TRENDS BOX

- Metabolic intermediates of biochemical pathways are able to act as intra- and extracellular signalling molecules affecting immune cell responses.
- The signalling effects of metabolites are concentration and localization dependent.
- Their functions go beyond self-regulatory mechanisms and include cell to cell communication as well as sensing of micro-environmental conditions to elicit stress responses and cellular adaptation.

Intermediates of metabolism: from bystanders to signalling molecules

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Summary

The integration of biochemistry into immune cell biology has immensely contributed to our understanding of immune cell function and the associated pathologies. So far, most studies have focused on the regulation of metabolic pathways during an immune response and their contribution to its success. More recently, novel signalling functions of metabolic intermediates are being discovered that might play important roles in the regulation of immunity. Here, we describe the three long-known small metabolites lactate, acetyl-CoA and succinate in the context of immuno-metabolic signalling. Functions of these ubiquitous molecules are largely dependent on their intra- and extra-cellular concentrations as well as their sub-compartmental localization. Importantly, the signalling functions of these metabolic intermediates extend beyond self-regulatory roles and include cell to cell communication and sensing of micro-environmental conditions to elicit stress responses and cellular adaptation.

Metabolite signaling in immunity

The metabolic regulation of immune cells during health and disease has gained a lot of attention as the active reconfiguration of immune cell metabolism enables these cells to sustain certain effector functions. The focus so far has been on the necessity of the main catabolic pathways glycolysis, fatty acid oxidation, the anaplerotic tricarboxylic acid (TCA) cycle and oxidative phosphorylation as well as amino acid metabolism (Figure 1) during activation, proliferation, differentiation and function as a response to extracellular signals.

It is now becoming increasingly evident that small molecule intermediates of these metabolic pathways, besides their anabolic and catabolic function, can act as intra- and extracellular signals that influence the outcome of an immune response. The roles of metabolite signalling stretch from regulation of cytokine production via indirect effects on the cellular redox state [1] or direct interaction with transcription factors binding the specific cytokine promoter elements [2] and modulating the activity of transmembrane ion channels [3], to interference with cell migration and differentiation. Interestingly, a few G-protein coupled receptors that are activated by intermediates of metabolism have recently been identified supporting a role for metabolites as extracellular signals [4, 5]. In this review, we discuss the three well known metabolites lactate, succinate and acetyl-CoA in more detail; identify their differences and similarities in signal transduction and effect on immunity and inflammation that defines them as novel signalling molecules in physiology and pathology.

Lactate is a signalling molecule

Lactate is a ubiquitous molecule, whose presence in the mammalian body was first observed in muscle tissue at the beginning of the 19th century [6]. Since its discovery, lactate has been intensely studied and it has been shown to have numerous metabolic functions (Figure 2, Key Figure), including as a central metabolite in the Cori cycle (also known as the lactic acid cycle), which mediates metabolic cross talk between the liver and the muscle. In the Cori cycle, muscle tissue metabolizes liver-derived glucose to lactate, which in turn is shuttled back to the liver and acts as a fuel source for hepatic gluconeogenesis [7]. By contrast, in the brain lactate acts as a metabolic signal and fuel for oxidative metabolism, which is the basis of the neuron-astrocyte lactate shuttle [8]. Briefly, the neurotransmitter glutamate induces high glycolytic activity in astrocytes, which secrete lactate into the synaptic cleft. The increased availability of extracellular lactate enables neurons to import it and use it as an alternative fuel source.

Although lactate has been known to biochemists for over two hundred years, it has been long neglected, seen as a by-product or a bio-marker at best rather than a bio-active molecule. As a consequence its potential functional effects have been under appreciated.

Recently, lactate is being rediscovered as an active signalling metabolite in multiple fields of biology and medicine that has two main ways of signal transduction – transporter and receptor mediated. Its direct regulation of global gene transcription [9, 10], endothelial and cancer cell migration [11, 12], cancer progression [13] and functional polarization of immune cells are being described [14-16].

Lactate production occurs mainly in the cytoplasm during hypoxia or aerobic glycolysis in proliferative cells and is then secreted through the plasma membrane. This transport is dependent on six so far described solute carrier transporters that perform proton – lactate symport (Mct1-4) or a sodium-dependent symport (Slc5a8, Slc5a12) [17, 18]. The MCT family harbours 14 members that all share conserved sequence motifs, yet differ in their substrate specificity, transport rate and expression pattern [17]. Indeed, only Mct1 (Slc16a1, Km 4.5) and Mct4 (Slc16a3, Km 28) have been shown to have a high specificity for lactate in concord with broad tissue expression [17, 19].

Similarly, sodium-coupled lactate transport is carried out by the ubiquitously expressed high affinity transporter Slc5a8 or the low affinity transporter Slc5a12 [18]. The transport direction of both systems depends on the intra- and extracellular concentration of lactate, favouring lactate import (even through low affinity transporters) only in the presence of high extracellular lactate concentrations.

The physiological lactate concentration is about 1.5mM – 3mM [18] in blood and healthy tissues, but can rise up to 10mM in inflammatory pathologies such as atherosclerotic plaques or rheumatic synovial fluid and even up to 20-30mM in cancerous tissue [14, 15, 20]. Far from being inert, accumulating lactate – a feature of most inflammatory sites – has tremendous effects on tissue resident or infiltrating immune cells as well as stromal cells. In the tumour microenvironment, lactate produced by tumour cells is taken up by macrophages where it promotes polarization towards Arginase 2 (Arg2) expressing M2-like phenotype via Hypoxia inducible factor 1α (Hif-1α) stabilization and the resulting increased production of vascular endothelial growth factor (VEGF). These effects further enhance tumour growth in a vicious loop [14]. The authors applied unbiased high-throughput platforms to look for hypothetical protein factors perpetuating such vicious loop, but surprisingly found lactate as the orchestrating factor. Similarly, an independent study recently found lactate to be the driving force behind tumour associated macrophage (TAM) development during epithelial to mesenchymal transition [16].

The involvement of Hif-1α in the response to lactate is currently under scrutiny. On the one hand, it has been demonstrated that targeting the lactate transporter Mct1 in endothelial cells or cervix squamous carcinoma cells rescues lactate-mediated Hif-1α activation and inhibits the consequential angiogenesis [21, 22]. On the other hand, these authors could show a lactate mediated Hif-1α independent induction of angiogenesis. Here, reactive oxygen species (ROS) induced the NF-κB pathway that led to IL-8 expression, a known chemotactic molecule that resulted in increased cell migration and tumour metastasis [23]. Additionally, a recent study demonstrates the Hif-1α independent, direct binding of lactate to NDRG3 during hypoxia. Upon lactate binding,

NDRG3 is stabilized and executes a Raf-ERK1/2 mediated signalling cascade promoting angiogenesis and cell growth [24]. Surprisingly, not only migration-promoting but also inhibiting functions of lactate have been described.

Activated T cells that infiltrate inflammatory sites are exposed to the increased lactate concentration that is commonly found in these sites (e.g., 10-12mM in arthritic synovium) [15]. Due to the high extracellular concentration, lactate internalization through the $CD8^+$ T cell specific transporter Mct1 and $CD4^+$ T cell specific transporter Slc5a12 is favoured. This inhibits glycolysis via inhibition of Pfk or downregulation of Hk1 [15, 25], causing T cells to lose their responsiveness to chemokines and effectively trapping them in the inflamed site. Of note, these effects are not only observed in *in vitro* assays; in an animal model of peritonitis, lactate levels and T cell numbers in the peritoneum are indeed increased 5 days after intra peritoneal (i.p.) injection with zymosan, a glucan commonly used to induce sterile inflammation. Of note, inhibition of lactate transporters re-establishes T cell migration not only *in vitro* but also in the peritonitis model [15]. These findings describe a mechanism explaining at least in part the well-known clinical observation that T cells are entrapped in inflamed tissue [15] (Figure 2).

In addition, lactate also triggers the production of the pro-inflammatory cytokine IL-17 in the $CD4^+$ subset and inhibits the cytolytic function of cytotoxic $CD8^+$ T cells (CTL) (Figure 2). The observed inhibition of CTL function was also reported in an earlier publication, showing that both proliferation and cytokine production in human CTLs is severely impaired in the presence of lactic acid [26].

The observed effects of T cell entrapment, CTL inhibition and increased production of pro-inflammatory cytokines are common features of many chronic inflammatory diseases that might be in part explained by lactate signalling. The detailed molecular mechanisms for lactate mediated inhibition of T cell migration and change in function, however, are yet to be elucidated.

Interestingly, the above effects of lactate/lactic acid on macrophages, endothelial cells and T cells are independent of the pH change that is caused by the acidic form of lactate [15, 16], yet protons are still required for the translocation of lactate into the cytoplasm [15].

Additional evidence establishing lactate as a signalling molecule comes from the identification of the lactate receptor Gpr81, first cloned in 2001 [27]. Seven years later, lactate was identified as the primary ligand for Gpr81, being involved in lactate-mediated reduction of lipolysis in adipocytes, the primary Gpr81-expressing cell type [4]. It is now known that Gpr81 is a G-protein coupled receptor that inhibits adenylyl cyclase via the Gi signalling pathway [28] and mediates the insulin-induced reduction of lipolysis [29, 30]. Interestingly, several reports identify lactate receptor activation as a critical survival signal for cancer cells [31, 32], and define it as a therapeutic target in ischemic brain injury [33] (Figure 2).

In contrast to the roles of lactate on cell signalling, its effect on regulating other metabolic pathways is better understood. Once in the cytoplasm, lactate is readily oxidized to pyruvate by lactate dehydrogenase (LDH). This reaction proceeds with a concomitant proton transfer from lactate to nicotinamide dinucleotide (NAD^+) thereby generating NADH and affecting the redox state of the cell (see Glossary). Although LDH is mainly considered a cytoplasmic enzyme, after years of controversy the existence of a mitochondrial LDH has finally been proven [34]. Thus, given the presence of Mct1 in the mitochondrial membrane [35], lactate metabolism is now being considered as an active part of mitochondrial metabolism. Recently, the LDH subunit B (LDHB) has also been shown to localize to peroxisomes in fibroblasts and HeLa cells [36]. This might be a hint towards the possible involvement of lactate in fatty acid oxidation or lipid metabolism in general as discussed in the following excellent review [37].

Contrarily, the effects on glycolysis remain questionable. It was shown that 10mM extracellular lactate inhibits glycolytic activity in T cells, which could be facilitated by downregulation of hexokinase 1 or direct inhibition of phosphofructokinase. By contrast, in heart tissue lactate

causes increased Glut1 and Glut4 expression on the plasma membrane, which is in general an indicator of increased glucose uptake and flux [38].

Taken together, lactate-induced signalling is an important pathway in health and disease. As discussed here, lactate has two primary means of relaying signals into the cell (receptor- and transporter-mediated), and lactate signalling has several possible outcomes that depend on the cell type. Moreover, the concentration of extracellular lactate that lie between 1.5mM in physiological and 10-30mM in pathological settings has immense impact on cell function as it will lead to the activation of different signalling pathways.

Effects beyond metabolism of TCA cycle intermediates: focus on succinate

It is now becoming clear that the reaction intermediates of the TCA cycle (Figure 1; Box 1) can act as proper signalling molecules once they are withdrawn from the cycle and redirected towards different functions. This occurs both in physiological and pathological conditions; for instance, citrate accumulates in the cytosol of Lipopolysaccharide (LPS) -treated macrophages where it is necessary for the synthesis of NO, ROS and prostaglandins [39]. Fumarate has been recently defined as a proto-oncometabolite [40, 41] because its accumulation, due to loss of function mutations in the fumarate hydratase (FH) (see Glossary) [42, 43], results in stabilization of Hif-1α via two different mechanisms: the inhibition of PHD2 (prolyl hydroxylase domain-containing protein 2), which targets HIF factors for degradation [44], and the amplification of ROS signalling through the consumption of reduced glutathione and NADPH [41]. Another TCA cycle metabolite that can regulate non-metabolic activities is NAD^+, an important cofactor for sirtuins, a family of deacetylases that targets important transcription factors of the inflammatory response, such as NF-kB [45] and AP1 [46], and controls mitochondrial quality and biogenesis [47].

For the rest of this section we focus on succinate, as in the past few years it has been reported to be a central metabolite in the biology of immune cells (such as macrophages) as well as

in cancer and other pathological contexts, providing a clear example of a new potential therapeutic target.

Succinate is synthesized from α-ketoglutarate (first converted to succinyl-CoA and then to succinate) and subsequently utilized as a substrate by succinate dehydrogenase (SDH) to produce fumarate (Figure 3). Inhibition of SDH results in accumulation of succinate, stabilization of Hif-1α, induction of Hif-1α transcriptional activity and oncogenic events. Stabilization of Hif-1α is due to the ability of succinate to inhibit PHD enzymes (prolyl hydroxylases). Hydroxylation of HIF by PHD enzymes is necessary for its binding to pVHL, part of an E3 ubiquitin ligase targeting HIF for degradation [48]. Thus, reduction of this hydroxylation results in HIF stabilization leading to the transcription of genes involved in proliferation, angiogenesis and metastasis [49] (Figure 3).

Stabilization of Hif-1α by succinate was also observed in LPS-treated macrophages and was associated with enhanced production of IL-1β [2]. The authors observed an accumulation of succinate in macrophages after Toll-like receptor 4 (TLR4) engagement by LPS, which was responsible for the stabilization of Hif-1α. The stabilization of Hif-1α in activated macrophages was directly linked to the increased transcription and production of IL-1β; indeed, the authors demonstrated that Hif-1α can bind the promoter of IL-1β, activating its transcription. LPS-treated macrophages undergo a switch from oxidative phosphorylation to glycolysis, lowering the activity of the TCA cycle, which raises questions as to the source of succinate accumulation. Indeed, the main source of succinate after LPS treatment appears to be glutamine, mainly via anaplerosis of α-ketoglutarate feeding into the TCA cycle and replenishing succinate, and, to a lesser extent, via the GABA (γ-aminobutyric acid) shunt (Figure 3). These findings suggest succinate is an inflammatory signal that is necessary to activate macrophages and stabilize a fundamental player of immune response, Hif-1α, leading to the production of IL-1β [2] (Figure 3).

A new depth of understanding of the metabolic rewiring of intracellular metabolism was obtained by a study that used metabolomics and transcriptomics to characterize, in detail, the changes that occur in macrophages during polarization towards M1 or M2 phenotypes [50]. With

regard to M1 polarization, they identified two TCA cycle break-points: the first at the conversion point of citrate to α-ketoglutarate, and the second after succinate synthesis at the conversion point of succinate to fumarate. The first break-point, due to the downregulation of isocitrate dehydrogenase, results in citrate accumulation, which is then redirected towards production of itaconic acid, an anti-microbial metabolite [51]. The authors also described a second break-point, at the TCA step of converting succinate into fumarate; succinate accumulation increased, as previously reported [2]. Despite the low efficiency of succinate to fumarate conversion, an accumulation of malate (produced from fumarate) was also observed. This was due to the upregulation of the arginosuccinate shunt, a series of reactions feeding first into fumarate and then malate. This shunt is important not only to replenish malate and subsequently citrate (to complete the cycle) but also to produce NO and IL-6, both necessary for appropriate activation of macrophages [50].

Ischaemia reperfusion (IR) also causes the specific accumulation of succinate and subsequent production of mitochondrial ROS. Production of ROS during ischaemia reperfusion has always been thought to be a nonspecific response due to reperfusion; however, a recent study demonstrates that succinate accumulation during reperfusion of ischaemic tissues is a selective response that drives the generation of ROS responsible for tissue damage [52]. Using an *in vivo* model of ischaemia in combination with unsupervised metabolomics analysis, succinate was found to specifically accumulate during ischaemia in different tissues (liver, kidney, heart and brain) and it was rapidly re-oxidized during reperfusion. To assess the source of succinate, they performed stable isotope tracing experiments and found that succinate derived mainly from the malate/aspartate shuttle (MAS) and the purine nucleotide cycle (PNC) (Figure 3). These two pathways led to the accumulation of fumarate which was then converted to succinate by the reversal of SDH. During reperfusion the accumulated succinate was rapidly re-oxidized to fumarate by SDH, leading to a massive production of mROS, mainly superoxide (Figure 3), due to the reverse electron transport (RET) through mitochondrial complex I (see Box 1) [52]. This observation describes succinate as a damage signal during reperfusion, making it an intriguing target for therapy development.

In addition to these intracellular non-metabolic effects of succinate, it also binds to a specific receptor localized onto the cytoplasmic membrane, which suggests it can signal as an extracellular molecule (Figure 3). The succinate receptor, GPR91 (also known as SUCNR), is a G protein-coupled receptor whose activation triggers intracellular calcium release and inhibits cAMP production. In mouse, it is expressed mainly in the kidney, liver, spleen and small intestine [5]. GPR91 is expressed on human and mouse dendritic cells (DC), where it enhances their immune-stimulatory capacity [53]. Specifically, succinate stimulation of GPR91 promotes migration of DC in a dose-dependent manner, and it cooperates with TLR ligands to induce cytokines via Erk1/2 phosphorylation. Furthermore, succinate sustains and empowers DC-mediated T cell activation. These effects were shown to be dependent on succinate stimulation of GPR91, as they were abrogated in Sucnr-/- mice [53].

Immune regulation by fatty acid oxidation: in search of a signalling role for lipid intermediates

Unlike glycolysis and the TCA cycle, the intermediates of fatty acid metabolism have not yet been shown to regulate T cell fate and functional specification (Figure 1 and Box 1). However, both the induction of fatty acid synthesis (FAS) and its inverse metabolic pathway, fatty acid oxidation (FAO), have been linked to T cell function. Specifically, while the induction FAS is known to be an integral part of the T cell activation program that is associated with increased glucose metabolism, which is essential for the differentiation of naïve T cells into their T effector subsets, FAO has been shown to be critical for the development of $CD8^+$ memory T cells and the induction of $CD4^+$ regulatory T cells [54, 55] (Figure 4).

Mice with a T cell-specific deletion of tumour necrosis factor (TNF) receptor-associated factor 6 (TRAF6) displayed a profound defect in $CD8^+$ memory T cell generation, and were unable to upregulate FAO after growth factors withdrawal during contraction phase of immune response. TRAF6-deficient $CD8^+$ T cells exhibited defective AMP-activated kinase (AMPK) activation, whereas

activation of AMPK with metformin was able to rescue both FAO and the generation of $CD8^+$ memory T cells, suggesting TRAF6 regulates a metabolic switch towards FAO important for generation of long-lived $CD8^+$ memory T cells [54]. Regulatory $CD4^+$ T cells (Tregs) have also been shown to rely primarily on FAO during their development [55, 56]. Naturally occurring Tregs display low levels of Glut1, and thus low rates of glycolysis, while having increased activation of AMPK. Treatment with etomoxir, an inhibitor of carnitine palmitoyl transferase (CPT1), the rate limiting enzyme in FAO, was sufficient to abrogate Treg development, suggesting that FAO is essential for Treg development [55].

Interestingly, it has recently been demonstrated that the increase in FAO in memory $CD8^+$ T is surprisingly the direct result of *de novo* FAS, rather than uptake of fatty acids from the extracellular environment [57]. After observing no increase in extracellular fatty acid uptake, the authors showed that extracellular glucose fuels mitochondrial fatty acid oxidation and oxidative phosphorylation (OXPHOS) indicating that fatty acids are synthesised for subsequent oxidation. Upon treatment with a fatty acid synthase inhibitor, memory T cell death increased suggesting fatty acid synthesis is necessary for their survival. Memory T cells lack typical fatty acid storage droplets; instead, lysosomal acid lipase (LAL) activity plays a role in non-classical fatty acid storage. LAL is required for lipolysis of stored fatty acids to generate available fatty acids for oxidation and necessary for memory T cell survival. These data support the phenomenon known as fatty acid "futile cycling" whereby intracellular fatty acids are catabolized rather than acquired from extracellular sources, for use in the mitochondria for fatty acid oxidation. With no net gain of ATP, this cycling of fatty acids is bio-energetically redundant. However, it has been suggested that fatty acid cycling in memory T cells may provide a mechanism to maintain their survival and, sustaining their glycolytic and mitochondrial metabolism, may enable rapid recall responses after antigen recognition.

Extracellular signalling by IL-7 and IL-15 has been shown to impact on T cell development by affecting FAO [58, 59] (Figure 4). The cytokine IL-15, which is critical for the development and maintenance of $CD8^+$ memory cells, enhanced the expression of the rate-limiting FAO enzyme CPT1,

and thus FAO. Importantly, blocking CPT1 with etomoxir impaired the mitochondrial spare respiratory capacity (see Glossary) and survival of $CD8^+$ memory T cells. Conversely, over-expression of CPT1 increased the formation of $CD8^+$ memory T cells following infection [58]. IL-7 is known to control $CD8^+$ memory T cell longevity and homeostasis [60, 61]. Recent findings demonstrate a new pathway in which IL-7 promotes survival via glycerol import, triglyceride synthesis and storage. Triglycerides are synthetized combining glycerol-3-phosphate and Acyl-CoAs (free fatty acids activated with a Coenzyme-A moiety), thus the amount of glycerol affects triglyceride synthesis. In this study IL-7 was shown to induce expression of the glycerol channel aquaporin 9 (AQP9), which was required for long term survival of $CD8^+$ memory cells. AQP9 deficiency resulted in impaired glycerol import, and thus esterification of fatty acids and reduced triglyceride synthesis and storage. These defects were rescued by ectopic expression of triglyceride synthases, which restored lipid stores and $CD8^+$ memory T cells [59].

Acyl-CoA is the main intermediate metabolite of lipid metabolism within the cell. FAO involves the sequential removal of 2-carbon units from a fatty acyl-CoA molecule to yield acetyl-CoA, which can be directly shuttled into the TCA cycle. While intermediate products of glycolysis and the TCA cycle have been shown to have active signalling roles, little research has been done into whether FAO metabolites may have direct effects on the cell fate decision of T cells. However, a recent paper has shown that long chain acyl-CoAs, which are the activated form of free fatty acids and represent the pre-step reaction for β-oxidation, can act as positive modulators of ion channels and exchangers [3]. Specifically, long chain acyl-CoAs were shown to be potent activators of TRPV1 cation channels independently of $Ca2^+$, and increasing the level of long chain acyl-CoAs in intact Jurkat T cells leads to a significant increase in agonist-induced $Ca2^+$ levels. This novel mechanism indicates that long chain acyl-CoAs could play an active role in T cell functions under both physiological and pathophysiological conditions that alter fatty acid transport and metabolism.

Acetyl-CoA, the end product of FAO, has also been implicated to have roles beyond the TCA cycle, as it can also act as a substrate for post-translational modifications such as acetylation [62].

One potential mechanism is through histone acetylation, which is known to be important for promoting gene transcription. In the context of immune cells, $CD8^+$ T cells histone acetylation occurs at specific loci and may be involved in determining the decision between memory and short-lived effector cell fate [63]. Similarly, different histone acetylation patterns have been shown in $CD4^+$ effector T cells compared to $CD4^+$ Tregs. Specifically, in Tregs there is increased acetylation of the Foxp3 locus [64], whereas in T effector cells the IL-13, IL-15 and IL-4 loci have all been shown to have increased acetylation induced by IL-4 [65].

Concluding remarks

The discovery of extra-metabolic functions of metabolic enzymes (referred to as moon-lighting) has been the first evidence that metabolism and its players have a role in the regulation of signalling pathways inside cells. Here, we have described how a similar concept applies to metabolites, which for decades were considered to be only the building blocks of biomasses. The observation that intermediates and end products of the main metabolic pathways can desert their metabolic roles to function, in certain circumstances, as transduction signals shows how these molecules can play an active and crucial role in regulating some of the most important biological processes. We currently have evidence that some metabolites play roles in the immune and inflammatory responses, and influence cytokine production, proliferation and angiogenesis, both in physiology and pathology. It remains to be established if other metabolites might display similar functions.

In this new scenario, the accumulation or the depletion of specific metabolites does not represent just a metabolic adaptation or a choice merely dictated by energy demand; it is rather an elected form of signalling-mediated regulation of biological processes. The loss of this intrinsic regulation is associated with several pathological conditions, and this might explain the current efforts to target metabolic pathways for therapeutic purpose. In doing so, we need to bear in mind

that some processes, such as ROS production, are necessary but must be tightly regulated to maintain homeostasis; hence in some cases mild intervention could be safer and even more beneficial than blunt blockade.

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BOX1 - Basics of TCA cycle, FAO and ETC/RET

TCA cycle

The tricarboxylic acid (TCA) cycle, also known as citric acid cycle or Krebs Cycle, is a series of biochemical reactions occurring into the mitochondrion that provide energy and reducing equivalents through the complete oxidation of acetate, in form of acetyl-CoA, produced from the breakdown of sugars, lipids and amino-acids (Figure 1).

FAO

Fatty acids are an alternative source of fuel for cells yielding large amounts of ATP during their oxidation. This catabolic process occurs in the mitochondria to generate acetyl-CoA, which directly enters into the TCA cycle (Figure 1).

ETC

The mitochondrial electron transport chain (ETC) is a series of protein complexes embedded into the inner mitochondrial membrane responsible for the transfer of electrons from donors to acceptors via redox reactions. The transfer of electrons through the chain is coupled to the pumping of protons into the intermembrane space. This proton gradient provides the proton-motive force necessary for the generation of energy in the form of ATP. In eukaryotes, ETC is composed of 4 complexes: complex I or NADH dehydrogenase, which oxidizes NADH and transfers electrons to ubiquinone Q (thus turned to ubiquinol QH2) while pumping four protons into the intermembrane space; complex II or succinate dehydrogenase, which oxidizes succinate to fumarate and transfers electrons to ubiquinone; complex III or cytochrome c oxidoreductase, which re-oxidizes ubiquinol to ubiquinone while reducing cytochrome c and pumping two protons across the membrane; and complex IV or cytochrome c oxidase, which oxidizes cytochrome c passing electrons to the final acceptor of the chain, molecular oxygen O_2, generating water and pumping four protons across the membrane.

Finally, ATP synthase utilizes the energy stored into the protons gradient to phosphorylate ADP to ATP. Complex I and II represent two independent entry points to the ETC.

RET

Reverse electron transfer (RET) is the flow of electrons from ubiquinone to NAD^+ catalysed by complex I in the presence of a reduced pool of QH2 and a high proton gradient forcing electrons backward from QH2 into complex I, which leads to the production of superoxide [66, 67].

GLOSSARY

REDOX STATE

The cellular redox state is often described as the balance of **red**uced and **ox**idized glutathione (GSH/GSSG), Nicotinamidedinuclotide (NAD^+/NADH) and Nicotinamidedinuclotide-phosphate ($NADP^+$/NADPH). These redox couples are central mediators for catabolic and anabolic reactions acting as cofactors and regulators for enzymes, scavengers for reactive oxygen species or substrates for the mitochondrial electron transport chain.

FUMARATE HYDRATASE (FH):

The TCA cycle enzyme responsible for the conversion of fumarate to malate.

SPARE RESPIRATORY CAPACITY:

The extra capacity available in cell to produce energy via mitochondrial respiration.

FIGURE LEGENDS

Figure 1 – Metabolic pathways and regulatory intermediates of metabolism. The main cellular catabolic pathways (glycolysis: blue; tricarboxylic acid (TCA) cycle: red; fatty acid oxidation (FAO): green) not only produce ATP, but also metabolic intermediates, such as lactate, acetyl-CoA and succinate, highlighted in colour. These are substrates for anabolic processes including lipid and nucleotide synthesis, but can also act as regulatory signalling molecules.

Figure 2, Key Figure – Lactate-mediated signalling pathways and their biological outcomes. Extracellular lactate (left side of the figure) has staggering functional effects on several cell types, including production of pro- and anti-inflammatory mediators by T cells and macrophages (Mϕ), or migratory changes and metabolic adaptation in T cells, endothelial cells (EC) and neurons. Intracellularly (right side of the figure), lactate can directly bind to proteins (i.e. NDRG3), influence the redox state via the lactate dehydrogenase (LDH) reaction, stabilize Hif-1α, induce reactive oxygen species (ROS) and act as an inhibitor of glucose breakdown. The occurrence of these effects might depend on the investigated cell type.

Figure 3 – Succinate signalling and its biological effects. Succinate exerts several biological responses. By stabilizing Hif-1α, it promotes proliferation, angiogenesis and cytokine production. Succinate can also regulate proteins activity via succinylation. During ischaemia, fumarate accumulates through the malate-aspartate shuttle (MAS) and the purine nucleotide cycle (PNC) and is converted into succinate by succinate dehydrogenase reversal (SDH); during reperfusion, the rapid re-oxidation of succinate to fumarate by SDH leads to the production of mitochondrial reactive oxygen species (mROS) responsible for tissue damage. Furthermore, succinate in the extracellular microenvironment can signal through its receptor (GPR91), sustaining cytokine production and migration of dendritic cells (DC). TCA, tricarboxylic acid cycle; AMP, adenosine monophosphate; IMP,

inosine monophosphate; PHD, prolyl hydroxylases; GABA, γ-aminobutyric acid; IR, ischeamia-reperfusion.

Figure 4 – Fatty acids oxidation and its role in signalling. FAO has been shown to be necessary for CD8+ memory T cells survival and CD4+ regulatory T cells induction. Moreover, upregulation of glycerol transporter AQP9 by cytokines is important to increase triglycerides synthesis and thus FAO. The futile cycling of lipolysis and subsequent FAO seems to be important for memory T cell survival.

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OUTSTANDING QUESTIONS BOX

- Lactate has two primary means of relaying signals into the cell, receptor- and transporter mediated, and depending on the cell type has several possible outcomes. The downstream pathways still need to be worked out and might illuminate new knowledge in the near future.
- Besides succinate, other metabolites of the TCA cycle might be directly regulating immune cell functions but this is yet to be fully investigated.
- Unlike glycolysis and the TCA cycle, whose intermediates have been shown to play important roles in determining specific T cell fates and functional specifications, the same has yet to be defined for the cellular metabolism of fatty acids.

Figure

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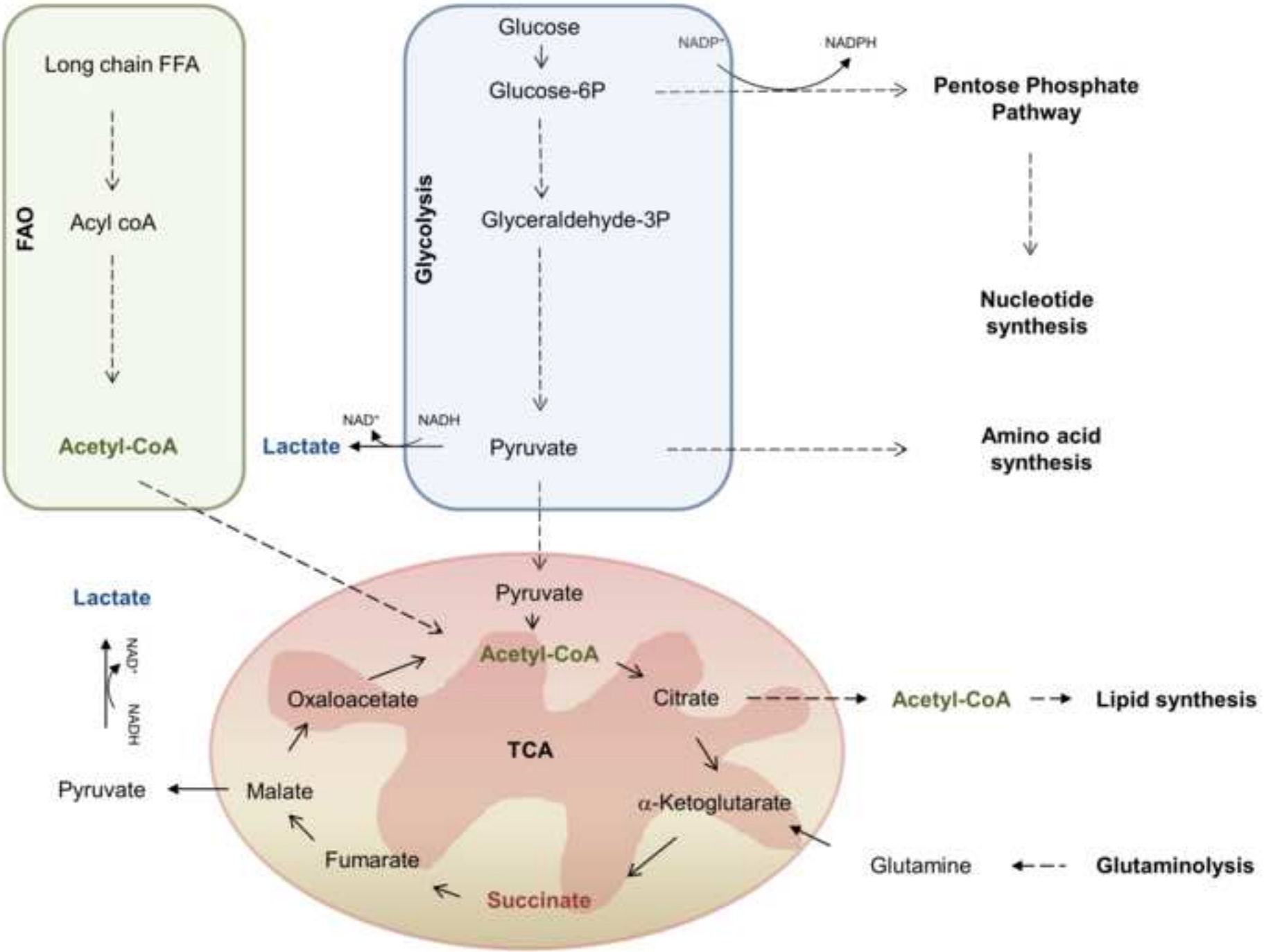

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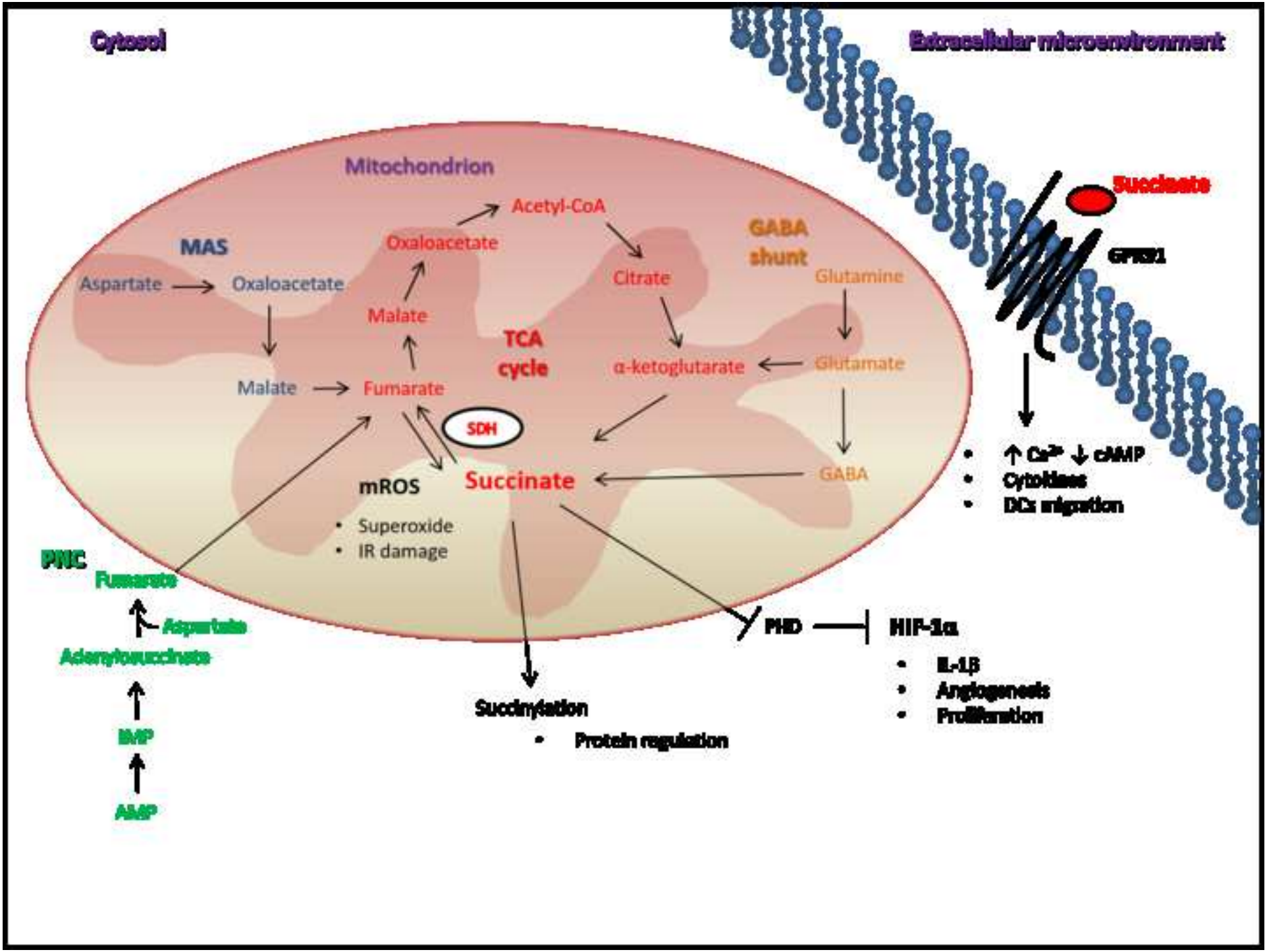

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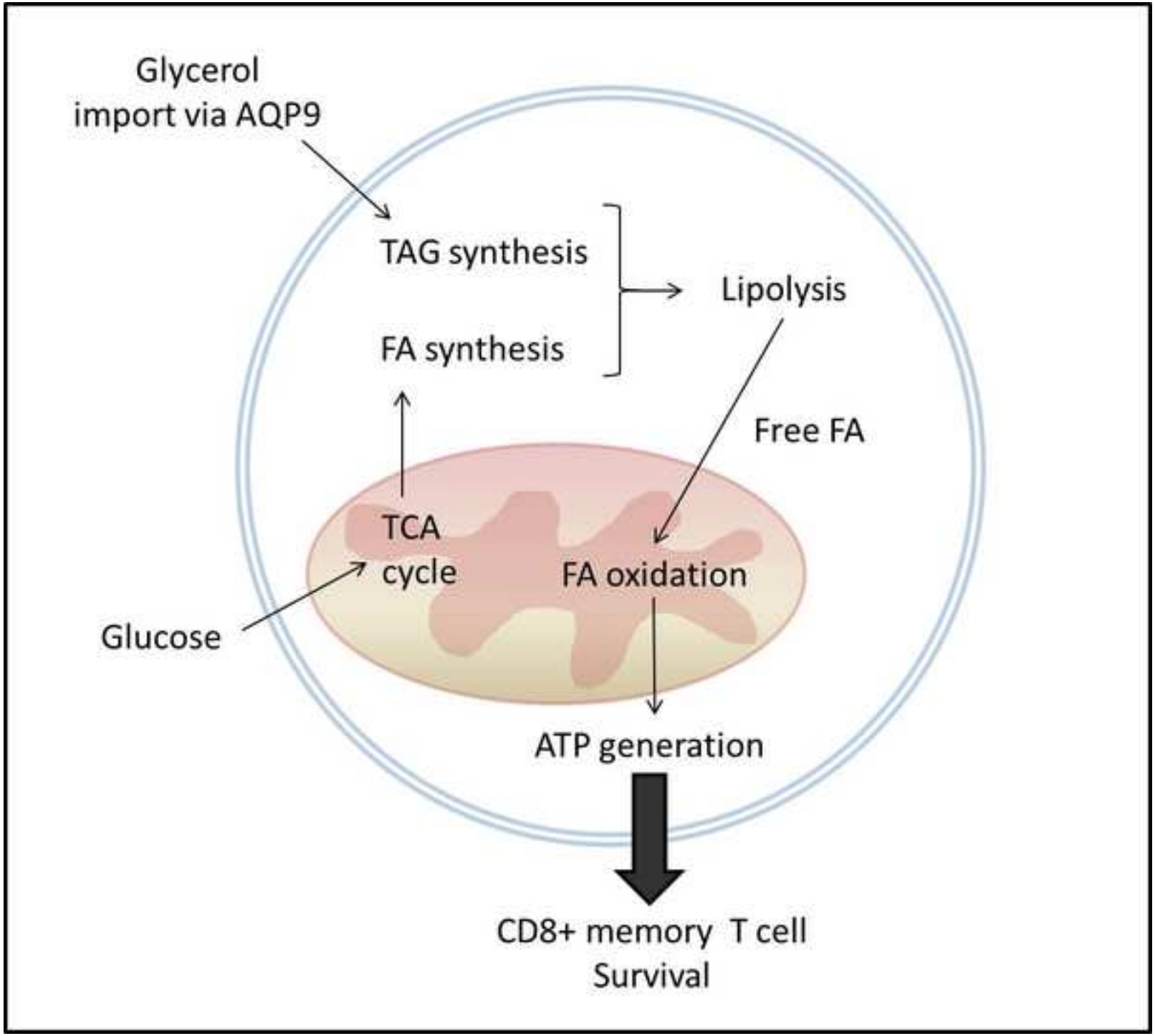

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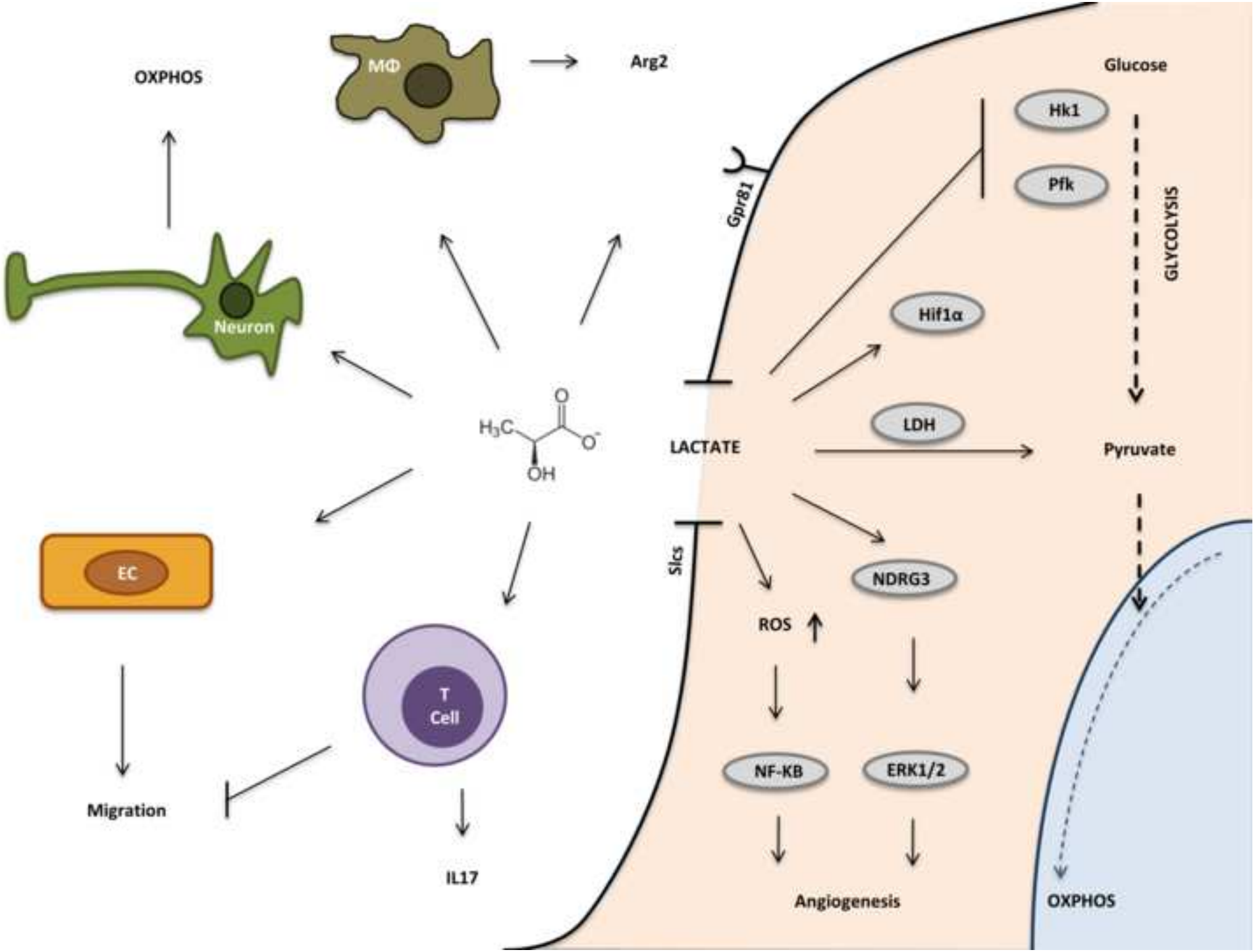

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