REVIEW

The impact of cellular metabolism on chromatin dynamics and epigenetics

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The substrates used to modify nucleic acids and chromatin are affected by nutrient availability and the activity of metabolic pathways. Thus, cellular metabolism constitutes a fundamental component of chromatin status and thereby of genome regulation. Here we describe the biochemical and genetic principles of how metabolism can influence chromatin biology and epigenetics, discuss the functional roles of this interplay in developmental and cancer biology, and present future directions in this rapidly emerging area.

Organismal metabolism begins with the ingestion of nutrients from food sources. It continues with the processing of these nutrients in the gut, which then interacts with the microbiome, liver, pancreas, muscle, and many other organs to result in a set of compounds that circulate in the plasma[1]. Cells take up these plasma-supplied nutrients along with other components provided by their microenvironment[2,3] and use them to create cellular metabolic networks that are organized through interconnected chemical reactions, with thousands of metabolites linked by commensurate numbers of reactions. Metabolic network activity is characterized by the concentrations of intermediate metabolites and rates (that is, fluxes) at which one metabolite is converted to another, and is mediated by genotype, epigenotype, and environmental inputs such as nutrient availability and the engagement of signalling pathways[3–6].

Chromatin is the intracellular structure that packages DNA in eukaryotic cells. The principal unit of chromatin organization is the nucleosome, which is formed by DNA wrapped around an octamer of histone proteins. Chromatin can exist in different stable states and is altered by covalent modifications on the histones and the presence of many other factors, such as long non-coding RNAs, protein chaperones, and chromatin remodelling enzymes[7–10]. These modifications influence chromatin structure and binding of chromatin remodelling enzymes and transcription factors in complex and often poorly understood ways[7–11]. They can also mark the existence of functional genomic elements (for example, promoters, enhancers and exons)[7–10,12–14]. Thus, there is tremendous potential for these posttranslational modifications to have profound effects on gene expression, and substantial ongoing efforts aim to understand the structure and function of chromatin modifications[10,11,15–19]. Chromatin and nucleic acid modifications, when inherited after cell division or in offspring after reproduction (for example, genomic imprinting), are often referred to as epigenetics[18,20].

As metabolites are the substrates used to generate chromatin modifications, there exists an intriguing but complex connection between metabolism and epigenetics. In this Review Article, we first introduce biochemical principles that enable the epigenome to respond to metabolic variation and then discuss the genetic basis for how this interaction may generate stable phenotypes. We next discuss recent advances in our understanding of this connection with particular emphasis on stem cell biology and tumorigenesis. Our aim is to provide both a foundation of the principles that govern the interaction between metabolism and chromatin state and a discussion of ongoing developments that are shaping our understanding of its role in biology.

Biochemical principles of the link from metabolism to chromatin

More than 100 distinct covalent modifications have been identified on chromatin, DNA and RNA, with many having substantially documented or emerging functional annotation[21–23]. Among them, methylation, phosphorylation, ubiquitination and acetylation are the most well-understood, but less studied modifications, including glycosylation, crotonylation and succinylation, are also known to be functionally important[24,25]. Addition and removal of these modifications are, with some exceptions[26], catalysed by enzymes of which the activities are mediated by the availability of substrates, cofactors and allosteric regulators that are derived from metabolic pathways. A key characteristic that defines the crosstalk between metabolism and chromatin is that the kinetic (for example, K_m values) and thermodynamic (for example, K_d values) properties of these interactions are commensurate with the dynamic range of physiological concentrations of the corresponding intermediates in metabolism (Table 1). For example, methylation and acetylation reactions often have substrates that have typical cellular concentrations that are commensurate with enzyme K_m values, and thus are

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Table 1 | Ranges of kinetic parameters and concentrations of substrates and cofactors of chromatin-modifying enzymes.

Enzyme	Substrates and cofactors	Kinetic parameter range [mM]	Substrate concentration range [mM]	[S]/K_m range	Refs
Histone acetyltransferases (e.g. HATs, KATs, GCN5, CBP, p300)	Acetyl-CoA	0.0002–0.046 (K_m)	0.002–0.013	0.04–65	82, 134–136
	CoA	0.00044–5.43 (K_i)	–	–	
Histone methyltransferases (e.g. COMPASS, MLLs, EZH2/PRC2, SETs, DOT1L)	SAM	0.0012–0.0345 (K_m)	0.0033–0.059	0.096–49	137–141
	SAH	0.00148–0.071 (K_i)	–	–	
	MTA	–	–	–	
DNA methyltransferases (e.g. DNMTs)	SAM	0.0001–0.021 (K_m)	0.0033–0.059	0.16–536	141–145
	SAH	0.000015–0.0024 (K_i)	–	–	
	MTA	–	–	–	
Histone deacetylases (e.g. SIRTs, HDACs)	NAD^+	0.0023–1.4 (K_m)	0.3–2	0.21–870	146–151
	Nicotinamide	0.029 –0.051 (K_i)	–	–	
	β-Hydroxybutyrate	2.4–5.3 (IC_{50})	0.01–10	–	
Histone demethylases (e.g. LSD1, JHDMs, JMJDs, JARIDs, UTX)	αKG	0.009–0.037 (K_m)	0.11–0.26	3–28.9	107, 152–158
	Oxygen	0.057–0.197 (K_m)	0.021–0.17	0.11–2.98	
	FAD	–	–	–	
	R-2HG	0.024–0.079 (IC_{50})	–	–	
	S-2HG	0.026–0.097 (IC_{50})	–	–	
	Succinate	0.32–0.8 (IC_{50})	–	–	
	Fumarate	1.5–2.3 (IC_{50})	–	–	
	Vitamin C	–	–	–	
	$FADH_2$	–	–	–	
DNA demethylases (e.g. TETs)	αKG	0.035–0.075 (K_m)	0.11–0.26	1.47–7.43	155–157, 159, 160
	Oxygen	0.02–0.04 (K_m)	0.021–0.17	0.52–8.5	
	R-2HG	4–5 (IC_{50})	–	–	
	S-2HG	1–1.6 (IC_{50})	–	–	
	Succinate	0.44–0.76 (IC_{50})	–	–	
	Fumarate	0.23–0.55 (IC_{50})	–	–	
	Vitamin C	–	–	–	

Kinetic parameters and concentrations of substrates and cofactors of chromatin-modifying enzymes. HATs, histone acetyltransferases; KATs, lysine acetyltransferases; GCN5, GCN5-related *N*-acetyltransferase; CBP, CREB-binding protein; p300, E1A binding protein p300; COMPASS, complex proteins associated with Set1; MLL, mixed-lineage leukaemia histone methyltransferases; EZH2/PRC2, enhancer of zeste 2 polycomb repressive complex 2; SETs, SET domain-containing methyltransferases; DOT1L, DOT1-like histone lysine methyltransferase; DNMTs, DNA methyltransferases; SIRTs, sirtuins; HDACs, histone deacetylases; LSD1, lysine demethylase 1; JHDMs; jumonji-domain-containing histone demethylases; JMJDs, jumonji C-domain-containing histone demethylases; JARIDs, jumonji and AT-rich interaction domain-containing histone demethylases; UTX, ubiquitously-transcribed X chromosome histone demethylase; TETs, ten-eleven translocation DNA demethylases; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; MTA, methylthioadenosine; NAD^+, nicotinamide adenine dinucleotide (oxidized); αKG, α-ketoglutarate; FAD, flavin adenine dinucleotide (oxidized); $FADH_2$, flavin adenine dinucleotide (reduced); *R*-2HG, (*R*)-2-hydroxyglutarate; *S*-2HG, (*S*)-2-hydroxyglutarate.

responsive to changes in metabolism[19,26–31] (Table 1, Fig. 1). In contrast, modifications such as phosphorylation and ubiquitination do not respond to changes in metabolism as kinases and E3 ligases that carry out phosphorylation and ubiquitination reactions use ATP as a metabolic substrate[27]. ATP levels (roughly mM in cells) do not reach physiological levels that limit the activities of these enzymes (K_m roughly μM) (Fig. 1a).

There are numerous chromatin- and nucleic-acid-modifying enzymes (Fig. 1b). Some examples that are non-exhaustive and have been reviewed extensively elsewhere[7–9,17,25,32–38] include histone methyltransferases, glycosyltransferases, demethylases, acetyltransferases, deacetylases, deacylases, and DNA and RNA methyltransferases and demethylases. As has been thoroughly reviewed[3,9,19,28,30,31,39–44], these enzymes utilize, as substrates and cofactors, metabolites derived from diverse metabolic pathways including serine–glycine–one–carbon (SGOC) metabolism and particularly the methionine cycle, the tricarboxylic acid cycle, β-oxidation, glycolysis, and hexosamine biosynthesis. In these metabolic networks, intermediate metabolites including *S*-adenosyl methionine (SAM), acetyl-coenzyme A (acetyl-CoA), NAD^+, α-ketoglutarate (αKG), uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), and others serve as substrates for enzymes that modify chromatin and nucleic acids. Additionally, metabolites such as *S*-adenosyl homocysteine (SAH), coA, β-hydroxybutyrate, fumarate, succinate, lactate, and *S* and *R* enantiomeric forms of 2-hydroxyglutarate (2HG) modify enzyme activity, often by competitively inhibiting substrate utilization. There is also emerging evidence that vitamin C may act as a cofactor for dioxygenases that modify chromatin and DNA[45–47]. Thus, each modification can be affected by metabolites from multiple metabolic pathways—for instance, enzymes involved in histone and DNA methylation and demethylation can be regulated by both methionine metabolism and the tricarboxylic acid (TCA) cycle—thus enabling the epigenome to respond to the status of the whole metabolic network.

Principles for the influence of metabolism on epigenomics

Epigenetic modifications are maintained over cellular and organismal generations even when the environmental stimuli triggering a

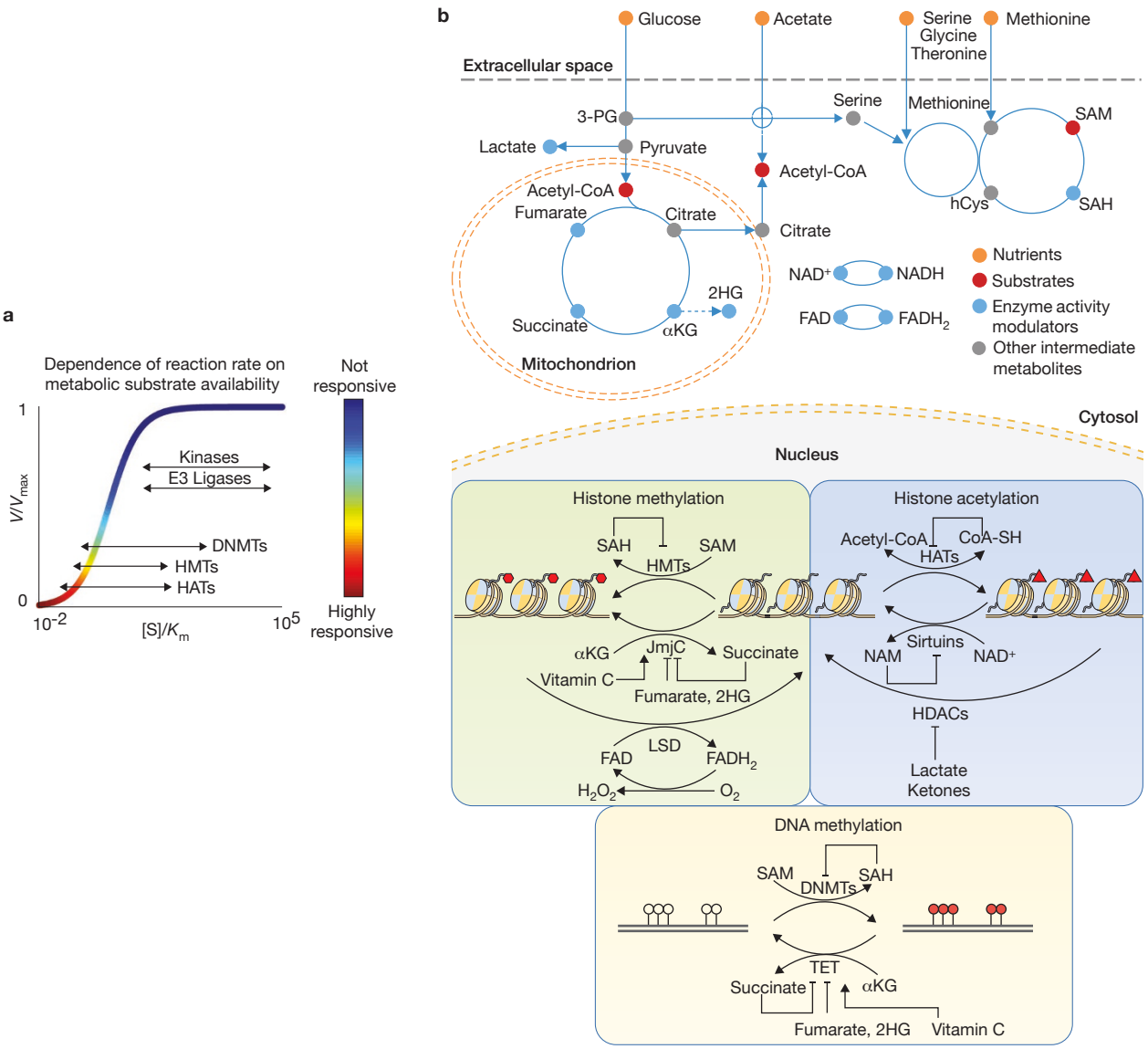

Figure 1 Biochemical basis of metabolite interaction with chromatin and metabolic pathways that contribute. (**a**) In contrast to kinases and E3 ligases, the physiological concentrations of substrates of chromatin-modifying enzymes such as DNA methyltransferase (DNMTs), histone methyltransferase (HMTs), and histone acetyltransferases (HATs) are much lower, thus limiting enzymatic activities. Consequently, the reaction rates of these enzymes are highly responsive to local changes in substrate availability. The ratio of substrate concentration to K_m value is shown on the x axis, and relative reaction rate on the y axis. Ranges of [S]/K_m for all five types of enzymes were estimated from K_m values in the BRENDA database (www.brenda-enzymes.org). (**b**) Uptake and catabolism of macronutrients—for example, glucose and amino acids—generate substrates such as acetyl-CoA and SAM, and activity modulators such as αKG, 2HG, succinate, fumarate, lactate, SAH, oxidized and reduced nicotinamide adenine dinucleotide (NAD^+, NADH), and oxidized and reduced flavin adenine dinucleotide (FAD, $FADH_2$) used by enzymes that modify chromatin. SAM is the major methyl donor for methylation of cytosine bases in DNA and histone residues by DNMTs and HMTs, respectively. Acetyl-CoA is an essential substrate for acetylation of histone residues carried out by HATs. Other metabolites such as αKG, NAD^+ and FAD are critical cofactors for the activity of chromatin-modifying enzymes. αKG is used by TET-family DNA demethylases (TETs) and JmjC-family histone demethylases (JmjCs) to facilitate removal of methyl groups from cytosine bases and histone residues, respectively. LSD-family histone demethylases (LSD) require FAD to demethylate histone residues. Sirtuins and other histone deacetylaces (HDACs) require NAD^+ to deacetylate histone residues. Additionally, metabolites such as 2HG, succinate, fumarate, lactate and SAH can inhibit the activity of chromatin-modifying enzymes.

particular epigenetic reprogramming mechanism are removed. For example, individuals exposed to famine during the Dutch Hunger Winter displayed altered DNA methylation patterns for over six decades[48]. Moreover, factors such as diet[49–52], microbiome[53], temperature[54], malnutrition[55], chemical exposure[56], and many others are able to induce heritable alterations in nucleic acid or histone methylation profiles that can be stably transmitted through more than ten generations[54]. How epigenomic states are stably inherited is unknown, but perturbations to chromatin-modifying enzymes in the germline in controlled laboratory settings have yielded comparable heritable effects to what is observed in settings of human exposure[57]. This suggests that changes to the activity of chromatin-modifying enzymes that are known to be affected by metabolism may be possible transient events that contribute to these phenotypic changes.

Furthermore, the epigenome can be used to characterize cellular state or type. Comparative analyses of epigenomic profiles have

identified cell- and tissue-type-specific chromatin and DNA methylation features[58]. Alterations to chromatin marks have been shown to serve as limiting steps to cell fate transitions, such as those occurring during nuclear transfer[59,60], indicating that the epigenome is causally implicated in the establishment of cellular states. A conceptual framework known as Waddington's landscape is often used to illustrate the relationship between the epigenome and cell states[61–66]. In the 1940s, Conrad Waddington developed the concept of an epigenomic landscape as a blueprint for the differentiation program during development[65–68]. Waddington's landscape is composed of valleys and summits, with valleys representing epigenotypes (in modern terms, stable chromatin modification profiles that define a phenotype) and summits corresponding to the barriers required for the maintenance of stable, heritable epigenomic states that prevent transitions between epigenotypes. Thus, valleys can represent different cell states (for example, pluripotent versus differentiated, normal versus cancerous), the transitions between which are limited by changes in chromatin status (Fig. 2a,b).

Two models are proposed for how epigenetics could be affected by metabolic alterations in the context of Waddington's landscape (Fig. 2c,d). One possibility (model I) is that metabolic reprogramming facilitates the transition from one cell type to another (for example, differentiation) by changing specific chromatin modifications. The stability of these cell states, and thus how probable it is for a cell that has transitioned to a new cell state to return to its previous cell type, depends on the height of the barrier. For example, a change in the levels of metabolites such as methionine or αKG would modulate the activity of methyltransferase or demethylase enzymes, thereby promoting the reorganization of specific epigenetic marks and facilitating cell differentiation across a barrier (Fig. 2b). Another possibility (model II) is that metabolic reprogramming reshapes Waddington's landscape and induces the formation of new stable epigenetic states. In this model, a change in cellular metabolism could either induce gene expression programs related to chromatin remodelling through any number of mechanisms known to allow for such effects, or could directly affect the availability of substrates and cofactors for chromatin-modifying enzymes. In both instances, the cell state transition would be irreversible as Waddington's landscape has changed. This may occur during germline transmission of an epigenomic phenotype due to a parental diet, or a germline mutation in a chromatin-modifying enzyme[49,57,69] (Fig. 2c). Although these proposed models are intriguing, more research is needed to reconstruct the structure of the epigenetic landscape under different metabolic conditions to investigate and distinguish between different possibilities.

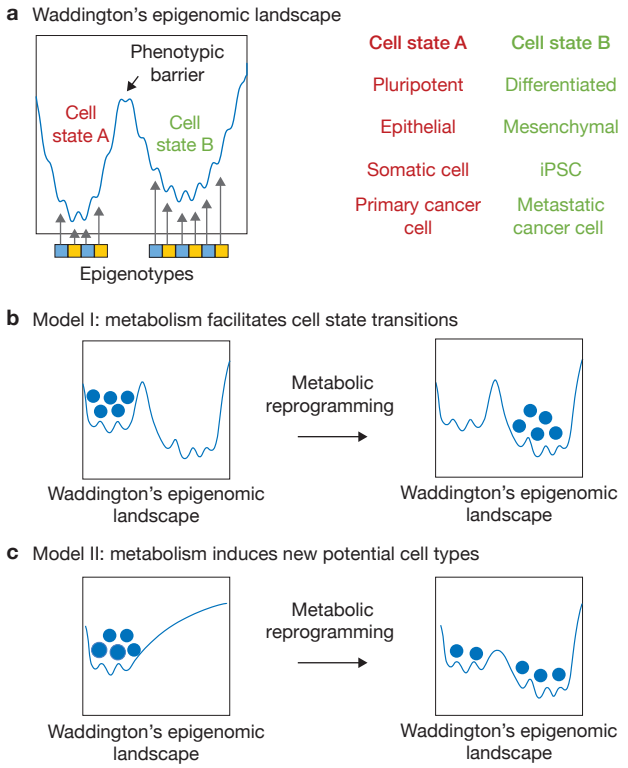

Figure 2 Metabolic reprograming and Waddington's epigenomic landscape. (**a**) Schematic representation of Waddington's landscape depicting cell states existing in valleys maintained by epigenotypes and the phenotypic barrier between two cell states, such as pluripotent and differentiated, epithelial and mesenchymal, somatic and induced pluripotent (iPSC), and primary and metastatic cancer cells. Examples of traits associated with each cell state are listed to the right of the plot. (**b**) Model of how metabolism could facilitate cell state transitions without affecting the shape of the epigenomic landscape, such as a change in metabolite level allowing for reorganization of specific chromatin marks. (**c**) Model of how metabolic reprogramming could reshape the entire epigenomic landscape, leading to new cell states in a case where a cell type has different metabolic requirements. Balls represent cells transitioning from one state to another after changes in metabolism-dependent chromatin remodelling alters the phenotypic barrier.

Metabolism and epigenetics in cell fate and development

In recent years, the link between cellular metabolism, cell fate and early organismal development has been an area of intense study. Early events in stem cell differentiation occur rapidly, and thus changes in metabolism as a driver of such events is an attractive hypothesis[40]. Indeed, the influence of metabolism in the maintenance of stem cell pluripotency has been extensively explored[5,40,70–74]. The effects of metabolism on stem cell fate have been recently reviewed in detail elsewhere[3,5,75] and so here we will touch on specific aspects that highlight their interplay.

Two subsets of embryonic stem cells (ESCs), termed naive and primed, have been defined based on their distinct pluripotency versus differentiation properties, and are also characterized by epigenetically distinct states[5,40]. Naive pluripotent stem cells are characterized by their ability to form all cell types without bias, whereas primed pluripotent stem cells are considered to be poised for lineage differentiation[5,40]. In general, high rates of glycolysis even in the presence of oxygen, a phenomenon known as the Warburg effect[28], is prevalent in pluripotency and appears to be dynamically regulated to facilitate the differentiation process[76]. For example, human naive pluripotent stem cells (PSCs) were shown to have higher glycolytic metabolism than human primed PSCs or differentiated cells[71], and loss of mitochondrial oxidative metabolism was shown to cause defects in mouse haematopoietic stem cell differentiation[77]. Of note, other reports have shown increased oxidative phosphorylation in human and mouse naive PSCs compared to their primed counterparts[78]. Together, these studies emphasize the temporal complexities of cellular metabolism in driving cell fate, and that glycolysis and oxidative metabolism may not be mutually exclusive when it comes to regulating pluripotency. Although metabolism unquestionably influences the pluripotent state, additional studies are needed to elucidate the exact mechanisms for how metabolic features contribute to pluripotency or differentiation.

In addition to the metabolic changes that occur during these cell fate transitions, it is also now widely appreciated that changes in metabolism are directly linked to changes in chromatin and DNA state. The levels of acetyl-CoA, the substrate for histone acetylation[79–83], have been shown to be critical for the maintenance of human and mouse stem cell pluripotency[70]. Reduced NAD^+ levels due to increased glycolytic metabolism have been shown to reduce NAD^+-dependent histone deacetylase activity and to promote mouse muscle stem cell differentiation[84]. αKG was shown to maintain mouse naive stem cell self-renewal by promoting histone and DNA demethylation through the activity of JmjC-family histone demethylases and TET-family DNA demethylases[85]. Increased αKG levels were also found to promote early differentiation of human primed PSCs and mouse epiblast stem cells[86]. Of significance, reduction of the αKG/succinate ratio was able to reverse the observed effects[85,86], indicating that the alterations in metabolic pathways drive chromatin dynamics.

The methionine cycle has also been shown to regulate histone methylation in mouse ESCs[74]. As with αKG, methionine-derived SAM appears to play multiple roles in mediating cell fate depending on context. Depletion of SAM through short-term methionine deprivation triggered differentiation of human primed ESCs[73]. Additionally, nicotinamide *N*-methyltransferase upregulation in human naive ESCs depleted SAM pools and maintained self-renewal, thus preventing the differentiation process[76]. Beyond the literature on pluripotency and metabolism, metabolism has been recently shown to maintain or induce specific adult stem cell lineages[87,88]. However, whether epigenetics may play a role in these settings remains to be determined. Together, these reports highlight that differing metabolomes are found in distinct cell states, and demonstrate the functional consequence of how changes in metabolism can affect and possibly specify cell fate.

The haematopoietic lineage is another well-studied system that exhibits cell state transitions. Recent reports highlight a critical role for metabolism in driving immune cell activation and differentiation[89,90]. For example, T cells undergo rapid changes in glycolysis during activation[91]. There are additionally well-established roles for epigenetics in immune cell fate[92]: enhanced glycolysis-dependent acetyl-CoA production in regulatory T cells has been shown to promote differentiation through increased histone acetylation[93], and αKG has been reported to regulate context-specific gene reprograming for helper T-cell differentiation[94]. Another example was observed in a *Caenorhabditis elegans* pathogen-feeding model, in which deletion of methionine synthase reduced the immune response to pathogens by preventing expression of protective genes dependent on histone methylation[95]. Future studies will undoubtedly uncover more links, and it will be interesting to examine how changes in nutrient availability as a result of metabolic competition with other cells[96,97] or metabolite exchange affects immune cell metabolism, chromatin biology, and function.

A remaining question concerns how metabolism-dependent cell state changes affect the overall development of the organism. Tissue-type specific mechanisms for how metabolic changes affect development have started emerging[98,99], and future studies will uncover how these may control cell fate, tissue morphogenesis and development through epigenetic mechanisms. Given that recent reports indicate systemic and distinct changes in histone methylation in early mammalian development[100–102], it will be exciting to explore the role metabolism and perhaps diet has in these contexts.

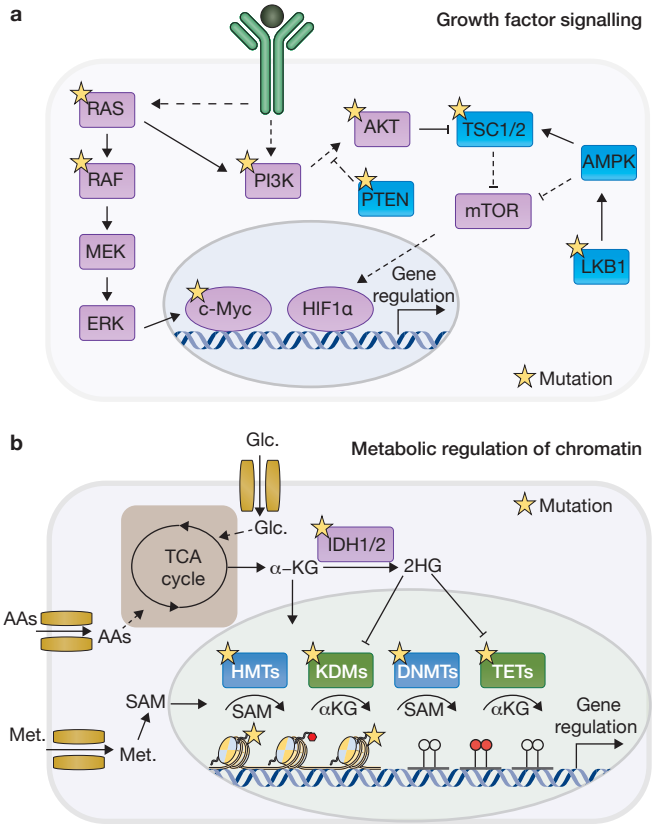

Figure 3 Analogy of cancer-associated mutations found in growth signalling with those in metabolism-dependent chromatin-modifying processes. (**a**) During oncogenesis, cells gain growth factor independence by frequently acquiring mutations that co-opt normal growth signalling. Ras and Raf are commonly mutated in cancer and drive downstream signalling through MEK and ERK, which can lead to gene regulation by c-Myc. Ras and growth factor signalling can activate the PI3K–AKT–mTOR signalling axis to promote cell growth and survival through downstream transcription factors such as HIF1α. Mutations to PI3K, AKT, PTEN, TSC and LKB1 are also common in cancer. Purple indicates oncogenes; blue indicates tumour suppressors; yellow star indicates common lesions in cancer; solid lines represent direct biochemical interactions; dotted lines represent indirect regulation. (**b**) Metabolism regulates normal physiological activity of chromatin-modifying enzymes, which are commonly mutated in cancer. Glucose (Glc.) and amino acids (AAs) feed into the TCA cycle, which generates regulators of chromatin modifying enzymes such as αKG. Methionine (Met.) produces the methyl-donor SAM in the methionine cycle. With exception of IDH1/2, mutations in metabolic enzymes are uncommon in cancer, yet cancer-associated mutations in chromatin modifiers such as DNMTs, TETs, HMTs, histone lysine demethylases (KDMs), and histones (H3K27 and H3K36) are prevalent, suggesting that cells may subvert the normal regulation of these enzymes by metabolism during transformation. Blue indicates enzymes that perform methylation reactions; green indicates enzymes that perform demethylation reactions; yellow star indicates common lesions in cancer.

Metabolism and epigenetics in cancer biology

Altered metabolism is a hallmark of cancer[2,4]. For almost a century, malignant cells have been known to exhibit nutritional differences compared to normal cells[28], and recent evidence supports that they also harbour epigenetic changes driven by their rewired cellular metabolism[39,41,103]. A major breakthrough in our understanding of the connection between metabolism and epigenetics in cancer was the discovery of gain-of-function mutations to the genes encoding isocitrate dehydrogenase 1 (IDH1) and IDH2 that cause an altered enzymatic activity

resulting in the production of the (*R*)-2-hydroxyglutarate (*R*-2HG) metabolite[104,105]. These mutations are recurrent and their consequences in tumorigenesis have been reviewed extensively elsewhere[44]. In brief, cells harbouring *IDH1/2* mutations display DNA and histone hypermethylation as accumulation of 2HG inhibits the activity of TET-family DNA and JmjC-family histone demethylase enzymes[106]. These mutations have been linked to pathogenesis of glioblastoma multiforme, acute myeloid leukaemia, chondrosarcoma, cholangiocarcinoma, and other human malignancies[44].

Furthermore, mutations in the genes encoding fumarate hydratase (FH) and succinate dehydrogenase (SDH), the enzymes that catabolize fumarate and succinate, have been identified in several sporadic and hereditary cancers and cause accumulation of their substrates[107]. High levels of fumarate and succinate can also inhibit αKG-dependent DNA and histone demethylases and loss of FH and SDH activity was shown to lead to hypermethylation of DNA and histone residues[107,108]. A recent report demonstrated that loss-of-function mutations in FH and the subsequent accumulation of fumarate promotes epithelial-to-mesenchymal-transition (EMT) through fumarate-dependent inhibition of TET demethylases and subsequent induction of genes necessary for EMT[109]. FH was also found to be *O*-GlcNAcylated, which caused changes in histone methylation[110] and provided evidence for additional layers of metabolic regulation of chromatin. Indeed, a recent study proposed that a substantial portion of variation in DNA methylation profiles across all human cancers could be explained by differences in the expression of enzymes related to methionine and the one-carbon network[111]. Taken together, these studies define clear and sometimes quantitative roles for metabolism in specifying aspects of the epigenome.

Cancer-specific deletions of other metabolic enzymes with implications in epigenetic regulation have also been reported. The gene encoding 5-methylthioadenosine phosphorylase (MTAP), a key enzyme in the methionine salvage pathway, is located near the ubiquitous tumour suppressor gene *CDKN2A* and the two are commonly co-deleted[112,113], with the loss of *MTAP* thought to be a passenger event in cancer progression. However, recent reports have established a collateral dependency in *CDKN2A*-deleted tumours in which loss of *MTAP* causes accumulation of methylthioadenosine, the metabolite cleaved by MTAP, leading to inhibition of the PRMT5 protein arginine methyltransferase, which is required for tumour growth[112,113]. The importance of methionine-derived SAM in regulating cell state and epigenetics[73,74] suggests that there could be instances where changes in SAM levels due to loss of the methionine salvage pathway have dramatic effects on chromatin state, which would support a more active role for MTAP in cancer development. However, whether *MTAP*/*CDKN2A*-deleted cancers display an altered chromatin state remains to be determined.

As discussed, although alterations in genes encoding metabolic enzymes have been identified in cancer, overall they are rare. In contrast, lesions in genes related to cancer-associated growth signalling pathways and downstream transcription factors are common[114]. Indeed, the ability of cancer cells to obtain growth factor independence by acquiring mutations that allow them to constitutively engage signalling pathways that control cell growth, survival, and proliferation is a recognized hallmark of oncogenesis. Mutations in enzymes that modify chromatin and DNA are recurrent and constitute a more recently defined class of cancer-associated mutations[66] that often result in aberrant chromatin and DNA epigenomic profiles. It is tempting to speculate that the function of these mutations is to uncouple their status from the interaction with metabolism and thus subvert this normal epigenetic regulation by nutrition and metabolism (Fig. 3). However, further studies are needed to better define the relationship between the normal metabolic regulation of chromatin and the cancer-associated chromatin mutations.

A major goal in studying metabolism-dependent epigenetic mechanisms in cancer is the hope of identifying targetable liabilities. Encouragingly, small molecules targeting mutant IDH1/2 are now very advanced clinically[115]. At the preclinical level, a study on nutrient heterogeneity of the tumour microenvironment reported that the core regions of melanoma tumours had enhanced histone methylation as a result of reduced αKG levels, which led to resistance to BRAF inhibitor treatment, and the combination of histone methyltransferase and BRAF inhibitors was sufficient to overcome resistance[116]. Separately, the SGOC network was shown to be upregulated in liver kinase B1 (LKB1)-deficient tumours with KRAS activation and could be coupled to SAM generation, causing enhanced DNA methyltransferase activity and elevated DNA methylation[117]. This study indicated that LKB1-deficiency could be a key vulnerability as DNA methyltransferase and serine metabolism inhibition reduced tumour growth[117]. A distinct line of work on the evolution of distant metastases of pancreatic ductal adenocarcinoma (PDAC) demonstrated that the oxidative branch of the pentose phosphate pathway (oxPPP) was a driving force for epigenome landscape reprogramming and the fitness of metastatic cells[118], suggesting that targeting the oxPPP could be effective in metastatic PDAC. Together, these studies represent a few examples of how advances in our understanding of metabolic effects on epigenetics can be translated into potential therapies.

Future directions

Much remains unanswered in each of the areas discussed in this Review Article. The key element of the biochemistry is that enzymatic parameters such as K_m, V_{max}, and allosteric and inhibitory binding constants must be tuned to values that can limit enzyme activity. Although there is ample evidence that this can be achieved in pathophysiological conditions such as the presence of IDH1/2 or FH mutations, resulting in millimolar concentrations of 2HG in the case of IDH1/2 (ref. 105), which is well above the inhibitory constant of dioxygenase enzymes, there is accumulating albeit far less evidence that such regulation occurs in physiological conditions[119] (Table 1).

Additional complications that limit our understanding are potential differences in enzymatic parameters measured under conditions *in vitro* versus *in vivo*, and the difficulty in obtaining accurate measurements of exact concentrations *in vivo*, especially when the relevant concentration is compartmentalized in cells. Thus, more studies are needed to define physiological conditions in which the concentration dynamics of relevant substrates and cofactors causally underlie a change in chromatin state. It will also be necessary to clarify the extent to which environmental variables such as diet, which have profound effects on cancer outcome[120] and cell fate[121], can modulate epigenetics by altering levels of the relevant metabolites to the needed concentrations.

A further complexity is that enzymes for both activating and repressive histone marks require metabolites. Thus, the precise input of cellular metabolism into the complex arrangement of multiple modifications on histones and DNA that have distinct functions remains an open

question. For example, how do changes in the levels of metabolites such as SAM lead to predictable changes in gene expression? In addition, poorly understood layers of regulation that define the specificity of the chromatin–metabolite link probably exist. For instance, the formation of multiprotein complexes in which enzyme activities are affected by dynamic protein interactions and their localization to specific sites of the genome would occur in parallel with metabolite changes that also affect enzyme activity, increasing the level of regulatory complexity that further work is expected to elucidate in the coming years. Finally, although expression levels of metabolic network components appear to be to some extent predictive of DNA methylation levels[111], how predictive metabolite levels are of the overall chromatin state and epigenetic phenotype remains largely unknown. As we know, many other factors, such as gene expression, influence chromatin state and much of the study of epigenetics and chromatin biology was historically conducted without consideration of metabolic influences. How the magnitude of the metabolic effects on the activity of chromatin-modifying enzymes compares relative to transcriptional programs that control the expression of these enzymes remains unknown.

Moreover, our understanding of the genetic basis for how stable chromatin states or traits can be established through metabolic changes is very limited. In addition, although certain architectural aspects of chromatin modifications, such as peak shape, are known to encode information about phenotype[12,13], the specific aspects of genomic architecture that may be affected by metabolites remain unknown. Our current knowledge of metabolic regulation of chromatin structure focuses on individual covalent chromatin marks, but the effect on higher-level chromatin structure such as genome folding and chromatin accessibility remains to be elucidated.

In stem and developmental biology, there are numerous examples of cell type transitions that show concomitant changes in metabolism and the chromatin landscape. Nevertheless, there are few examples that show that a metabolic change leads to a biological outcome due to a specific effect on chromatin or DNA modifications and independently of all other effects that may occur alongside this change in metabolite levels. This complexity results from the fact that metabolites involved in epigenetics are also connected to larger metabolic networks that affect nearly all aspects of cellular physiology. New CRISPR–Cas9-based technologies that can engineer posttranslational modifications at specific genomic loci, when combined with defined metabolic perturbations, may address some of these challenges[122–124].

In cancer, although there is much interest in targeting both altered metabolism and altered epigenetics, whether these two hallmarks confer dependencies in tumours synergistically is unknown, with the exception of a few examples[115,116,117,118]. The same difficulties in establishing causal links apply also in this setting. In that respect, exploring metabolic dependencies in settings where a genetic lesion modifies chromatin, as in MLL-rearranged leukaemias[125,126] or paediatric brain tumours and sarcomas with histone mutations[127,128], might prove fruitful as these cases could be particularly susceptible to a disruption in metabolism.

Although our understanding remains at a very early stage, rapid progress is expected, especially considering the techniques that are available for chromatin and metabolic state characterization, and cell culture methods, including organoid systems, that can model and manipulate physiological metabolism more effectively[119,129–133]. This wealth of technology available to probe and interpret both chromatin status and metabolism and the collective interest in both subjects raise optimism that rapid progress will continue to be made.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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