated SAM; DLD = dihydrolipoamide dehydrogenase; Enoph1 = Enolase-phosphatase 1; fMet-tRNA = N-formylmethionine-tRNA; FTCD = Formimidoyltransferase cyclodeaminase; GART = phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase; GCSH = glycine cleavage system protein H; GLDC = Glycine decarboxylase; Gly = glycine; Met = methionine; Mri1 = Methylthioribose-1-phosphate isomerase; MTA = methylthioadenosine; Mtap = S-methyl-5'-thioadenosine phosphorylase; MTOBUTA = 4-Methylthio-2-oxobutanoic acid; MTRP = S-Methyl-5-thio-D-ribose 1-phosphate; MTRUP = S-Methyl-5-thio-D-ribulose 1-phosphate; Ser = serine; SHMT = Serine hydroxymethyl transferase; Sms = Spermine synthase; Srm = Spermidine synthase; Tat = Tyrosine aminotransferase; TYMS = Thymidylate synthase; CHDH = choline dehydrogenase.

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CONFLICT OF INTEREST STATEMENT

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NOTE

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REFERENCES

Afgan E, Baker D, Batut B, van den Beek M, Bouvier D, Cech M, Chilton J, Clements D, Coraor N, Grüning BA, et al. (2018) The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic Acids Res 46:W537-W544.

Atwood A, DeConde R, Wang SS, Mockler TC, Sabir JS, Ideker T, and Kay SA (2011) Cell-autonomous circadian clock of hepatocytes drives rhythms in transcription and polyamine synthesis. Proc Natl Acad Sci U S A 108:18560-18565.

Bailey LB and Berry RJ (2005) Folic acid supplementation and the occurrence of congenital heart defects, orofacial clefts, multiple births, and miscarriage. Am J Clin Nutr 81:1213S-1217S.

Bar-Nun A and Hartman H (1978) Synthesis of organic compounds from carbon monoxide and water by UV photolysis. Origins of Life 9:93-101.

Beaudet R and Mackenzie RE (1976) Formiminotransferase cyclodeaminase from porcine liver. An octomeric enzyme containing bifunctional polypeptides. Biochim Biophys Acta 453:151-161.

Brody LC, Conley M, Cox C, Kirke PN, McKeever MP, Mills JL, Molloy AM, O'Leary VB, Parle-McDermott A, Scott JM, et al. (2002) A polymorphism, R653Q, in the trifunctional enzyme methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthetase is a maternal genetic risk factor for neural tube defects: report of the Birth Defects Research Group. Am J Hum Genet 71:1207-1215.

Cantoni GL (1975) Biological methylation: selected aspects. Annu Rev Biochem 44:435-451.

Carmel R and Jacobsen DW (2001) Homocysteine in health and disease. Cambridge (UK): Cambridge University Press.

Chen Z, Karaplis AC, Ackerman SL, Pogribny IP, Melnyk S, Lussier-Cacan S, Chen MF, Pai A, John SW, Smith RS, et al. (2001) Mice deficient in methylenetetrahydrofolate reductase exhibit hyperhomocysteinemia and decreased methylation capacity, with neuropathology and aortic lipid deposition. Hum Mol Genet 10:433-443.

Clare CE, Brassington AH, Kwong WY, and Sinclair KD (2019) One-carbon metabolism: linking nutritional biochemistry to epigenetic programming of long-term development. Annu Rev Anim Biosci 7:263-287.

Cleaves HJ (2008) The prebiotic geochemistry of formaldehyde. Precambrian Res 164:111-118.

Cooper GJT, Surman AJ, McIver J, Colón-Santos SM, Gromski PS, Buchwald S, Suárez Marina I, and Cronin L (2017) Miller-Urey spark-discharge experiments in the deuterium world. Angew Chem Int Ed Engl 56:8079-8082.

Crider KS, Bailey LB, and Berry RJ (2011) Folic acid food fortification-its history, effect, concerns, and future directions. Nutrients 3:370-384.

Cronstein BN and Aune TM (2020) Methotrexate and its mechanisms of action in inflammatory arthritis. Nat Rev Rheumatol 16:145-154.

Dai Y, Zhu J, Meng D, Yu C, and Li Y(2016) Association of homocysteine level with biopsy-proven non-alcoholic fatty liver disease: a meta-analysis. J Clin Biochem Nutr 58:76-83.

de Hoon MJ, Imoto S, Nolan J, and Miyano S (2004) Open source clustering software. Bioinformatics 20:1453-1454.

Dickinson ME, Flenniken AM, Ji X, Teboul L, Wong MD, White JK, Meehan TF, Weninger WJ, Westerberg H, Adissu H, et al. (2017) Corrigendum: high-throughput discovery of novel developmental phenotypes. Nature 551:398.

Ducker GS and Rabinowitz JD (2017) One-carbon metabolism in health and disease. Cell Metab 25:27-42.

Duong HA and Weitz CJ (2014) Temporal orchestration of repressive chromatin modifiers by circadian clock Period complexes. Nat Struct Mol Biol 21:126-132.

Eckel-Mahan KL, Patel VR, Mohney RP, Vignola KS, Baldi P, and Sassone-Corsi P (2012) Coordination of the transcriptome and metabolome by the circadian clock. Proc Natl Acad Sci U S A 109:5541-5546.

Eisen MB, Spellman PT, Brown PO, and Botstein D (1998) Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci U S A 95:14863-14868.

Etchegaray JP, Yang X, DeBruyne JP, Peters AHFM, Weaver DR, Jenuwein T, and Reppert SM (2006) The polycomb group protein EZH2 is required for mammalian circadian clock function. J Biol Chem 281:21209-21215.

Fustin JM, Doi M, Yamaguchi Y, Hida H, Nishimura S, Yoshida M, Isagawa T, Morioka MS, Kakeya H, Manabe I, et al. (2013) RNA-methylation-dependent RNA processing controls the speed of the circadian clock. Cell 155:793-806.

Fustin JM, Ye S, Rakers C, Kaneko K, Fukumoto K, Yamano M, Versteven M, Grünewald E, Cargill SJ, Tamai TK, et al. (2020) Methylation deficiency disrupts biological rhythms from bacteria to humans. Commun Biol 3:211.

Gonen N and Assaraf YG (2012) Antifolates in cancer therapy: structure, activity and mechanisms of drug resistance. Drug Resist Updat 15:183-210.

Greco CM, Cervantes M, Fustin JM, Ito K, Ceglia N, Samad M, Shi J, Koronowski KB, Forne I, Ranjit S, et al. (2020) S-adenosyl-l-homocysteine hydrolase links methionine metabolism to the circadian clock and chromatin remodeling. Sci Adv 6:eabc5629.

Guo T, Chang L, Xiao Y, and Liu Q (2015) S-adenosyl-L-methionine for the treatment of chronic liver disease: a systematic review and meta-analysis. PLoS ONE 10:e0122124.

Hoffbrand AV and Weir DG (2001) The history of folic acid. Br J Haematol 113:579-589.

Ilisso CP, Delle Cave D, Mosca L, Pagano M, Coppola A, Mele L, Caraglia M, Cacciapuoti G, and Porcelli M (2018) S-Adenosylmethionine regulates apoptosis and autophagy in MCF-7 breast cancer cells through the modulation of specific microRNAs. Cancer Cell Int 18:197.

Jewison T, Su Y, Disfany FM, Liang Y, Knox C, Maciejewski A, Poelzer J, Huynh J, Zhou Y, Arndt D, et al. (2014) SMPDB 2.0: big improvements to the Small Molecule Pathway Database. Nucleic Acids Res 42:D478-D484.

Johnson AP, Cleaves HJ, Dworkin JP, Glavin DP, Lazcano A, and Bada JL (2008) The Miller volcanic spark discharge experiment. Science 322:404.

Kanehisa M (2019) Toward understanding the origin and evolution of cellular organisms. Protein Sci 28:1947-1951.

Kanehisa M and Goto S (2000) KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28:27-30.

Kanehisa M, Sato Y, Furumichi M, Morishima K, and Tanabe M (2019) New approach for understanding genome variations in KEGG. Nucleic Acids Res 47:D590-D595.

Kapoor V, Watson NF, and Ball L (2021) Chronic insomnia in the setting of MTHFR polymorphism. J Clin Sleep Med. Epub ahead of print December 2. doi:10.5664/jcsm.9794.

Katada S and Sassone-Corsi P (2010) The histone methyltransferase MLL1 permits the oscillation of circadian gene expression. Nat Struct Mol Biol 17:1414-1421.

Kluyver AJ and van Niel CB (1956) The microbe's contribution to biology. Cambridge (MA): Harvard University Press.

Kohls D, Sulea T, Purisima EO, MacKenzie RE, and Vrielink A (2000) The crystal structure of the formiminotransferase domain of formiminotransferase-cyclodeaminase: implications for substrate channeling in a bifunctional enzyme. Structure 8:35-46.

Koike N, Yoo SH, Huang HC, Kumar V, Lee C, Kim TK, and Takahashi JS (2012) Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. Science 338:349-354.

Kozich V and Kraus JP (1992) Screening for mutations by expressing patient cDNA segments in E. coli: homocystinuria due to cystathionine beta-synthase deficiency. Hum Mutat 1:113-123.

Kraus JP, Janosik M, Kozich V, Mandell R, Shih V, Sperandeo MP, Sebastio G, de Franchis R, Andria G, Kluijtmans LA, et al. (1999) Cystathionine beta-synthase mutations in homocystinuria. Hum Mutat 13:362-375.

Krishnaiah SY, Wu G, Altman BJ, Growe J, Rhoades SD, Coldren F, Venkataraman A, Olarerin-George AO, Francey LJ, Mukherjee S, et al. (2017) Clock regulation of metabolites reveals coupling between transcription and metabolism. Cell Metab 25:1206.

Laurino P and Tawfik DS (2017) Spontaneous emergence of S-adenosylmethionine and the evolution of methylation. Angew Chem Int Ed Engl 56:343-345.

Lawrance AK, Racine J, Deng L, Wang X, Lachapelle P, and Rozen R (2011) Complete deficiency of methylenetetrahydrofolate reductase in mice is associated with impaired retinal function and variable mortality, hematological profiles, and reproductive outcomes. J Inherit Metab Dis 34:147-157.

Lippi G and Plebani M (2012) Hyperhomocysteinemia in health and disease: where we are now, and where do we go from here? Clin Chem Lab Med 50:2075-2080.

Longnecker DS (2002) Abnormal methyl metabolism in pancreatic toxicity and diabetes. J Nutr 132:2373S-2376S.

Maclean KN, Sikora J, Kozich V, Jiang H, Greiner LS, Kraus E, Krijt J, Crnic LS, Allen RH, Stabler SP, et al. (2010) Cystathionine beta-synthase null homocystinuric mice fail to exhibit altered hemostasis or lowering of plasma homocysteine in response to betaine treatment. Mol Genet Metab 101:163-171.

Martin JL and McMillan FM (2002) SAM (dependent) I AM: the S-adenosylmethionine-dependent methyltransferase fold. Curr Opin Struct Biol 12:783-793.

Meller E, Rosengarten H, Friedhoff AJ, Stebbins RD, and Silber R (1975) 5-methyltetrahydrofolic acid is not a methyl donor for biogenic amines: enzymatic formation of formaldehyde. Science 187:171-173.

Miller MW, Duhl DM, Winkes BM, Arredondo-Vega F, Saxon PJ, Wolff GL, Epstein CJ, Hershfield MS, and Barsh GS (1994) The mouse lethal nonagouti (a(x)) mutation deletes the S-adenosylhomocysteine hydrolase (Ahcy) gene. EMBO J 13:1806-1816.

Miller SL (1953) A production of amino acids under possible primitive earth conditions. Science 117:528-529.

Minton DR, Nam M, McLaughlin DJ, Shin J, Bayraktar EC, Alvarez SW, Sviderskiy VO, Papagiannakopoulos T, Sabatini DM, Birsoy K, et al. (2018) Serine catabolism by SHMT2 is required for proper mitochondrial translation initiation and maintenance of formylmethionyl-tRNAs. Mol Cell 69:610-621.e5.

Mischoulon D, Nierenberg AA, Schettler PJ, Kinkead BL, Fehling K, Martinson MA, and Hyman Rapaport M (2015) A double-blind, randomized controlled clinical trial comparing eicosapentaenoic acid versus docosahexaenoic acid for depression. J Clin Psychiatry 76:54-61.

Mishanina TV, Koehn EM, and Kohen A (2012) Mechanisms and inhibition of uracil methylating enzymes. Bioorg Chem 43:37-43.

Mompean C, Marin-Yaseli MR, Espigares P, González-Toril E, Zorzano MP, and Ruiz-Bermejo M (2019) Prebiotic chemistry in neutral/reduced-alkaline gas-liquid interfaces. Sci Rep 9:1916.

Moore RY and Eichler VB (1972) Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res 42:201-206.

Morscher RJ, Ducker GS, Li SH, Mayer JA, Gitai Z, Sperl W, and Rabinowitz JD (2018) Mitochondrial translation requires folate-dependent tRNA methylation. Nature 554:128-132.

Mosca L, Minopoli M, Pagano M, Vitiello F, Carriero MV, Cacciapuoti G, and Porcelli M (2020) Effects of S-adenosyl-L-methionine on the invasion and migration of head and neck squamous cancer cells and analysis of the underlying mechanisms. Int J Oncol 56:1212-1224.

Naj AC, Beecham GW, Martin ER, Gallins PJ, Powell EH, Konidari I, Whitehead PL, Cai G, Haroutunian V, Scott WK, et al. (2010) Dementia revealed: novel chromosome 6 locus for late-onset Alzheimer disease provides genetic evidence for folate-pathway abnormalities. PLoS Genet 6:e1001130.

Najm WI, Reinsch S, Hoehler F, Tobis JS, and Harvey PW (2004) S-adenosyl methionine (SAMe) versus celecoxib for the treatment of osteoarthritis symptoms: a double-blind cross-over trial. [ISRCTN36233495]. BMC Musculoskelet Disord 5:6.

Nelson DL, Cox MM, and Lehninger AL (2017) Lehninger principles of biochemistry. New York (NY): W.H. Freeman and Company.

Oh G, Ebrahimi S, Carlucci M, Zhang A, Nair A, Groot DE, Labrie V, Jia P, Oh ES, Jeremian RH, et al. (2018) Cytosine modifications exhibit circadian oscillations that are involved in epigenetic diversity and aging. Nat Commun 9:644.

Panda S (2016) Circadian physiology of metabolism. Science 354:1008-1015.

Pang Z, Chong J, Zhou G, de Lima Morais DA, Chang L, Barrette M, Gauthier C, Jacques PÉ, Li S, and Xia J (2021) MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. Nucleic Acids Res 49:W388-W396.

Parker ET, Cleaves HJ, Callahan MP, Dworkin JP, Glavin DP, Lazcano A, and Bada JL (2011a) Enhanced synthesis of alkyl amino acids in Miller's 1958 H2S experiment. Orig Life Evol Biosph 41:569-574.

Parker ET, Cleaves HJ, Callahan MP, Dworkin JP, Glavin DP, Lazcano A, and Bada JL (2011b) Prebiotic synthesis of methionine and other sulfur-containing organic compounds on the primitive Earth: a contemporary reassessment based on an unpublished 1958 Stanley Miller experiment. Orig Life Evol Biosph 41:201-212.

Parker ET, Cleaves HJ, Dworkin JP, Glavin DP, Callahan M, Aubrey A, Lazcano A, and Bada JL (2011c) Primordial synthesis of amines and amino acids in a 1958 Miller H2S-rich spark discharge experiment. Proc Natl Acad Sci U S A 108:5526-5531.

Parle-McDermott A, Pangilinan F, O'Brien KK, Mills JL, Magee AM, Troendle J, Sutton M, Scott JM, Kirke PN, Molloy AM, et al. (2009) A common variant in MTHFD1L is associated with neural tube defects and mRNA splicing efficiency. Hum Mutat 30:1650-1656.

Patel BH, Percivalle C, Ritson DJ, Duffy CD, and Sutherland JD (2015) Common origins of RNA, protein and lipid precursors in a cyanosulfidic protometabolism. Nat Chem 7:301-307.

Petrossian TC and Clarke Sg (2009a) Bioinformatic identification of novel methyltransferases. Epigenomics 1:163-175.

Petrossian TC and Clarke SG (2009b) Multiple Motif Scanning to identify methyltransferases from the yeast proteome. Mol Cell Proteomics 8:1516-1526.

Petrossian TC and Clarke SG (2011) Uncovering the human methyltransferasome. Mol Cell Proteomics 10:M110000976.

Pinto JP, Gladstone GR, and Yung YL (1980) Photochemical production of formaldehyde in Earth's primitive atmosphere. Science 210:183-185.

Poirier LA, Herrera LAC, and Wise C (2003) On the chemical causation of methyl deficiency and its attendant pathologies. Regul Res Perspect 3:1.

Powner MW, Gerland B, and Sutherland JD (2009) Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions. Nature 459:239-242.

Ramani K and Lu SC (2017) Methionine adenosyltransferases in liver health and diseases. Liver Res 1:103-111.

Reich M, Liefeld T, Gould J, Lerner J, Tamayo P, and Mesirov J (2006) GenePattern 2.0. Nat Genet 38:500-501.

Schubert HL, Blumenthal RM, and Cheng X (2003) Many paths to methyltransfer: a chronicle of convergence. Trends Biochem Sci 28:329-335.

Schwahn BC and Rozen R (2001) Polymorphisms in the methylenetetrahydrofolate reductase gene: clinical consequences. Am J Pharmacogenomics 1:189-201.

Schwahn BC, Chen Z, Laryea MD, Wendel U, Lussier-Cacan S, Genest J Jr, Mar MH, Zeisel SH, Castro C,

Garrow T, et al. (2003) Homocysteine-betaine interactions in a murine model of 5,10-methylenetetrahydrofolate reductase deficiency. FASEB J 17:512-514.

Sherriff JL, O'Sullivan TA, Properzi C, Oddo JL, and Adams LA (2016) Choline, its potential role in nonalcoholic fatty liver disease, and the case for human and bacterial genes. Adv Nutr 7:5-13.

Shimizu-Saito K, Horikawa S, Kojima N, Shiga J, Senoo H, and Tsukada K (1997) Differential expression of S-adenosylmethionine synthetase isozymes in different cell types of rat liver. Hepatology 26:424-431.

Steinman G, Smith AE, and Silver JJ (1968) Synthesis of a sulfur-containing amino acid under simulated prebiotic conditions. Science 159:1108-1109.

Stephan FK and Zucker I (1972) Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proc Natl Acad Sci U S A 69:1583-1586.

Stover P and Schirch V (1990) Serine hydroxymethyltransferase catalyzes the hydrolysis of 5,10-methenyltetrahydrofolate to 5-formyltetrahydrofolate. J Biol Chem 265:14227-14233.

Sunden SL, Renduchintala MS, Park EI, Miklasz SD, and Garrow TA (1997) Betaine-homocysteine methyltransferase expression in porcine and human tissues and chromosomal localization of the human gene. Arch Biochem Biophys 345:171-174.

Swanson DA, Liu ML, Baker PJ, Garrett L, Stitzel M, Wu J, Harris M, Banerjee R, Shane B, and Brody LC (2001) Targeted disruption of the methionine synthase gene in mice. Mol Cell Biol 21:1058-1065.

Teng YW, Mehedint MG, Garrow TA, and Zeisel SH (2011) Deletion of betaine-homocysteine S-methyltransferase in mice perturbs choline and 1-carbon metabolism, resulting in fatty liver and hepatocellular carcinomas. J Biol Chem 286:36258-36267.

Tibbetts AS and Appling DR (2010) Compartmentalization of Mammalian folate-mediated one-carbon metabolism. Annu Rev Nutr 30:57-81.

Valekunja UK, Edgar RS, Oklejewicz M, van der Horst GTJ, O'Neill JS, Tamanini F, Turner DJ, and Reddy AB (2013) Histone methyltransferase MLL3 contributes to genome-scale circadian transcription. Proc Natl Acad Sci U S A 110:1554-1559.

Van Trump JE and Miller SL (1972) Prebiotic synthesis of methionine. Science 178:859-860.

Vugrek O, Beluzic R, Nakic N, and Mudd SH (2009) S-adenosylhomocysteine hydrolase (AHCY) deficiency: two novel mutations with lethal outcome. Hum Mutat 30:E555-565.

Waddell TG, Eilders LL, Patel BP, and Sims M (2000) Prebiotic methylation and the evolution of methyl transfer reactions in living cells. Orig Life Evol Biosph 30:539-548.

Wang X, Jiang B, Gu L, Chen Y, Mora M, Zhu M, Noory E, Wang Q, and Lin C (2021) A photoregulatory mechanism of the circadian clock in Arabidopsis. Nature Plants 7:1397-1408.

Wang Y, Sun Z, and Szyf M (2017) S-adenosyl-methionine (SAM) alters the transcriptome and methylome and specifically blocks growth and invasiveness of liver cancer cells. Oncotarget 8:111866-111881.

Watkins D and Rosenblatt DS (1989) Functional methionine synthase deficiency (cblE and cblG): clinical and biochemical heterogeneity. Am J Med Genet 34:427-434.

Weber AL (2002) Chemical constraints governing the origin of metabolism: the thermodynamic landscape of carbon group transformations under mild aqueous conditions. Orig Life Evol Biosph 32:333-357.

Wollrab E, Scherer S, Aubriet F, Carré V, Carlomagno T, Codutti L, and Ott A (2016) Chemical analysis of a "Miller-type" complex prebiotic broth: part I: chemical diversity, oxygen and nitrogen based polymers. Orig Life Evol Biosph 46:149-169.

Wu G, Anafi RC, Hughes ME, Kornacker K, and Hogenesch JB (2016) MetaCycle: an integrated R package to evaluate periodicity in large scale data. Bioinformatics 32:3351-3353.

Xia J, Psychogios N, Young N, and Wishart DS (2009) MetaboAnalyst: a web server for metabolomic data analysis and interpretation. Nucleic Acids Res 37:W652-660.

Xu J, Chmela V, Green NJ, Russell DA, Janicki MJ, Góra RW, Szabla R, Bond AD, and Sutherland JD (2020) Selective prebiotic formation of RNA pyrimidine and DNA purine nucleosides. Nature 582:60-66.

Xu J, Green NJ, Gibard C, Krishnamurthy R, and Sutherland JD (2019) Prebiotic phosphorylation of 2-thiouridine provides either nucleotides or DNA building blocks via photoreduction. Nat Chem 11:457-462.

Xu J, Green NJ, Russell DA, Liu Z, and Sutherland JD (2021) Prebiotic photochemical coproduction of purine ribo- and deoxyribonucleosides. J Am Chem Soc 143:14482-14486.

Xu J, Ritson DJ, Ranjan S, Todd ZR, Sasselov DD, and Sutherland JD (2018) Photochemical reductive homologation of hydrogen cyanide using sulfite and ferrocyanide. Chem Commun (Camb) 54:5566-5569.

Xu J, Tsanakopoulou M, Magnani CJ, Szabla R, Šponer JE, Šponer J, Góra RW, and Sutherland JD (2017) A prebiotically plausible synthesis of pyrimidine beta-ribonucleosides and their phosphate derivatives involving photoanomerization. Nat Chem 9:303-309.

Yang XM and MacKenzie RE (1993) NAD-dependent methylenetetrahydrofolate dehydrogenase-methenyltetrahydrofolate cyclohydrolase is the mammalian homolog of the mitochondrial enzyme encoded by the yeast MIS1 gene. Biochemistry 32:11118-11123.

Zhang R, Lahens NF, Ballance HI, Hughes ME, and Hogenesch JB (2014) A circadian gene expression atlas

in mammals: implications for biology and medicine. Proc Natl Acad Sci U S A 111:16219-16224.

Zhong X, Yu J, Frazier K, Weng X, Li Y, Cham CM, Dolan K, Zhu X, Hubert N, Tao Y, et al. (2018) Circadian clock regulation of hepatic lipid metabolism by modulation of m(6)A mRNA methylation. Cell Rep 25:1816-1828.e4.

Zhu X, Song J, Mar MH, Edwards LJ, and Zeisel SH (2003) Phosphatidylethanolamine N-methyltransferase (PEMT) knockout mice have hepatic steatosis and abnormal hepatic choline metabolite concentrations despite ingesting a recommended dietary intake of choline. Biochem J 370:987-993.