

# The engine driving the ship: metabolic steering of cell proliferation and death

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**Abstract** | Metabolic activity is a crucial determinant of a cell's decision to proliferate or die. Although it is not fully understood how metabolic pathways such as glycolysis and the pentose phosphate pathway communicate to cell cycle and apoptotic effectors, it is clear that a complex network of signalling molecules is required to integrate metabolic inputs. D-type cyclins, cyclin-dependent kinases, the anaphase-promoting complex, p53, caspase 2 and B cell lymphoma 2 proteins, among others, have been shown to be regulated by metabolic crosstalk. Elucidating these pathways is of great importance, as metabolic aberrations and their downstream effects are known to contribute to the aetiology of cancer and degenerative disorders.

**Pentose phosphate pathway**  
A metabolic pathway that generates NADPH and pentose sugars from glucose-6-phosphate. NADPH is important for the biosynthesis of many cell components and serves as a major cellular antioxidant.

A wide range of metabolites participate in cellular energy commerce; glucose, lipids, amino acids and nucleic acids are extensively broken down by the cell and remodelled to form countless metabolic intermediates and byproducts. The number of potential metabolic signalling molecules is staggering, and research is only beginning to uncover how these metabolites communicate with the cell cycle and the cell death machinery to affect cell fate.

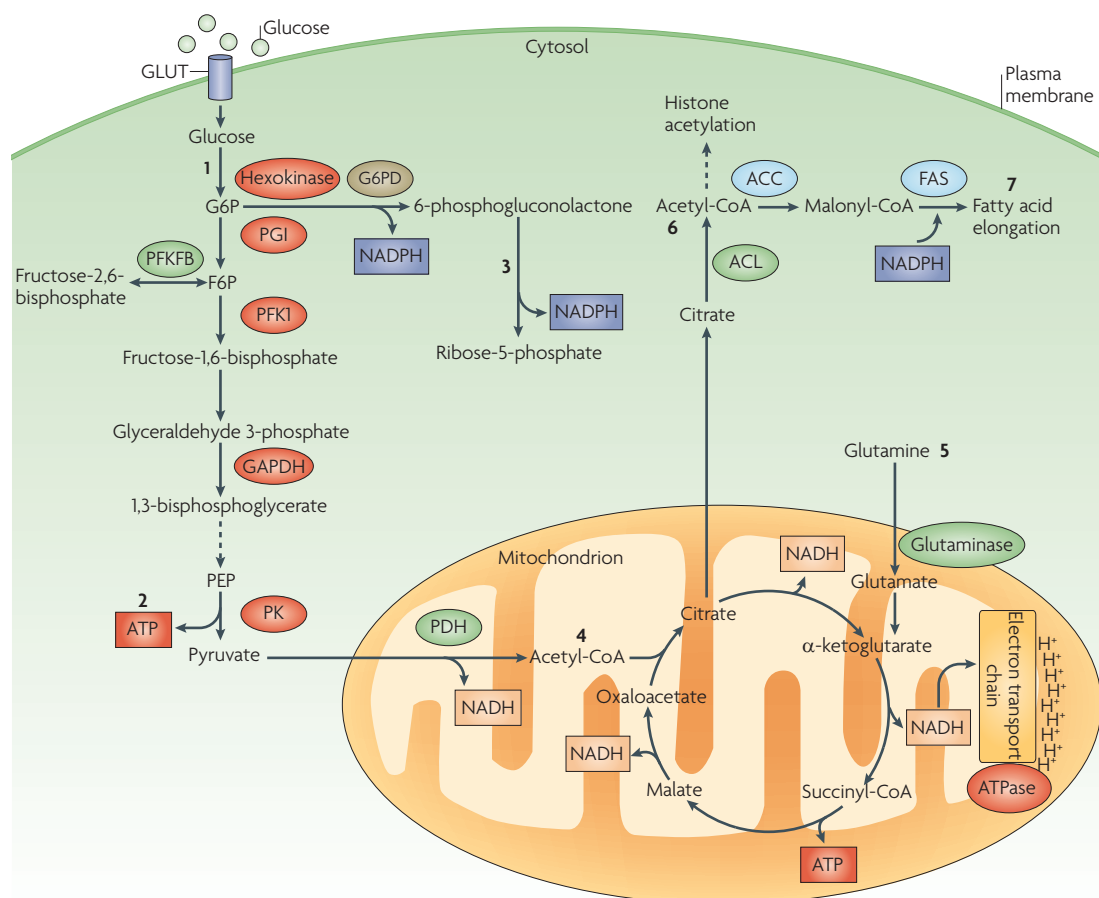
Of importance to this Review are several central metabolic pathways, including glycolysis, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway (PPP) and a range of biosynthetic pathways, most notably those that culminate in fatty acid biosynthesis (FIG. 1). Glucose enters the cell through glucose transporters (GLUTs) and, once intracellular, is phosphorylated to glucose-6-phosphate (G6P) by hexokinases. G6P can then proceed through glycolysis to produce ATP, the coenzyme NADH and pyruvate, or through the PPP, resulting in the production of ribose-5-phosphate and NADPH. In addition to hexokinases, glycolytic enzymes that are crucial for linking metabolism and the cellular events discussed in this Review include phosphofructokinase 1, which catalyses the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate, and pyruvate kinase, which yields pyruvate and ATP in the final step of glycolysis.

Pyruvate produced by glycolysis is converted to acetyl-CoA, which enters the TCA cycle and undergoes a series of oxidative reactions, ultimately resulting in the production of two ATP molecules and six NADH molecules per glucose. NADH is then used in mitochondrial oxidative phosphorylation, which produces abundant ATP from nutrients in the cell. In addition to glucose, amino acids

can also funnel into the TCA cycle, as their catabolism results in the production of TCA cycle intermediates ( $\alpha$ -ketoglutarate, succinyl-CoA, fumarate, oxaloacetate and acetyl-CoA). For example, glutamine is converted to glutamate by glutaminase, and glutamate in turn may be converted to  $\alpha$ -ketoglutarate to feed the TCA cycle. In addition to participating in the TCA cycle, acetyl-CoA is as a key precursor for fatty acid biosynthesis. Acetyl-CoA cannot cross the inner mitochondrial membrane, but intramitochondrial acetyl-CoA and oxaloacetate combine to form citrate, which is transported out of the mitochondria and broken back down into its constituents by ATP citrate lyase (ACL). Acetyl-CoA is converted to malonyl-CoA by acetyl-CoA carboxylase (ACC), and acetyl-CoA and malonyl-CoA are then both used by the multi-subunit enzyme fatty acid synthase (FAS) for the synthesis and elongation of fatty acid chains. Cytosolic and nuclear acetyl-CoA is also a precursor for the post-translational modification of proteins (for example, histones) by acetylation.

In the PPP, G6P is converted to ribose-5-phosphate while producing two molecules of NADPH. The enzyme that governs entry of G6P into this pathway is glucose-6-phosphate dehydrogenase (G6PD), which is regulated by the availability of its substrate (for example, the expression of the gene encoding G6PD increases when animals transition from a fasting to a fed state) and the NADPH to NADP<sup>+</sup> ratio<sup>1</sup>. NADPH is both a major cellular antioxidant, maintaining glutathione in a reduced state to prevent oxidative damage, and a required cofactor in the reductive biosynthesis of fatty acids, nucleotides and amino acids.

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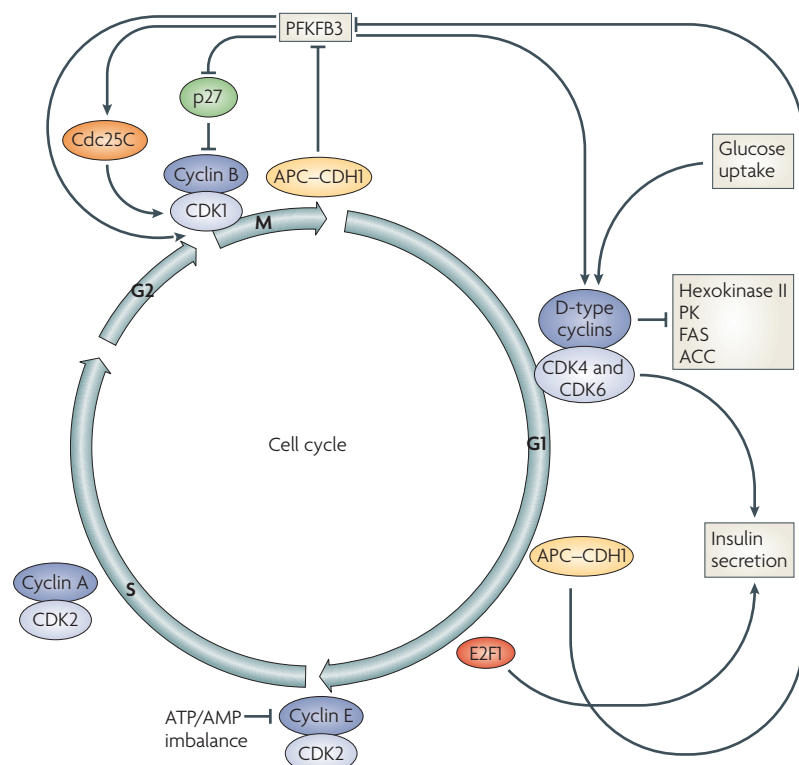
**Figure 1 | Overview of metabolism.** Metabolic pathways important to this Review include glycolysis, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway (PPP) and fatty acid biosynthesis. Glucose enters the cell and is phosphorylated to glucose-6-phosphate (G6P) by hexokinase (1). G6P either proceeds through glycolysis to produce ATP, NADH and pyruvate (2), or through the PPP, producing ribose-5-phosphate and NADPH (3). G6P dehydrogenase (G6PD) dictates entry of G6P into the PPP, and G6P oxidation produces NADPH. NADPH is an important cellular antioxidant and is a cofactor in the reductive biosynthesis of fatty acids, nucleotides and amino acids. Pyruvate produced by glycolysis is converted to acetyl-CoA, which enters the TCA cycle and produces two ATP molecules and six NADH molecules per glucose (4). NADH is then used in mitochondrial oxidative phosphorylation for ATP production. Glutamine and other amino acids also feed into the TCA cycle; glutamine, for example, is converted to glutamate by glutaminase, and glutamate can be converted to  $\alpha$ -ketoglutarate (5). Acetyl-CoA is also an important precursor for fatty acid biosynthesis. Intramitochondrial acetyl-CoA and oxaloacetate combine to form citrate, which is transported out of the mitochondria and broken back down by ATP citrate lyase (ACL) (6). Acetyl-CoA can be used as a precursor for post-translational acetylation of proteins and can also be converted to malonyl-CoA by acetyl-CoA carboxylase (ACC), resulting in the synthesis and elongation of fatty acid chains (7). GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT, glucose transporter; F6P, fructose-6-phosphate; FAS, fatty acid synthase; PDH, pyruvate dehydrogenase; PEP, phosphoenolpyruvate; PFK1, phosphofructokinase 1; PFKFB, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase; PGI, phosphoglucose isomerase; PK, pyruvate kinase.

#### Anaphase-promoting complex

A large multisubunit E3 ubiquitin ligase with a RING-containing subunit (APC11) that ubiquitylates, among other proteins, several proteins that are crucial for the transition from M phase to G1.

This Review provides a glimpse into the ways in which the metabolic pathways introduced briefly above influence key cell cycle and apoptotic effectors to promote cell survival or death. Molecular responses to nutrient flux are complex, and many reviews on the topic have focused on upstream, multimodal nutrient-sensing pathways such as those initiated by phosphoinositide 3-kinase (PI3K)–AKT, 5'-AMP-activated protein kinase (AMPK) and mammalian target of rapamycin<sup>2–5</sup>. In this Review we dissect the modulation of cell cycle and apoptotic effectors by metabolism, taking a bottom-up approach to examine how nutrient-initiated signalling pathways impinge on key

cellular processes. The effects of metabolism on cell cycle targets are discussed first, with specific emphasis on cyclins and cyclin-dependent kinases (CDKs), CDK inhibitors, the anaphase-promoting complex (APC) and chromatin remodelling. Crosstalk between metabolism and apoptosis, and the roles of p53, caspase 2, B cell lymphoma 2 (BCL-2) family proteins, cytochrome *c* and the apoptosome at the interface of these cellular processes, is also examined. The identification and understanding of metabolic effectors in the cell cycle and cell death machinery provides a platform for future inquiry and for rational drug design to specifically target metabolism-responsive effectors.



**Figure 2 | Crosstalk between cell cycle transitions and metabolism.** Transitions between phases of the cell cycle (G1, S, G2 and M) are orchestrated by cell cycle activators and inhibitors, including cyclin–cyclin-dependent kinase (CDK) pairs, Cdc25C, the anaphase-promoting complex (APC) and p27. Signalling by metabolic enzymes (including 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase isoenzyme 3 (PFKFB3), hexokinase II, pyruvate kinase (PK), fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC)) and small molecule metabolites (for example, glucose and fructose-2,6-bisphosphate), influences cell cycle transitions to determine cell fate. For example, PFKFB3, which promotes fructose-2,6-bisphosphate production and helps to drive glycolysis, dictates cell cycle progression by upregulating cyclin D3 and Cdc25C and downregulating the cell cycle inhibitor p27, all of which contribute to an increase in CDK1 activity. In a reciprocal manner, APC–Cdc20 homologue 1 (CDH1) ubiquitylates PFKFB3, targeting it for degradation, thereby moving metabolic intermediates from glycolysis to the pentose phosphate pathway. Further crosstalk from the cell cycle back to metabolism is exemplified by the observation that cyclin D1 overexpression results in decreased activity of the key glycolytic enzyme hexokinase II, and cyclin D1 knockdown promotes increases in PK, FAS and ACC, which influence glycolysis and fatty acid metabolism. Furthermore, in *Drosophila melanogaster*, cyclin E is degraded in response to a decrease in ATP following mitochondrial dysfunction, which results in cell cycle arrest. In pancreatic  $\beta$ -cells, glucose uptake ultimately signals for cyclin D2 expression, and increased CDK4 activity in the G1 phase promotes E2F1-mediated expression of cell components involved in glucose-mediated insulin release.

### Powering on: metabolism and division

To proliferate, a cell must cycle through interphase, which consists of G1, S and G2 phases, and then mitosis, the stage at which a cell divides into two daughter progeny. Given the energy required to replicate the entire contents of the cell, including DNA as well as organelle and membrane components, cells must be able to assess whether there are adequate carbohydrates, nucleotides, amino acids and fatty acids to initiate and complete these processes. From the perspective of a single cell, if nutrients were unavailable, it would be unwise to expend energy on division instead of the housekeeping

functions required for continued survival. Thus, information on metabolic status must be taken into account by the cell cycle machinery when deciding when to divide and when to conserve energy and abandon the path towards mitosis (FIG. 2; TABLE 1).

### Cyclins–CDKs and metabolism to fuel interphase.

Dynamic regulation of the cell cycle machinery ensures that progression is unidirectional and DNA is replicated only once per cycle. This process is tightly controlled by the master regulatory proteins cyclins and CDKs. The cyclin–CDK interaction confers activity to the pair, and specific cyclin–CDK combinations predominate during different phases of the cell cycle to orchestrate accurate cell cycle progression. CDKs are constitutively expressed and regulate cell cycle substrates by phosphorylation, whereas the activity of cyclins is primarily determined by oscillatory changes in their protein expression in response to molecular cues<sup>6</sup>. Phosphorylation of downstream targets by cyclins–CDKs alters their activity to promote controlled entry into the next phase of the cell cycle<sup>6,7</sup>.

Following mitosis, cells must decide whether to commit to G1 phase and prepare for subsequent division or whether to exit the cycle into G0 phase. G0 is a metabolically conservative phase in which cells display decreased gene expression and lowered activity of proteins involved in nucleotide, carbohydrate and lipid biosynthesis, the products of which are required in subsequent cell cycle phases for division<sup>8,9</sup>. Given the importance of this commitment to division in G1, it is perhaps not surprising that recent literature emphasizes new links between cell metabolism and G1 phase regulators. Of note, D-type cyclins, which act in G1, have emerged as crucial targets in metabolism–cell cycle crosstalk<sup>10–14</sup>. Overexpression of nuclear 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase isoenzyme 3 (PFKFB3) was recently shown to promote proliferation through its effects on central cell cycle regulators<sup>11</sup>. PFKFBs are bifunctional enzymes that interconvert fructose-6-phosphate and fructose-2,6-bisphosphate (Fru-2,6-BP), the latter of which is an essential co-activator of the rate-limiting glycolytic enzyme phosphofructokinase 1. Accordingly, cytoplasmic PFKFB3, which is an isoform with a high kinase to phosphatase activity ratio (740/1), seems to have a mainly glycolytic role<sup>11,15,16</sup>. However, overexpression of PFKFB3 results in increased expression of several central cell cycle regulators, including cyclin D3 (REF. 11). In addition to promoting G1 phase by upregulating cyclin D3, nuclear Fru-2,6-BP was also shown to increase the expression of the M phase-promoting phosphatase Cdc25C and decrease the expression of the CDK1 inhibitor p27 (REF. 11). Surprisingly, Fru-2,6-BP had direct effects on CDK1-mediated p27 phosphorylation, as addition of exogenous metabolite to cell lysates promoted Thr187 phosphorylation, which is a cue for p27 degradation. Levels of Fru-2,6-BP may reflect the robustness of glucose uptake and glycolysis, resulting in an accumulation of active cyclin–CDK1 complexes and an efficient drive towards proliferation when glucose is readily available and Fru-2,6-BP levels are high<sup>11</sup>.

Table 1 | Crosstalk between cell cycle and metabolism

Metabolic pathway	Proximal metabolic or cell cycle factor	Cell cycle or metabolic target	Effector or metabolic outcome	Cell cycle outcome	Refs
Glycolysis	PFKFB3	Cyclin D3	↑ Cyclin D3 expression	Promotes G1	11
		CDK1	↑ CDK1 expression	Promotes mitotic entry	11
		Cdc25C	↑ Cdc25C expression	Promotes mitotic entry	11
		APC-CDH1	↓ PFKFB3 levels (↑ APC-CDH1-mediated degradation)	↓ Glycolysis and ↓ G1 progression	21,22
	Fructose-2,6-bisphosphate	p27	↓ p27 levels (↑ Thr187 phosphorylation and protein degradation) and ↑ CDK1 activity	Promotes G1	11
	Cyclin D1	Hexokinase II	↑ Cyclin D1 causes ↓ hexokinase II levels and ↓ glycolysis	NA	12
		Pyruvate kinase	Cyclin D1 knockdown causes ↑ pyruvate kinase levels	NA	12
	Histones	PFK1 and hexokinase II	↑ PFK1 levels; hexokinase II transcription causes ↑ glycolysis	NA	25
Glucose uptake and glycolysis	ACL	Histones	↑ Acetyl-CoA; promotes global histone acetylation	Promotes S phase	25
Glucose uptake	Intracellular glucose	Cyclin D2	↑ Cyclin D2 transcription (↓ cyclin D2 repressor BCL-6 levels)	Promotes G1-S transition	14
	Histones	GLUT4	↑ GLUT4 transcription causes ↑ insulin-regulated glucose uptake	NA	25
Anaerobic glycolysis	Histones	LDHA	↑ LDHA transcription causes ↑ anaerobic glycolysis	NA	25
Insulin secretion	CDK4	KIR6.2*	↑ CDK4 activity at G1 promotes ↑ insulin release	NA	27
Fatty acid synthesis	Cyclin D1	FAS and ACC	Cyclin D1 knockdown causes ↑ ACC and ↑ FAS	NA	12
Respiration	ATP/AMP imbalance	Cyclin E	SCF-mediated cyclin E degradation	G1-S arrest	18,19

ACC, acetyl-CoA carboxylase; ACL, ATP citrate lyase; APC, anaphase-promoting complex; BCL-6, B cell lymphoma 6; CDK, cyclin-dependent kinase; CDH1, Cdc20 homologue 1; FAS, fatty acid synthase; GLUT4, glucose transporter 4; LDHA, L-lactate dehydrogenase A chain; NA, not applicable; PFK1, phosphofructokinase 1; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; SCF, SKP1-cullin 1-F-box protein. \* A component of  $K_{ATP}$  mitochondrial channels.

However, it is not yet clear on a molecular level how nuclear Fru-2,6-BP promotes changes in the abundance and activity of cell cycle regulators.

Not only do metabolic intermediates control G1 and S phase progression, cyclins that promote the G1 to S transition can also provide feedback to the metabolic machinery. For example, cyclin D1 overexpression decreases the abundance and activity of the key glycolytic enzyme hexokinase II in breast cancer cells<sup>12</sup>. Similarly, cyclin D1 knockdown results in increased levels of pyruvate kinase, thereby promoting glycolysis, and increased levels of FAS and ACC, which are crucial for fatty acid biosynthesis<sup>12</sup>. Further analysis of gene expression from cyclin D1-null mice revealed that cyclin D1 inhibits numerous target genes involved in glycolysis, lipogenesis and mitochondrial activity, potentially leading to downregulation of metabolic activity<sup>12</sup>. The effects of cyclin D1 outside of the cell cycle have also been studied by profiling cyclin D1 interacting proteins using epitope-tagged cyclin D1 knock-in mice to isolate binding partners<sup>13</sup>. Although this work primarily focused on a transcriptional role for cyclin D1 in promoting the expression of the transmembrane receptor Notch1, examination of extensive

published data uncovered interactions between cyclin D1 and numerous metabolic proteins, such as FAS and ACC, as well as the mitochondrial electron transport chain components cytochrome *c* oxidase and ATP synthase<sup>13</sup>. The consequences of these interactions have not yet been delineated, but these binding partners suggest that cyclin D1 may play a new part in metabolic regulation in addition to its duties in driving the cell cycle. Indeed, cyclin D1 expression is high during G1 phase; however, once the G1 to S transition has occurred, cyclin D1 levels drop as DNA replication is initiated<sup>17</sup>. If metabolic activity increases cyclin D1 expression and cyclin D1 suppresses metabolism, it is attractive to speculate that an increase in cyclin D1 levels during G1 phase steadily tempers metabolic activity, thus supporting a negative feedback loop to help prevent re-initiation of G1 until the G1 to S transition is complete. Furthermore, a drop in cyclin D1 levels during S phase might increase the availability of metabolic intermediates, providing energy for the crucial tasks of DNA replication and repair.

Work in model systems has recently provided additional insight into links between metabolism and cyclins-CDKs. For example, in *Drosophila melanogaster* it



has been shown that cyclin E is a crucial effector that ensures G1 to S arrest in the setting of mitochondrial dysfunction and imbalances in the ATP/AMP ratio<sup>18,19</sup>. Mutation of the cytochrome *c* oxidase subunit Va, which leads to decreased ATP levels, did not seem to compromise cell survival, but induced a loss of cyclin E and resulted in cell cycle arrest<sup>19</sup>. Subsequent work showed that a decrease in mitochondrial ATP production promotes downstream transcription of the F-box protein archipelago through an AMPK- and p53-dependent pathway<sup>18</sup>. Archipelago then recruits cyclin E to the SKP1–cullin 1–F-box protein (SCF) ubiquitin ligase complex, resulting in cyclin E degradation and a stall at the G1 to S transition until ATP levels are restored and cyclin E reaccumulates<sup>18</sup>.

**APC substrates in the metabolic cascade.** APC is a central regulator of the cell cycle, controlling the ubiquitylation of key substrates to affect both exit from M phase and transition through G1. Degradation of specific substrates by the APC depends on one of two activators, Cdc20 or Cdc20 homologue 1 (CDH1)<sup>20</sup>. APC–CDH1 is active from late mitosis and throughout G1 phase and ubiquitylates mitotic substrates for proteasomal degradation, thus preventing premature entry into S phase and inhibiting proliferation<sup>20</sup>. Interestingly, it was recently reported that loss of APC–CDH1 activity affects cell proliferation and also enhances glycolysis, suggesting that the APC might control components of metabolic pathways as well as cell cycle regulators<sup>21</sup>. Indeed, an examination of PFKFB3 dynamics in rat cortical neurons and astrocytes revealed that the differential abundance of PFKFB3 in these cells (detectable in astrocytes, undetectable in cortical neurons) was due to a difference in the activity of APC–CDH1 (low in astrocytes, high in cortical neurons). In fact, this complex was found to be directly responsible for ubiquitylating PFKFB3 (REFS 21,22). Examination of the effects of APC–CDH1 in other cell lines suggested that CDH1 overexpression abrogates glycolytic flux, whereas depletion of CDH1 by small hairpin RNA promotes glycolysis and entry into S phase<sup>21</sup>. Interestingly, this study confirmed the nuclear localization of PFKFB3 and suggested that inhibition of APC–CDH1-mediated nuclear PFKFB3 destruction increases the levels of nuclear and cytoplasmic PFKFB3 and enhances glycolysis and cell proliferation.

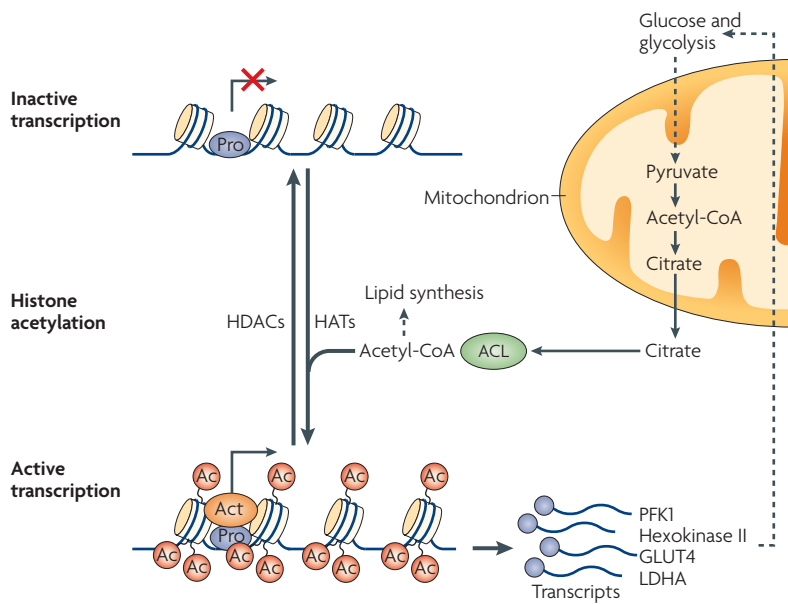
In addition to linking glycolysis and proliferation in cycling cells, APC–CDH1 seems to provide a crucial link between metabolism and oxidant-induced apoptosis in terminally differentiated neurons by regulating PFKFB3 expression<sup>22</sup>. As mentioned above, the activity of APC–CDH1 is high in terminally differentiated neurons to help prevent cell cycle progression. Accordingly, neurons contain undetectable levels of PFKFB3, which is continuously targeted for ubiquitylation<sup>22</sup>. This dampening of glycolysis is beneficial to neurons because high levels of glycolysis can move the shared intermediate, G6P, away from the antioxidant-producing PPP and towards glycolysis, thus promoting oxidative stress-induced apoptosis<sup>22</sup>. In neurons,

APC–CDH1 helps to maintain a reducing intracellular environment, protecting cells from apoptosis<sup>22</sup>. These examples highlight the fact that interactions between the cell cycle machinery and metabolic intermediates vary among different cell types. In cycling cells, PFKFB3 promotes proliferation, as evidenced by increased cyclin D3, CDK1 and Cdc25C levels; however, in terminally differentiated neurons, APC–CDH1 serves primarily as a locus of control to ensure that PFKFB3 is completely degraded so that the cell can funnel glucose into the PPP to promote cell survival<sup>11,21,22</sup>.

**Linking chromatin structure to metabolism.** DNA must be made accessible to the replication machinery to be replicated in S phase. This can be achieved by global core histone acetylation, which remodels chromatin structure, thereby freeing DNA for replication and transcriptional upregulation and enabling cell cycle progression<sup>23,24</sup>. ACL has recently emerged as an integral link between glucose metabolism and global histone acetylation<sup>25</sup> (BOX 1). Increased glycolysis and pyruvate production results in the generation of the downstream metabolite citrate, which can be used for fatty acid synthesis when converted to acetyl-CoA in the cytoplasm by ACL<sup>26</sup>. ACL is crucial for citrate-derived acetyl-CoA production, which, in addition to its use in lipid synthesis, has recently been shown to act in the nucleus to promote core histone acetylation, global transcriptional upregulation and S phase progression<sup>25</sup>. These findings reveal an important role for acetyl-CoA availability as determined by changes in glucose uptake, in histone acetyl transferase activity and histone acetylation. Furthermore, ACL-dependent histone acetylation promotes a positive feedback metabolic circuit by driving the transcription of genes encoding glycolytic proteins, such as phosphofructokinase 1, hexokinase II, L-lactate dehydrogenase A chain (LDHA) and GLUT4 (REF. 25). Therefore, ACL-mediated acetyl-CoA production serves as an inducer of chromatin remodelling, providing an additional means for cells to coordinate proliferation and metabolic flux.

**Cyclins–CDKs and metabolic crosstalk in  $\beta$ -cells.** Crosstalk between metabolism and cyclins–CDKs is readily evident in cells that are exquisitely sensitive to metabolite levels, such as pancreatic  $\beta$ -cells. These cells continuously sample extracellular glucose to determine appropriate insulin release and maintain organismal glucose homeostasis. Thus, their proliferation and metabolic machinery must be tightly coupled. A study of pancreatic  $\beta$ -cells revealed that glucose uptake promotes cell proliferation mediated by the downstream effector cyclin D2 (REF. 14). Specifically, it was determined that glucose activates the upstream targets PI3K and AKT<sup>14</sup>, which in turn inactivate forkhead box O (FOXO) transcription factors, thus decreasing the expression of the cyclin D2 repressor BCL-6 (REF. 14). An increase in cyclin D2 transcription supports  $\beta$ -cell proliferation and provides a link between glucose metabolism and pancreatic islet expansion<sup>14</sup>. Similarly, crosstalk has also been discovered in  $\beta$ -cells between

Box 1 | Effects of ATP citrate lyase on cell cycle and metabolism



Global core histone acetylation remodels chromatin structure to increase DNA replication and transcription. Interestingly, histone acetylation has recently been linked to metabolism through the function of the enzyme ATP citrate lyase (ACL) (see the figure), an important contributor to fatty acid biosynthesis that acts in the cytoplasm to convert the tricarboxylic acid (TCA) cycle intermediate citrate into the fatty acid synthetic precursor acetyl-CoA. In addition to its role in fatty acid synthesis, acetyl-CoA is also an important source of acetyl for histone acetylation, which results in global transcriptional upregulation and S phase progression. It was recently discovered that ACL activity also links upstream glucose metabolism with downstream replication and transcription, and this signalling pathway also feeds back to metabolism by regulating the transcription of metabolic enzymes. Increased citrate levels from glycolytic flux drive ACL-mediated acetyl-CoA production and histone acetylation, thereby promoting DNA replication and cell cycle progression. Among the downstream transcriptional targets affected by ACL are glucose transporter 4 (GLUT4), hexokinase II, phosphofructokinase 1 (PFK1) and L-lactate dehydrogenase A chain (LDHA), which are further involved in determining the metabolic status of the cell by regulating glucose uptake, glycolysis and anaerobic glycolysis. Therefore, ACL-mediated acetyl-CoA production influences chromatin remodelling and the expression of metabolic genes, helping to coordinate proliferation and metabolism. Act, activator; HAT, histone acetylase; HDAC, histone deacetylase; Pro, promoter.

**Initiator caspase**

A caspase lying at the apex of apoptotic signalling cascades (for example, caspase 2, caspase 8 and caspase 9). These cleave and activate executioner caspases.

**Executioner caspase**

A caspase (caspase 3, caspase 6 and caspase 7) that cleaves a range of cellular substrate proteins, resulting in apoptotic cell death. Also termed effector caspases.

**Senescence**

A cellular state of prolonged G1 cell cycle arrest with characteristic metabolic, morphological and protein expression alterations.

the CDK4–retinoblastoma (RB)–E2F pathway at the G1 to S transition and glucose-mediated insulin secretion<sup>27</sup>. In this model, increased CDK4 activity in G1 releases the transcription factor E2F1 from RB repression, allowing it to bind target promoters<sup>6,27</sup>. In  $\beta$ -cells, E2F1 activates the transcription of *Kir6.2* (also known as *KCNJ11*), the product of which is a subunit of the ATP-sensitive potassium ( $K_{ATP}$ ) mitochondrial channels involved in glucose-mediated insulin release<sup>27,28</sup>. This study describes the mechanisms linking upstream nutrient-sensing pathways, intermediate cell cycle CDK4–E2F1 activity and downstream endocrine signalling through insulin release and  $\beta$ -cell responsiveness. Given the interplay between cell cycle mediators and metabolism in  $\beta$ -cells, it will be interesting to determine whether similar mechanisms exist in other insulin-responsive cell types, such as skeletal muscle cells and adipocytes.

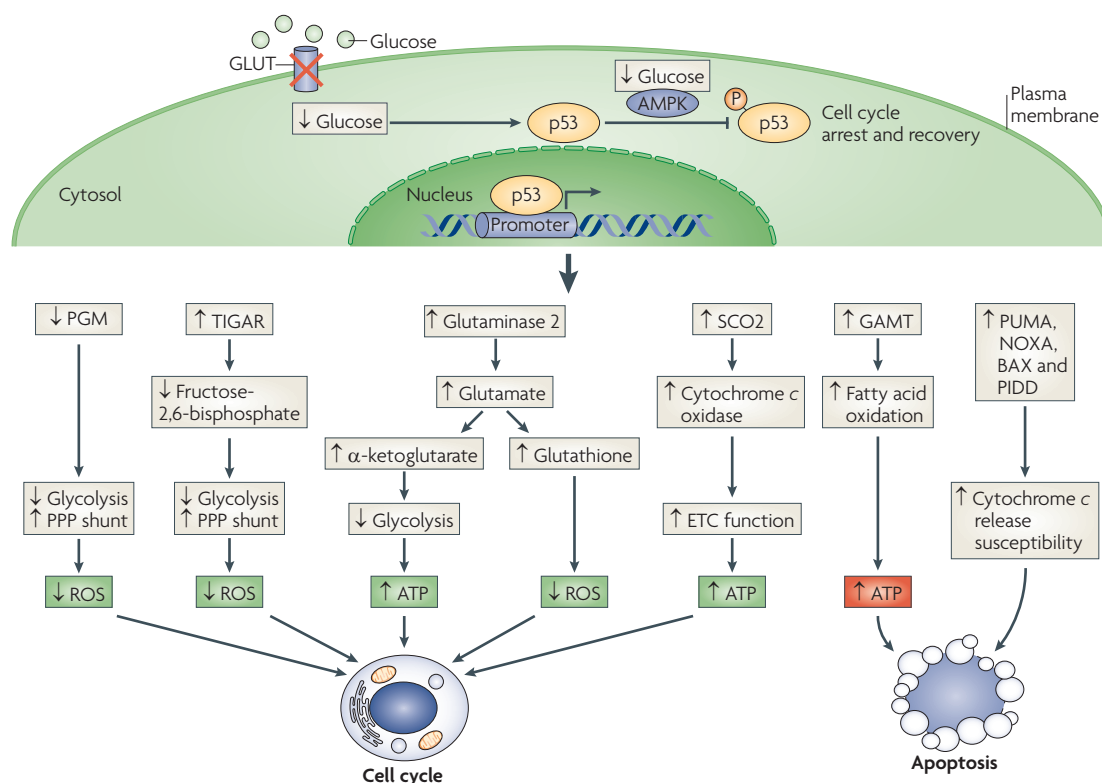
**Powering off: metabolism and apoptosis**

Apoptosis is a form of programmed cell death that uses the caspase family of Cys proteases to orchestrate signal-mediated cell destruction<sup>29,30</sup>. Following receipt of pro-apoptotic signals, such as DNA damage and oxidative stress, the cell undergoes rapid and orderly demolition that is marked by chromatin condensation, DNA fragmentation and membrane blebbing<sup>29</sup>. Membrane-enclosed cell components are ultimately engulfed by phagocytes, and the intracellular milieu of the dying cell is never exposed to the extracellular environment<sup>31</sup>. Under normal physiological conditions, apoptosis of damaged or unneeded cells is balanced by cell regeneration, thus maintaining proper homeostasis. A defect in the apoptotic process can manifest pathologically as cancer or autoimmunity, whereas excessive apoptosis is seen in neurodegenerative and immunodeficiency disorders<sup>29</sup>.

Canonical apoptosis proceeds through either an extrinsic or intrinsic signalling pathway. In the extrinsic pathway, plasma membrane death receptors are engaged by pro-apoptotic ligands to activate downstream caspase-dependent signalling. By contrast, the intrinsic death pathway is initiated by intracellular pro-apoptotic stimuli and involves the convergence of initiator caspases and BCL-2 family proteins (see below) on mitochondria to promote cytochrome *c* release from the intermembrane space. Cytochrome *c* molecules then form a complex with the adaptor protein apoptotic protease-activating factor 1 (APAF1) to recruit and activate pro-caspase 9 in the apoptosome complex<sup>32–34</sup>. Active caspase 9 promotes apoptosis by activating executioner caspases, which cause the demise of the cell by cleaving and inactivating substrates that are important for normal cell function or by cleaving substrates to produce fragments with new functions<sup>35,29,30</sup>.

When glucose is available and metabolic activity is robust, cells can often withstand apoptotic stimuli. Conversely, when nutrients wane, cells may be unable to carry out crucial tasks and become more vulnerable to apoptosis. There are many points when metabolism can impinge on apoptotic signalling pathways; we highlight here some of the important effectors that communicate signals between metabolism and apoptosis.

**Fuel depletion: connecting p53 to cell death.** The tumour suppressor p53 integrates intracellular signals and induces the transcription of target genes to help determine cell fate and promote cell cycle arrest, senescence or apoptosis. p53 is itself modified in response to metabolism; it is phosphorylated by AMPK when intracellular glucose levels are low, and this phosphorylation is required for the p53-mediated G1–S cell cycle arrest that allows cells to recover following a period of low ambient glucose<sup>36</sup>. Sustained activation of AMPK-mediated p53 phosphorylation during conditions of prolonged glucose deprivation can, however, lead to senescence<sup>36</sup>. p53 has an established role as a pro-apoptotic effector in response to genotoxic stress as it upregulates the transcription of pro-apoptotic molecules such as the BCL-2 homology 3 (BH3)-only proteins PUMA



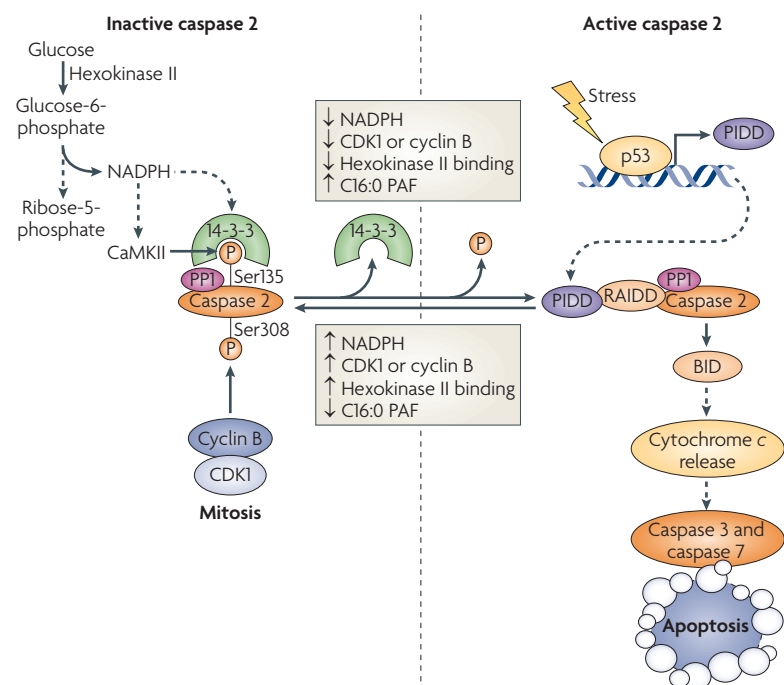
**Figure 3 | p53 activation and its effects on metabolism and cellular fate.** Active p53 induces the transcription of many target substrates, several of which are closely involved in metabolism. For example, p53 influences metabolism, and consequently cell fate, by upregulating guanidinoacetate N-methyltransferase (GAMT), synthesis of cytochrome c oxidase 2 (SCO2), glutaminase 2 and TP53-induced glycolysis and apoptosis regulator (TIGAR), and downregulating phosphoglycerate mutase (PGM). Glutaminase 2 converts glutamine to glutamate, which feeds either into the tricarboxylic acid cycle when converted to  $\alpha$ -ketoglutarate, or into glutathione synthesis to help dictate the antioxidant status of the cell. Increased glutaminase 2 expression by p53 therefore results in increased ATP production and decreased levels of reactive oxygen species (ROS), promoting cell cycle progression and protecting the cell from apoptosis. The p53-mediated regulation of PGM, SCO2 and GAMT also promotes cell cycle progression by decreasing ROS, or increasing ATP, as indicated. Note that GAMT can also cause an increase in ATP through increased fatty acid oxidation to provide sufficient energy for apoptosis. p53 also directly regulates apoptosis by upregulating the expression of the pro-apoptotic proteins PUMA, NOXA, BAX and PIDD. Interestingly, p53-mediated PUMA expression is suppressed by high glucose, suggesting an additional layer of complexity in the crosstalk between p53, metabolism and apoptosis. AMPK, 5'-AMP-activated protein kinase; ETC, electron transport chain; GLUT, glucose transporter; PPP, pentose phosphate pathway.

(also known as BBC3) and NOXA (also known as PMAIP1), the BCL-2-family member BAX and the caspase 2-activating adaptor protein PIDD (also known as LRDD)<sup>37–41</sup>. It has also been suggested that p53 acts as a metabolic sensor to control PUMA expression. Consistent with this, p53-mediated PUMA induction is suppressed in the presence of abundant glucose, and glucose can suppress the induction of PUMA that would normally occur after growth factor withdrawal<sup>42</sup>. In a transcriptionally independent manner, p53 can also promote mitochondrial outer membrane permeabilization (MOMP) and apoptosis by forming a complex with the anti-apoptotic proteins BCL-X<sub>L</sub> and PUMA<sup>40,43</sup>. The metabolic crosstalk between p53 and metabolism is shown in FIG. 3.

In addition to activating cell death proteins, p53 influences metabolic pathways by upregulating the expression of guanidinoacetate N-methyltransferase (GAMT), synthesis of cytochrome c oxidase 2 (SCO2),

glutaminase 2 and TP53-induced glycolysis and apoptosis regulator (TIGAR), and decreasing the expression of phosphoglycerate mutase (PGM)<sup>44–49</sup>. p53 modulates creatine biosynthesis during both genotoxic and nutrient stress by upregulating GAMT<sup>44</sup>, which converts the glycine metabolite guanidinoacetate to creatine for ADP/ATP energy metabolism and promotes genotoxic- and glucose starvation-induced apoptosis<sup>44</sup>. Interestingly, upregulation of GAMT by p53 in the setting of glucose deprivation not only affects creatine biosynthesis but actually provides an alternative source of ATP by increasing fatty acid oxidation. This consequence of GAMT upregulation may be important for providing sufficient ATP to execute apoptosis, particularly under conditions in which energy generation by glycolysis is compromised.<sup>44</sup>

The effect of p53 on the cytochrome c oxidase complex assembly protein SCO2 also promotes alterations in cell respiration and oxygen consumption<sup>46</sup>.



**Figure 4 | Metabolic regulation of caspase 2.** The initiator caspase, caspase 2, is intricately involved in the crosstalk between cell metabolism and apoptosis. Caspase 2 is inhibited in response to pentose phosphate pathway glucose flux and abundant NADPH, thereby preventing cell death. Caspase 2 inhibition by NADPH is mediated by  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase II (CaMKII)-directed phosphorylation at Ser135 (in *Xenopus laevis*), which promotes the association of the phosphorylated Ser-binding protein 14-3-3. As NADPH levels wane, 14-3-3 is released from caspase 2, leaving the inhibitory phosphorylation vulnerable to removal by the constitutively bound and active protein phosphatase 1 (PP1). Once dephosphorylated, caspase 2 is poised for activation by induced proximity oligomerization through its adaptor proteins RIP-associated protein with a death domain (RAIDD) and PIDD. In addition to NADPH, caspase 2 is also sensitive to the lipid metabolite 1-O-hexadecyl-2-acetyl-sn-glycerol-3-phosphocholine (C16:0 PAF) and the glycolytic enzyme hexokinase II; caspase 2 is required for C16:0 PAF-dependent neurotoxicity, and caspase 2-mediated cell death can be enhanced by detachment of hexokinase II from mitochondria. Caspase 2 signalling is suppressed during mitosis by cyclin-dependent kinase 1 (CDK1) through inhibitory phosphorylation at Ser308 (in *X. laevis*). Thus, caspase 2 has emerged as a central integrator of cell metabolism and apoptosis that is also responsive to cell cycle status. BID, BH3-interacting domain death agonist.

p53 increases SCO2 expression and upregulates aerobic mitochondrial respiration; conversely, loss of p53 compromises cytochrome *c* oxidase function and supports a switch to glycolytic metabolism, which contributes to the metabolic phenotype observed in cancer cells, in which glycolytic pathways are used for ATP generation and aerobic respiration is downregulated<sup>46</sup>.

p53 also influences glucose flux by regulating the expression of PGM and TIGAR. p53 activity decreases the expression of PGM, which produces the glycolytic substrate 2-phosphoglycerate; this results in a drop in glycolysis and entry into senescence<sup>45</sup>. Thus, loss of functional p53, as is common in cancer cells, might drive increased PGM expression, thereby propelling aerobic glycolysis<sup>45</sup>. This change promotes glycolysis and the PPP and renders cells resistant to oxidative stress in the absence of p53, thereby forestalling senescence. In contrast to the effects of PGM on glycolysis, TIGAR

expression decreases intracellular Fru-2,6-BP levels, concomitantly decreasing glycolysis<sup>47</sup>. As described previously, a decrease in glycolysis allows the diversion of G6P into the PPP, in which it generates reducing equivalents and limits oxidative stress-induced cell death<sup>22</sup>. Therefore, TIGAR might help the cell to assess the appropriate response to p53-dependent stressors by metabolically fine-tuning the cell's reaction to apoptotic stimuli.

Recent publications have delineated interesting connections between p53, cell metabolism and antioxidant responses<sup>48,49</sup>. As mentioned above, p53 promotes an increase in glutaminase 2 expression, which drives the conversion of glutamine to glutamate. Glutamate can support mitochondrial respiration and ATP production by its interconversion into the TCA cycle substrate  $\alpha$ -ketoglutarate, and can also participate in glutathione synthesis to help regulate the antioxidant status of the cell. By upregulating glutaminase 2, p53 influences both mitochondrial respiration and intracellular levels of reactive oxygen species. Therefore, glutaminase 2 upregulation and glutamate production may be mechanisms by which p53 protects against apoptosis by promoting an intracellular environment conducive to recovery from subthreshold stressors<sup>48,49</sup>.

#### Caspase 2: metabolism and cell death at mitochondria.

In the past several years caspase 2 has emerged as a crucial locus for communication between metabolic and cell death pathways upstream of mitochondria. In response to specific stimuli, such as DNA damage and heat shock, caspase 2 is activated by induced proximity oligomerization mediated by adaptor proteins<sup>50,51</sup>. Following oligomerization, caspase 2 is autocatalytically processed to amplify its enzymatic activity and can then cleave the pro-apoptotic protein BH3-interacting domain death agonist (BID) to promote mitochondrial cytochrome *c* release and cell death<sup>50,51</sup>.

Caspase 2 was known to be important for programmed oocyte death during mouse development, and this observation has been extended to *Xenopus laevis* oocytes, in which caspase 2 has been extensively characterized as a metabolism-regulated apoptotic effector<sup>52–54</sup> (FIG. 4). *X. laevis* egg cell-free extracts spontaneously undergo apoptosis, marked by successive initiator and executioner caspase activation and cytochrome *c* release, when they are incubated at room temperature over time<sup>55</sup>. Apoptosis in this case is initiated by the depletion of a key PPP byproduct, NADPH<sup>53</sup>, which is primarily produced by glucose flux through the PPP. NADPH is an important reducing agent in the cell and is also involved in the reductive biosynthesis of fatty acids and nucleotides. An endogenous drop in NADPH levels over time promotes caspase 2 activation and downstream apoptosis, and caspase 2 activation is inhibited by supplementing the extract with either G6P, to ectopically increase NADPH levels through the PPP, or malate, which produces NADPH through a non-PPP mechanism (through the malic enzyme (also known as malate dehydrogenase))<sup>53</sup>. High PPP flux and abundant NADPH production is communicated to effector



caspase 2 through  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase II (CaMKII), which phosphorylates caspase 2 at Ser135 to prevent binding of the caspase 2 adaptor RIP-associated protein with a death domain (RAIDD; also known as CRADD) and thus caspase 2 activation<sup>53</sup>. Therefore, NADPH and glucose flux through the PPP dictate the viability of *X. laevis* oocytes by maintaining caspase 2 in an inhibitory state when nutrient levels are high<sup>53</sup>.

Interestingly, caspase 2 activation in the setting of low NADPH levels is also regulated by metabolism in *X. laevis* egg extracts<sup>54</sup>. As PPP metabolism slows down, NADPH begins to drop, and apoptosis occurs following caspase 2 dephosphorylation<sup>53,54</sup>. It was shown that caspase 2 activation depends on the metabolism-regulated release of the phosphorylated Ser-binding protein 14-3-3 (REF. 54). 14-3-3 release from caspase 2 allows the inhibitory phosphate on Ser135 to be removed by the constitutively active protein phosphatase 1 (PP1), thus freeing caspase 2 for induced proximity activation<sup>54</sup>. In the presence of excess G6P to prevent a decrease in NADPH levels, 14-3-3 remains bound to phosphorylated caspase 2, resulting in inhibition of caspase 2 activation and apoptosis<sup>54</sup>. The mechanism of 14-3-3 removal by changes in metabolic intermediates is being investigated, and elucidation of these details will help to complete the story of how caspase 2 is regulated by NADPH.

An exciting extension of the work on NADPH and caspase 2 recently showed that caspase 2 activity, as visualized by real-time oligomerization, might also be regulated by metabolism<sup>56</sup>. This study used bimolecular fluorescence complementation to examine induced proximity oligomerization of recombinant caspase 2 constructs fused to non-fluorescent halves of Venus fluorescent protein (VFP)<sup>56</sup>. Treatment of cells with dehydroepiandrosterone (DHEA), which inhibits the rate-limiting enzyme of the PPP and leads to a decrease in NADPH, induced caspase 2 oligomerization as observed by VFP fluorescence visualization, further revealing a role for the PPP in caspase 2 activation<sup>56</sup>.

Caspase 2 is also involved in the crosstalk between cell cycle and apoptotic machinery in *X. laevis* egg extracts<sup>57</sup>. Caspase 2 is directly suppressed during mitosis by the M phase-promoting kinase CDK1 (REFS 6,57). CDK1–cyclin B1 phosphorylates caspase 2 at Ser308 in *X. laevis*, thus inhibiting its activation and ensuring that caspase 2-mediated apoptosis will not be aberrantly initiated during normal mitosis<sup>57</sup>. In investigating PPP- and mitosis-mediated caspase 2 suppression, it emerged that abundant NADPH was sufficient to prevent caspase 2 activation in mitotic *X. laevis* egg extracts<sup>57</sup>. Furthermore, high CDK1–cyclin B1 activity was also sufficient to prevent metabolism-mediated caspase 2 activation<sup>57</sup>. This detailed crosstalk between metabolism, mitosis and apoptosis in *X. laevis* egg extracts suggests that caspase 2 is a central metabolic effector in the decision of whether to proliferate or die.

In addition to its role as an integrator of the PPP and mitotic signals, caspase 2 potentiates apoptosis in the setting of glucose and lipid signalling<sup>58,59</sup>. Caspase 2

modulates mitochondrial activity and the apoptotic threshold by cooperating with hexokinase II at mitochondria<sup>58</sup>. Hexokinase II catalyses the conversion of glucose to G6P, which feeds into both glycolysis and the PPP, and the interaction between hexokinase II and the mitochondrial voltage-dependent anion channel (VDAC) is important for mitochondrial function and cell survival<sup>60</sup>. Detachment of hexokinase II from mitochondria enhances caspase 2-dependent apoptosis that is induced by the platinum-based chemotherapeutic cisplatin, suggesting an interplay between caspase 2, BCL-2 family members and metabolic machinery at the mitochondria<sup>58</sup>. In another interesting paradigm, alterations in lipid signalling, and specifically in the choline-containing lipid alkylacylglycerophosphocholine, was shown to promote caspase 2-mediated apoptosis in a  $\beta$ -amyloid oligomer model of Alzheimer's disease<sup>59,61</sup>. Using an unbiased lipidomics approach, it was determined that metabolic alterations in the alkylacylglycerophosphocholine second messenger 1-O-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (C16:0 PAF) signal for cell death in response to toxic  $\beta$ -amyloid oligomers<sup>59</sup>. C16:0 PAF mediated neurotoxicity was also shown to depend on caspase 2, as caspase 2 inhibition protects neurons from  $\beta$ -amyloid- and C16:0 PAF-induced apoptosis<sup>59,62</sup>. This new link between lipid metabolism and caspase 2 mediated  $\beta$ -amyloid neurotoxicity opens the possibility of using metabolism-targeted treatments to modulate the course of Alzheimer's disease as well as that of other caspase 2-dependent neurodegenerative disorders.

Given the apparent role of caspase 2 in integrating metabolic signals and progression of apoptosis, it is interesting that the only obvious phenotype of caspase 2 deficient mice is an excess of oocytes (indicative of a failure of apoptosis in these cells)<sup>52</sup>. This may be because caspase 2 is more important for cell death in response to cell stress than in response to developmental cues or because of compensatory upregulation of other caspases in the caspase 2-null setting. However, given the potentially important role of caspase 2 in linking metabolism and cell death, an alternative explanation is that oocytes depend more on stockpiled internal energy stores than other cells in the body, and it is advantageous to immediately eliminate any oocytes that are nutrient deficient as they would not have sufficient energy stores to successfully undergo fertilization and initial cell divisions. Thus, the brake on apoptosis in oocytes may primarily depend on the metabolic suppression of caspase 2 in a way that does not occur in cells that use glucose as their primary source of energy.

**BCL-2 family proteins: balancing metabolism and death.** Mitochondria use downstream metabolites from cytosolic pathways, such as glycolysis and the PPP, in addition to housing the TCA cycle, glutamine metabolism, fatty acid  $\beta$ -oxidation and oxidative phosphorylation. Importantly, mitochondria are also responsible for integrating cell death signals to initiate cytochrome *c* release from their intermembrane space, triggering apoptosis. Mitochondria are therefore prime candidates

#### Bimolecular fluorescence complementation

A method for detecting protein–protein interactions using non-fluorescent protein halves fused to the proteins of interest. When the proteins of interest interact, the non-fluorescent halves associate to form a fluorescent complex.

Table 2 | **Crosstalk between BCL-2 family members and metabolism**

Metabolic pathway	Proximal metabolic factor or BCL-2 protein	BCL-2 protein or metabolic target	Effector or metabolic outcome	Apoptotic outcome	Refs
Growth factor stimulation and glucose uptake	GSK3	MCL1	AKT inhibits GSK3-mediated MCL1 ubiquitylation and degradation	Anti-apoptotic	76,77
	AKT and mitochondrion-associated PKA	BAD	BAD phosphorylation and inhibition	Anti-apoptotic	65–68
Sphingolipid metabolism	Ceramide	BCL-2	↑ Ceramide causes PP2A-mediated BCL-2 dephosphorylation	Pro-apoptotic	98
	Ceramide	BAK	Ceramides cooperate with BAK to promote MOMP	Pro-apoptotic	99
	BAK	Long-chain ceramides	↑ Long-chain ceramide production	Pro-apoptotic	100
Glucose uptake and glycolysis	Glucose-responsive p53	PUMA	↓ PUMA expression causes ↓ BAX activation	Anti-apoptotic	42
	AKT	BAX	↓ BAX activity (BAX held in inactive conformation)	Anti-apoptotic	72,73
Glucose deprivation	MCL1–NOXA interaction	NOXA	↓ MCL1 causes ↑ NOXA-induced cell death	Pro-apoptotic	71
Glycolysis	Hexokinase II	BAX	Hexokinase II binding to VDAC limits BAX's ability to promote MOMP	Anti-apoptotic	60
Glycolysis and mitochondrial respiration	BAD	Glucokinase	↓ BAD causes ↓ glucokinase activity and ATP production	Anti-apoptotic	70

BAD, BCL-2 antagonist of cell death; BAK, BCL-2 antagonist/killer; BCL-2, B cell lymphoma 2; GSK3, glycogen synthase kinase 3; MCL1, myeloid leukaemia cell differentiation 1; MOMP, mitochondrial outer membrane permeabilization; PKA, protein kinase A; PP2A, protein phosphatase 2A; VDAC, voltage-dependent anion channel.

for regulating metabolic and apoptotic crosstalk. In particular, BCL-2 family proteins dynamically interact with mitochondria, and the balance between pro- and anti-apoptotic BCL-2 proteins is a central determinant of downstream caspase activation. Intriguingly, BCL-2 family members are extensively regulated by nutrients to create a metabolic threshold for MOMP, a process that is required for the activation of downstream caspases in the intrinsic apoptotic pathway<sup>63</sup> (TABLE 2). Furthermore, BCL-2 family proteins have also been shown to have a role in modulating metabolism and in particular in mitochondrial respiration.

The BCL-2 family members are generally classified as either the anti-apoptotic BCL-2, BCL-X<sub>L</sub> and myeloid leukaemia cell differentiation 1 (MCL1) proteins, the pro-apoptotic BAX and BCL-2 antagonist/killer (BAK) proteins, or the pro-apoptotic BH3-only proteins, which include BID, BCL-2 antagonist of cell death (BAD), BCL-2-interacting mediator of cell death (BIM), PUMA and NOXA<sup>64</sup>. Anti-apoptotic BCL-2, BCL-X<sub>L</sub> and MCL1 interact with and inhibit the activity of BAX and BAK, thereby preventing cytochrome *c* release. When active, the pro-apoptotic BH3-only proteins sequester BCL-2, BCL-X<sub>L</sub> and MCL1 from BAX and BAK, leaving them free to interact with mitochondrial membrane proteins and induce MOMP. In addition, some BH3-only proteins, such as BID and BIM, also serve as direct activators of BAK and BAX (for a general BCL-2 family review, see REF. 64). We touch briefly here on the crosstalk between metabolism and BCL-2 proteins.

Pro-apoptotic BH3-only proteins such as BAD have emerged as central metabolic effectors that dictate downstream BCL-2 family protein activation. Growth

factor stimulation (for example, by interleukin-3 (IL-3)) results in increased glucose uptake and metabolism as well as AKT-mediated inhibitory BAD phosphorylation, which protects cells exposed to enough nutrients from undergoing apoptosis<sup>65–67</sup>. Interestingly, BAD is also phosphorylated and inhibited in response to IL-3 by mitochondrion-associated protein kinase A (PKA)<sup>68</sup>. Dissociation of PKA from mitochondria disrupts the PKA–BAD interaction, promotes BAD hypophosphorylation and is thought to sensitize the cell to apoptosis<sup>68</sup>. Indeed, BAD phosphorylation is crucial for its anti-apoptotic functions, as replacement of endogenous BAD with a non-phosphorylatable variant reduces the threshold for MOMP<sup>69</sup>. BAD itself can influence mitochondrial metabolism by interacting with a membrane-associated holoenzyme containing glucokinase, which catalyses the first committed step in glycolysis<sup>70</sup>. BAD is required for holoenzyme assembly and efficient glucokinase activity, as *Bad*<sup>−/−</sup> hepatocytes show decreased glucokinase function and compromised mitochondrial respiration<sup>70</sup>.

Paradoxically, growth factor stimulation of T cells, which promotes glucose uptake and metabolism, has also been shown to upregulate the pro-apoptotic BH3-only protein NOXA<sup>71</sup>. When glucose is readily available, the anti-apoptotic BCL-2 protein MCL1 binds and inhibits NOXA, whereas the expression of NOXA renders these cells poised for death in the event of glucose insufficiency<sup>71</sup>. The authors of this study termed this NOXA–MCL1 pair a glucose-sensitive apoptotic ‘rheostat’: when glucose levels are low, MCL1 degradation is accelerated, thereby liberating the pre-existing pool of NOXA to trigger mitochondrial cytochrome *c* release and apoptosis, whereas when there is

sufficient glucose, NOXA is inhibited by MCL1 (REF. 71). Loss of glucose uptake following growth factor withdrawal has also been shown to induce the expression of the p53-regulated, pro-apoptotic BH3-only protein PUMA<sup>42</sup>. Interestingly, maintenance of glucose uptake, even in the absence of growth factor stimulation, is sufficient to downregulate PUMA levels, thus abrogating BAX activation and downstream caspase induction<sup>42</sup>. These studies suggest that PUMA, which is induced by glucose-responsive p53, is a metabolism-regulated apoptotic effector<sup>42</sup> (FIG. 3). Pro-apoptotic BAX has also been shown to be directly regulated by glucose levels, as BAX is maintained in an inactive conformation by AKT signalling in the presence of glucose, thereby preventing apoptosis<sup>72,73</sup>. Apoptosis is further linked to metabolism by the interaction between hexokinase II and VDAC, which is thought to prevent cytochrome *c* release both in the presence and absence of BAX or BAK signalling<sup>60,74,75</sup>.

The anti-apoptotic proteins BCL-2, BCL-X<sub>L</sub> and MCL1 have also been shown to receive metabolic signals and influence mitochondrial physiology; for example, MCL1 is also regulated by the AKT signalling axis to promote cell survival<sup>76,77</sup>. When active, the AKT substrate glycogen synthase kinase 3 (GSK3) phosphorylates MCL1, targeting it for ubiquitylation and proteasomal degradation, thus predisposing the cell to cytochrome *c* release<sup>76,77</sup>. In response to growth factor stimulation, AKT inhibits GSK3, thereby preserving MCL1 levels and contributing to cell survival<sup>76,77</sup>. The interplay detailed above between metabolic and apoptotic proteins localized to the mitochondria raises the question of whether additional crosstalk at the level of mitochondria might remain to be discovered.

As an interesting side note, rho-zero ( $\rho^0$ ) cells, which lack mitochondrial DNA, die by apoptosis despite the absence of respiratory chain function. Moreover, this apoptosis can be inhibited by BCL-2 expression, highlighting the fact that the survival function of BCL-2 does not depend on mitochondrial respiration<sup>78</sup>. Analysis of mouse embryos deficient for transcription factor A mitochondrial (TFAM) or mouse hearts with a tissue-specific TFAM deficiency, revealed that cells lacking mitochondrial respiration *in vivo* are even more susceptible to apoptotic stimuli than their normal counterparts<sup>79</sup>.

#### **Cytochrome *c*: at the metabolism–apoptosis crossroads.**

Cytochrome *c* is at the crossroads of energy generation and the apoptotic cascade<sup>80–82</sup>. Thus, it is not difficult to imagine a scenario in which upstream metabolic messages affect electron transport chain fitness and modulate cell death signalling through cytochrome *c*-dependent pathways. In fact, cells that rely heavily on glucose utilization, such as neurons and cancer cells, have been shown to inhibit the pro-apoptotic functions of cytochrome *c* in a glucose-dependent manner<sup>83–85</sup>. The pro-apoptotic activity of cytochrome *c* is influenced by its redox state, with reduced cytochrome *c* lacking the ability to promote cell death<sup>85</sup>. Glucose flux through the PPP to produce abundant

NADPH is crucial in determining the redox state of cytochrome *c*, and inhibition of the PPP by DHEA or incubation of cells in glucose-free media sensitizes the cells to cytochrome *c*-induced apoptosis<sup>85</sup>. This mechanism couples glucose flux through the PPP with the potency of pro-apoptotic cytochrome *c*, providing an additional layer of crosstalk between metabolism and cell death.

Outside of the canonical intrinsic pathway of apoptosis, glycolysis and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity can protect cells from caspase-independent cell death, a type of cell death that can occur following MOMP even when downstream caspases are inhibited<sup>86–88</sup>. In CICD, caspase inhibition is not sufficient to prevent MOMP-induced cell death, most likely owing to compromised mitochondrial membrane potential and the release of pro-apoptotic mediators from the intermembrane space<sup>88,89</sup>. MOMP is typically accompanied by a loss of GAPDH levels and decreased glycolysis and membrane potential<sup>86,90</sup>. However, overexpression of GAPDH was found to rescue cell death following MOMP and cytochrome *c* release, not only by inducing ATP production, but also by triggering autophagy, presumably resulting in the removal of damaged mitochondria<sup>86</sup>. Thus, upregulation of GAPDH following mitochondrial damage may provide a cell with the ability to recover following MOMP<sup>86,91</sup>. Additional studies have demonstrated the importance of mitochondrial respiratory chain dysfunction, rather than release of pro-apoptotic mitochondrial constituents, in promoting MOMP-induced caspase-independent cell death<sup>92</sup>. Specifically, MOMP results in progressive loss of complex I and complex IV activities by 8 hours after mitochondrial permeabilization, with a concomitant decrease in oxidative phosphorylation. These events lead to cell death despite the absence of caspase activity<sup>92</sup>.

Evidence of crosstalk between metabolism and the apoptosome outside of cytochrome *c* regulation is limited, although it is certainly plausible that inhibiting executioner caspase activity in conditions of nutrient abundance would contribute to cell survival. Despite the paucity of evidence for regulation of the apoptosome by metabolism at the post-translational level, some interesting work has emerged that suggests an interplay between lipid metabolism and caspase 9 regulation at the post-transcriptional level<sup>93</sup>. The caspase 9 splice variant caspase 9b has an inhibitory role in the apoptotic cascade in contrast to the canonical pro-apoptotic caspase 9 isoform<sup>93,94</sup>. Ceramides are lipid molecules involved in bilayer cell membrane structure, as well as various cell signalling pathways, and are known to induce apoptosis when present in excess. Excess ceramide promotes a decrease in the caspase 9b to caspase 9 ratio by affecting the proteins responsible for alternative splicing, thus sensitizing cells to apoptosis by the intrinsic pathway<sup>93</sup>. This crosstalk between lipid molecules and caspase 9 mRNA represents a mechanism by which ceramide-producing death stimuli sensitize the cell to apoptosis and communicate pro-death signals by a metabolic messenger<sup>93</sup>.

#### **Autophagy**

A process in which intracellular contents are destroyed by bulk enclosure of cytoplasmic material in membrane-enclosed vesicles that are then targeted for lysosomal degradation.

# Conclusion and perspectives

The metabolic signalling networks communicating with cell cycle and apoptotic machinery are complex and have crucial roles in determining the fitness of the cell. It is likely that most metabolic pathways impinge at some point on the machinery that controls cell proliferation and death. We have not in this Review been able to touch on all of the many metabolism-regulated factors that modulate effectors of cell proliferation and cell death. For example, there is an emerging literature on sirtuins (a class of proteins that have either histone deacetylase or mono-ribosyltransferase activity) as potential bridges between metabolism and the apoptotic machinery, and continued characterization of new microRNAs is also likely to reveal additional means of linking metabolism with cell death and division targets (REFS 95,96). Moreover, broad-based genetic, RNA

interference and proteomic screens are likely to reveal new interactions between the cell cycle, the apoptotic and the metabolic machinery. For example, a recent genome-wide screen for genes important in DNA damage-induced apoptosis in *D. melanogaster* S2 cells identified many proteins involved in metabolic regulation, although it remains to be determined where and how they impinge on cell death pathways<sup>97</sup>. Agents that can alter pathways linking metabolism and cell proliferation and/or cell death, identified both through high-throughput small molecule screening and rational drug design, may become viable therapeutic agents for altering the course of metabolism-driven diseases. The potential for usurping metabolic crosstalk to alter disease progression is exciting, and a complete understanding of metabolic effectors will help to reach this goal.

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#### Competing interests statement

The authors declare no competing financial interests.

#### FURTHER INFORMATION

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