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How metabolites modulate metabolic flux

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Adaptation to metabolic needs and changing environments is a basic requirement of every living system. These adaptations can be very quick and mild or slower but more drastic. In any case, cells have to constantly monitor their metabolic state and requirements. In this article we review general concepts as well as recent advances on how metabolites can regulate metabolic fluxes. We discuss how cells sense metabolite levels and how changing metabolite levels regulate metabolic enzymes on different levels, from specific allosteric regulation to global transcriptional regulation. We thereby focus on local metabolite sensing in mammalian cells and show that several major discoveries have only very recently been made.

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Introduction

In recent years, interest in cellular metabolism as a possible intervention point to treat diseases has been renewed, which has led to the discovery of new links between metabolism and the pathogenesis of various diseases [1]. However, identifying regulatory nodes within the metabolic network is challenging due to its complex structure. Since most of the anabolic and catabolic pathways share a common set of metabolites, complex regulatory mechanisms have evolved to ensure that metabolites are moved through the correct pathways at the correct rate to match the cell's ever-changing metabolic requirements.

Regulation of cellular metabolism may occur at several different levels (Figure 1), beginning at the gene encoding different enzyme isoforms, followed by the

transcriptional level that selects which genes are activated. Subsequently, alternative splicing, mRNA stability, translation, and protein degradation control enzyme abundances. Since all of these mechanisms are long-term regulations (hours to days), they are classified as 'coarse' regulations [2]. Usually, coarse regulations are a response to long-term environmental changes or hormonal stimulation and require newly synthesized proteins.

More rapid adjustments (seconds to minutes) of cellular metabolism, however, change the activity of enzymes already present in the cell. Usually, they are triggered by changes in local concentrations of metabolites leading to an allosteric or post-translational regulation of enzyme activity. These mechanisms are classified as 'fine' regulations and provide short-term regulation of pathways that need to be constitutively active [2].

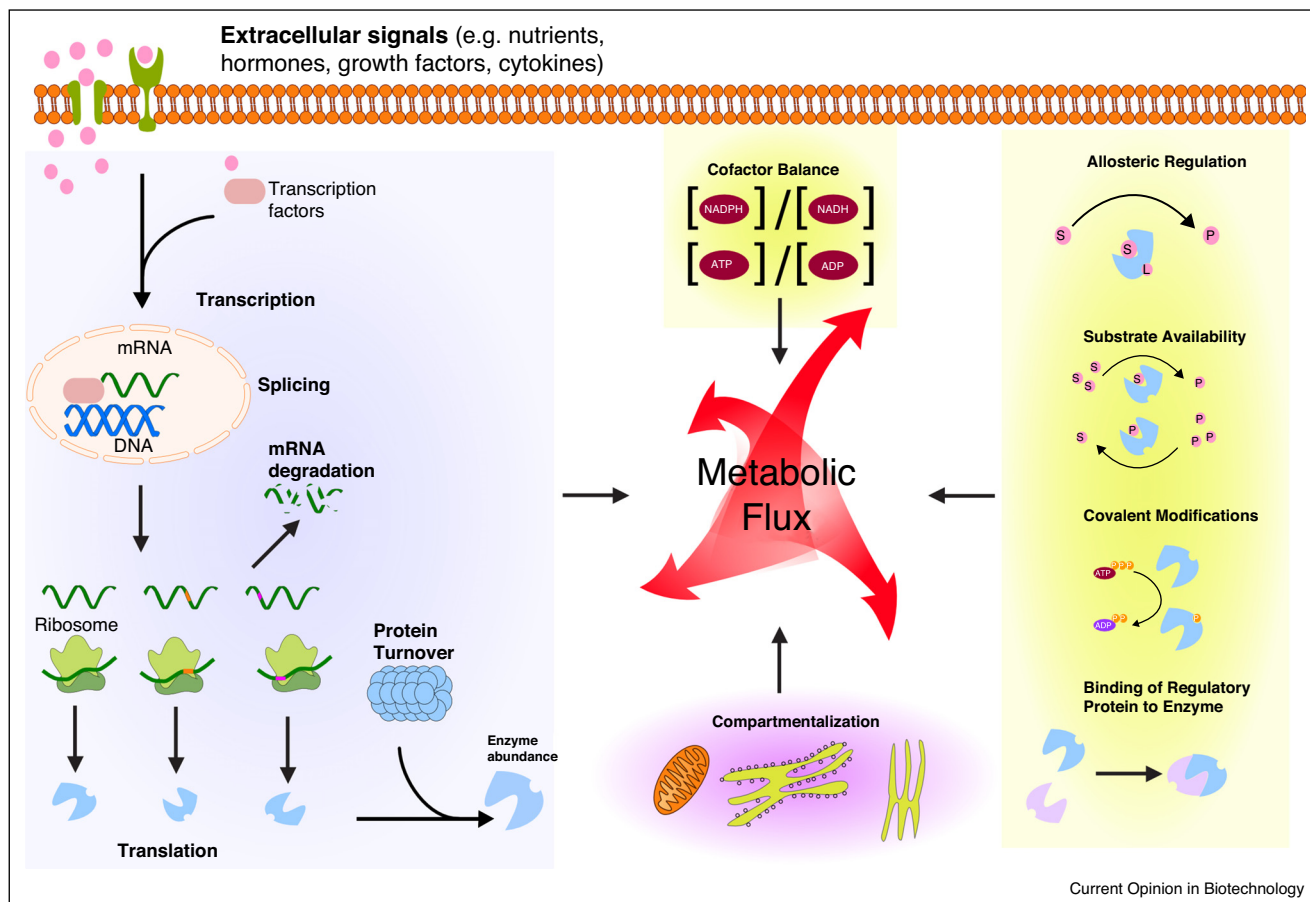
In this article we critically review how metabolite levels control the regulatory mechanisms depicted in Figure 1. To this end, we first need to define what we consider as a regulatory role of a metabolite. We consider a metabolite to have a role in regulating cellular metabolism if it fulfills the following two conditions: first, the concentration of the metabolite must be able to fluctuate in response to internal or external perturbations; second, and more importantly, this concentration change must trigger a response which leads to a change in activity of one or more specific enzymes. In the following we will review general mechanisms that fulfill these two conditions, accompanied by specific examples of metabolites recently shown to regulate mammalian cellular metabolism.

Although we here only review local sensing mechanisms, it should be noted that in complex organisms metabolite sensing occurs in dedicated cell types which initiate specific systemic hormonal signaling which is then integrated in a cell-type specific manner [3].

Substrate and product concentrations

All metabolic reactions are dependent both on the concentration of the substrate and product. Most metabolic reactions are catalyzed by enzymes. Enzymes bind their substrate with a characteristic affinity and a parameter K_M . The K_M value describes the concentration of the substrate for a given enzyme concentration at which the reaction is at half maximum speed and is of fundamental importance for the following reason: in case the substrate's concentration is much higher than the K_M value,

Figure 1



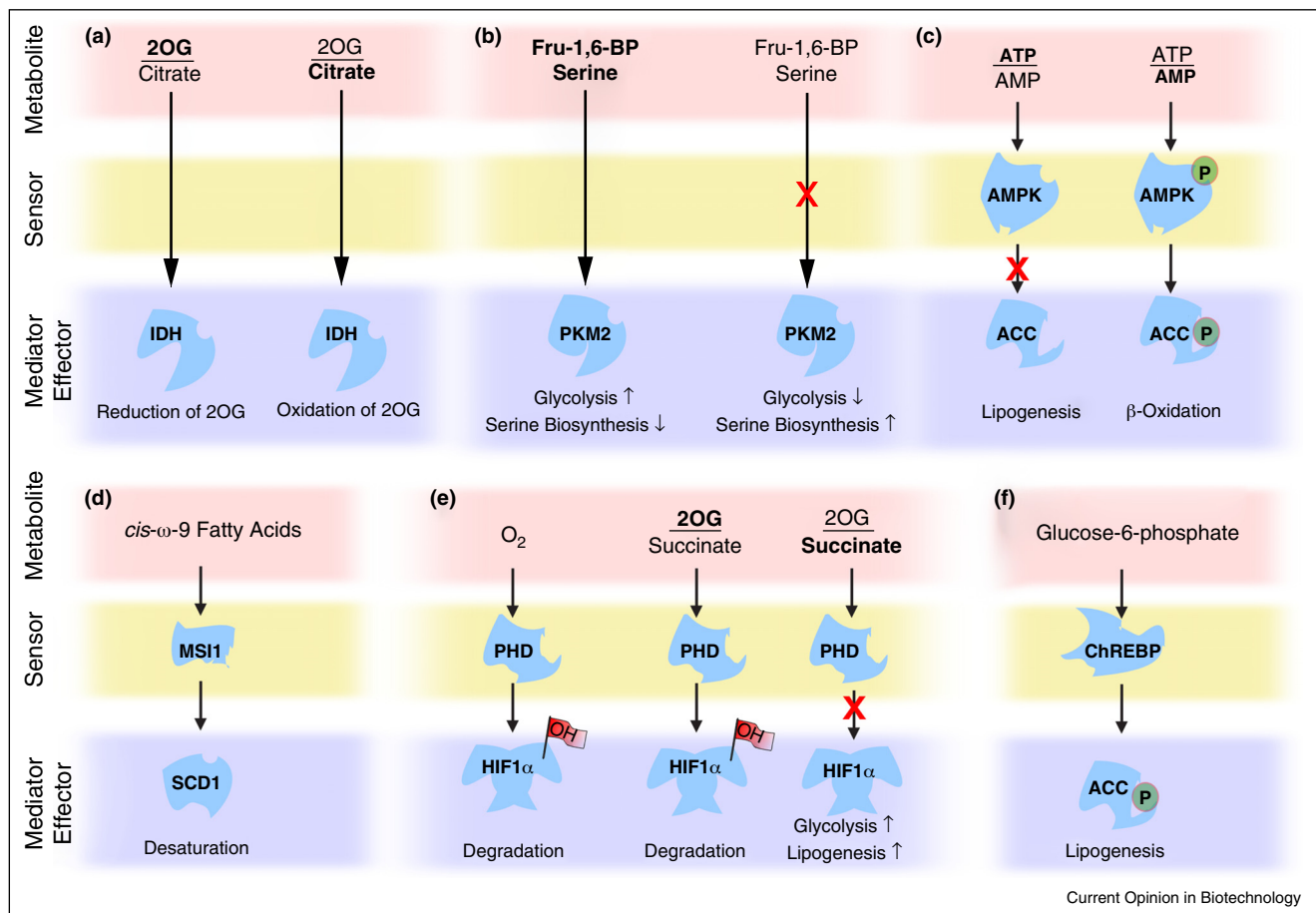
Enzyme activity, and thus metabolic fluxes can be modulated by varying the number of enzyme molecules within the cell (blue background), the enzyme's subcellular location (purple background) or by changing the activity of the enzyme molecules already present in a cell (yellow background).

the enzyme is mostly saturated and changes in substrate concentration will not significantly influence its activity. Within a cell, concentrations of most substrates of central carbon metabolism are below or close to K_M , except for those enzymes that catalyze initial steps of amino and nucleic acid degradation [2,4]. Therefore, small changes in substrate concentrations *in vivo* can in fact induce significant changes in metabolic fluxes. Enzymes following Michaelis–Menten kinetics show a hyperbolic saturation kinetic, meaning that with increasing substrate concentration the reaction speed asymptotically converges to the maximal speed and, thus, the flux change per concentration change converges to zero. That being said, a significant flux increase through an enzymatic reaction where the substrate concentration is higher than the K_M value would require physiologically infeasible concentration changes.

Recently, reductive glutamine metabolism was suggested to be initiated by changes in concentrations of substrate

and product of isocitrate dehydrogenase (IDH), specifically 2-oxoglutarate (2OG) and citrate [5^{*},6,7^{**}]. 2OG can either be oxidatively decarboxylated to succinyl-CoA via 2OG dehydrogenase (OGDH), or it can be reductively carboxylated via IDH to eventually generate citrate. The latter metabolic pathway is of particular importance in different cancer types and under hypoxic conditions for lipogenesis, when glucose oxidation is suppressed [6,8–10]. The accumulation of 2OG with a concomitant decrease in citrate was shown to be the driving force to switch from oxidative phosphorylation to reductive glutamine metabolism [5^{*},6,7^{**}] (Figure 2A). However, a recent report by Mullen and coworkers showed that knockdown of OGDH increases the 2OG to citrate ratio, without inducing reductive glutamine metabolism [11^{*}]. The authors concluded that initiation of this metabolic pathway depends on an increasing NADH/NAD⁺ ratio. Therefore, silencing of the NAD⁺ dependent OGDH prevented an increase in the NADH/NAD⁺ ratio and thus, increased reductive carboxylation of 2OG.

Figure 2



Regulation of metabolic fluxes by different mechanisms (see text). 2OG: 2-oxoglutarate; Fru-1,6-BP: fructose-1,6-bisphosphate; AMPK: 5' AMP-activated protein kinase; IDH: isocitrate dehydrogenase; PKM2: pyruvate kinase (M2 isoform); ACC: acetyl-CoA carboxylase; MSI1: musashi1; PHD: prolyl hydroxylase domain-containing protein; HIF1 α : hypoxia inducible factor 1 α ; SCD1: stearoyl CoA-desaturase-1; ChREBP: carbohydrate-responsive element-binding protein.

Allosteric regulation of enzymes

Although substrate concentrations provide fast and the most direct means to control enzymatic reactions, they can affect several metabolic pathways simultaneously and, thus, cannot specifically increase a flux to produce a certain metabolite. For instance, high glucose levels will induce a high glycolytic flux. Since serine is derived from the glycolytic intermediate glycerate 3-phosphate, increased glycolysis would increase the flux to serine biosynthesis, assuming all enzymes are solely regulated by substrate concentrations. However, the end product of both pathways is either used for anabolic or catabolic reactions. Hence, the fluxes through both pathways should be regulated based on the current metabolic needs of the cell.

To that end, allosteric regulation of enzyme activity provide a further means to finely control cellular metabolism on a short time scale. Mechanistically, an allosteric

regulator binds an allosteric enzyme at a binding pocket distinct from the active site, which induces a conformational change modulating the enzyme's activity. Allosteric enzymes show reaction kinetics that deviate from classical Michaelis–Menten kinetics, changing from hyperbolic to sigmoidal saturation. As such, the activity of an allosteric enzyme can either be more sensitive to concentration changes of its substrate in case of a positive regulator or its sensitivity can be reduced in the case of a negative regulator.

Even though allosteric regulation is well known for around 50 years and many of the pacemaker enzymes of central carbon metabolism are known to be allosterically regulated by metabolites [2], serine was recently identified as a new allosteric effector of a key glycolytic enzyme. In addition to fructose-1,6-bisphosphate, serine was shown to allosterically activate the M2 isoform of pyruvate kinase (PKM2), which catalyzes the final step of

glycolysis converting phosphoenolpyruvate to pyruvate with the concomitant generation of ATP [12,13**]. Although serine was already identified as a potent activator of PKM2 in the last century [14], the exact mechanism was only elucidated recently. Chaneton and colleagues identified a previously uncharacterized binding pocket specific for serine [13**]. Based on stable isotope labeling experiments they showed, that serine deprivation significantly lowered the flux from phosphoenolpyruvate to pyruvate, leading to an accumulation of upstream metabolites. The allosteric regulation of PKM2 enables the cell to tightly regulate the bifurcation of glucose-derived carbon flux into biosynthetic and energy producing pathways (Figure 2B). If serine levels are low, PKM2 activity is reduced to shuttle glucose-derived carbon towards serine biosynthesis. However, if serine levels are high, PKM2 is fully active, promoting high rates of glycolysis. This metabolic switch is highly important for tumor development, as shown by Anastasiou and coworkers [15**]. They showed that expression of PKM1 or constitutively activated PKM2 inhibits tumor growth.

Reversible covalent modifications of enzymes

Reversible covalent modifications of specific enzyme residues provide another means to fine control cellular metabolism by controlling the enzyme's activity, subcellular localization, or stability. Post-translational modifications switch enzymatic activity on or off and therefore can have a much higher impact than allosteric effectors.

AMP-activated protein kinase (AMPK) is known as key regulator of metabolism in response to changing ATP/AMP ratios. If the cellular energy load is low, AMPK is active and can phosphorylate its targets. Activity of AMPK itself is regulated by phosphorylation. Whether ADP or AMP was the actual allosteric regulator to facilitate AMPK phosphorylation by upstream kinases was controversially discussed in the literature [16–18]. Recent work by Gowans *et al.* aimed to answer this open question. Although the cellular concentration of AMP is usually below that of ADP, the authors showed that AMP is the true allosteric regulator of AMPK for both, its phosphorylation and its kinase activity [18].

Phosphorylation of AMPK depends on the binding of AMP or ATP. At high ATP/AMP ratios, ATP binds to the regulatory domain, resulting in the inactivation of kinase activity by dephosphorylation of AMPK. If the ATP/AMP ratio drops, the bound ATP is replaced by AMP. This leads to a conformational change, favoring phosphorylation of AMPK by upstream kinases and subsequent induction of AMPK activity. When the ATP levels rise again, bound AMP is replaced by ATP resulting in dephosphorylation and thus, inactivation of AMPK. When AMPK is activated it modulates metabolic fluxes: energy

producing processes are induced and energy consuming processes are repressed [19] (Figure 2C).

A well-known downstream target of AMPK is acetyl-CoA carboxykinase (ACC) [20]. ACC is the key enzyme in fatty acid synthesis because it catalyzes malonyl-CoA production. Malonyl-CoA is the substrate for fatty acid biosynthesis and acts as an inhibitor of carnitine palmitoyltransferase (CPT) which imports fatty acids into the mitochondrion for β -oxidation. Activated AMPK phosphorylates ACC and thereby, prevents inhibition of CPT. This enables the cell to drive oxidation of lipids into the mitochondrion to match current energy demands.

Interestingly, AMPK activation reaches beyond regulation of energy metabolism. It has also been shown to control energy intensive processes such as cell-cycle progression, protein synthesis or neuronal excitation [19,21]. In summary, AMPK represents an example of how metabolite concentrations can be key regulators of cellular metabolism at the enzyme level by inducing PTMs.

Translational regulation

Metabolites can also control mRNA translation. Positive regulation can initiate biosynthesis if precursors are accumulating and negative regulation is important to prevent unnecessary production of enzymes if the reaction products are already accumulating.

Negative translational regulation occurs, for example, in the case of fatty acid desaturation. Unsaturated fatty acids bind to a translation activator (Musashi-1) and induce a conformational change. Thereby RNA binding is prevented and mRNA translation of SCD1, a fatty acid desaturase, is inhibited [22] (Figure 2D).

Besides the indirect regulation of translation via translation factors, translation can also be controlled by direct interaction of metabolites with mRNA. Riboswitches are regulatory domains which can specifically bind certain metabolites. Binding of the effector induces a conformational change in the mRNA to activate or inactivate translation. This sensing is well known in bacteria, and recently a few examples have also been found in eukaryotes [23]. Potential riboswitches in mammals remain elusive. However, it would not be surprising if they were present there as well, as they provide a direct means for metabolic control of mRNA translation.

Transcriptional regulation

Complex transcriptional regulation is one of the things that separates eukaryotes from prokaryotes and provides the basis for many dedicated cell types within a multicellular organism. Since global changes in gene expression are energetically expensive, they are usually triggered by long term environmental changes that

require drastic adaptations of cellular metabolism. For example, different oxygen levels require distinct sets of metabolic enzymes, as oxidative phosphorylation is the most important energy producing pathway under normoxia. The activity of the transcription factor hypoxia inducible factor 1 (HIF1) is modulated by oxygen availability. In the presence of oxygen, HIF1 α becomes hydroxylated, which marks the protein for ubiquitination and proteasomal degradation [24]. In the absence of oxygen, hydroxylation of the protein is impaired and proteasomal degradation is avoided. The hydroxylation is directly controlled by prolyl hydroxylase 2 (PHD2), a member of the family of dioxygenases. Aside from oxygen, PHD2 relies on Fe²⁺ and 2OG to mediate hydroxylation, with the simultaneous production of succinate and CO₂ from 2OG. In addition to an oxygen dependent regulation, it has been shown that the activity of PHD2 as well as other 2OG dependent dioxygenases can be regulated by the level of succinate and 2OG, as well as by fumarate and the oncometabolite 2-hydroxyglutarate (2HG) [25,26*,27,28,29,30]. When local concentrations of succinate, fumarate, or 2HG increase, protein hydroxylation is inhibited. In the case of HIF1 α , this leads to a stabilization independent of the oxygen supply. Stabilized HIF1 α forms a complex with HIF1 β , and subsequently translocates to the nucleus, where it binds to HIF response elements (HRE) and initiates the transcription of target genes. This leads to metabolic rearrangements such as aerobic glycolysis (Warburg effect) and reductive carboxylation of glutamine derived 2OG [5*,7**,8,9] (Figure 2E). Interestingly, Sun and Denko recently reported that HIF1 induces the expression of the ubiquitinating enzyme SIAH2 which in turn destabilizes a small subunit of OGDH [7**], resulting in increased 2OG concentrations. In this case, induction of reductive carboxylation of 2OG by mass action is caused by transcriptional activation of SIAH2 and subsequent inhibition of OGDH. The observations made by Sun and Denko were made in hypoxic cells. Therefore, HIF1 α was stabilized independent of increasing 2OG concentrations and SIAH2 transcription could be induced. As described above, reductive carboxylation of 2OG can also be increased independent of HIF1 solely by changes in the co-factor ratio, as reported by Mullen and his co-workers [11*].

An example of a more direct relationship between a metabolite and a transcription factor is the carbohydrate-responsive element-binding protein (ChREBP). ChREBP is known to be affected by different glucose levels in hepatocytes and acts as a transcription factor by directly binding to carbohydrate responsive elements (ChREs) [31]. ChREBP regulates the expression of glycolytic and lipogenic enzymes, including pyruvate kinase, fatty acid synthase, and acetyl-CoA carboxylase [32–34]. To efficiently adjust the activatory effect of ChREBP, a

mechanism to sense intracellular carbohydrate levels is required (Figure 2F). Three metabolites have been reported to modulate ChREBP activity: xylulose-5-phosphate, glucose-6-phosphate, and more recently fructose-2,6-bisphosphate. Due to a predicted binding pocket, glucose-6-phosphate is the most promising candidate to directly interact with ChREBP [35].

In addition to changing the localization or binding affinity of the transcription factors, the accessibility of transcription factor binding sites can be altered by chromatin remodeling. In particular, acetylation of histones has been implicated in facilitating the binding of transcription factors and thereby the expression of specific genes. Since histone acetylation reactions utilize acetyl-CoA as a substrate, it has been speculated that histone acetylations are regulated as a function of the metabolic state, and more specifically by intracellular acetyl-CoA levels [36–38]. Most of these speculations are based on a study by Wellen and colleagues [39] reporting that acetyl-CoA generated via ATP-citrate lyase (ACL) is necessary for histone acetylations. Indeed, this study shows that in the absence of ACL, total histone acetylation levels are reduced and that nutrient availability changes histone acetylation levels. However, whether the acetyl-CoA concentration is the driving force behind the increased histone acetylation levels or just a simple correlation remains to be elucidated. In this context, it would be of interest to investigate if histone acetyltransferases (HATs) are in fact sensitive to physiological fluctuations in acetyl-CoA concentrations.

Conclusion

In this article we discussed how metabolites can regulate enzymes on various levels, beginning with very direct mechanisms, such as substrate and product concentrations and allosteric modulations to more intricate and complex transcriptional regulations. Since cellular metabolism is regulated on many different levels, it is sometimes not clear whether changing metabolite concentrations are cause or consequence of the regulation. To obtain a more mechanistic understanding of metabolite regulation, instead of correlation based assumptions, the cell's direct metabolite sensing mechanisms need to be elucidated. To that end, interdisciplinary approaches and new experimental methods are required. For example, compartment-specific metabolite concentration measurements in combination with computational modeling to detect putative binding pockets in proteins and nucleic acids will accelerate our understanding of how metabolites modulate cellular metabolism.

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