[Frontiers in Bioscience 19, 1003-1014, June 1, 2014]

Burn to cycle: Energetics of cell-cycle control and stem cell maintenance

Laurie A. Mans[1], Anna-Pavlina G. Haramis[1]

[1]Institute of Biology, Leiden University, Sylviusweg 72, 2333 BE Leiden, The Netherlands

TABLE OF CONTENTS

1. ABSTRACT

Stem cells have the unique ability to both maintain the stem cell population via self-renewal and give rise to differentiated cells. The balance between these options is very delicate and important for the short- and long-term maintenance of tissue homeostasis in an organism. Pathways involved in integrating environmental cues and in directing energy metabolism play an important role in the fate decisions of stem cells. In this review, we give an overview of the effects of cellular and systemic metabolic states on stem-cell fate in both embryonic and in adult stem cell populations, with a particular emphasis on cell-cycle regulation. We discuss the major pathways implicated in sensing energetic status and regulating metabolism, including: the mTOR pathway, Forkhead-box-O transcription factors (FoxOs), Sirtuins, reactive oxygen species (ROS), AMP-activated kinase (AMPK) and LKB1, the mTOR pathway and hypoxia inducible factors (HIFs). Given the importance of a correct balance between self-renewal and differentiation, understanding the mechanisms that drive stem-cell fate in different metabolic conditions will provide more insight in stem cell biology in both health and disease.

2. INTRODUCTION

During embryonic and postnatal development stem cells give rise to all tissues in an organism. Furthermore, in adult life stem cells are critical to ensure tissue homeostasis, particularly in rapidly self-renewing tissues such as the skin or intestine, as well as in repair upon injury or tissue-damage. There are different "types" of stem cells; the embryonic stem cells which are pluripotent (have the potential to give rise to all tissues in an organism) and stem cells that reside in the adult tissues that can be either unipotent (giving rise to one specialized differentiated cell lineage) or multipotent (giving rise to several cell lineages).

An essential feature of all stem cells is their ability to self-renew; Self-renewal during embryogenesis is important to ensure expansion of the stem-cell pool and during adulthood to maintain the stem-cell pool. Stem cells that divide give rise to daughter cells with similar developmental potential as the mother cell, or differentiated cells that lack this potential. Precise differentiation into downstream lineages is necessary to provide sufficient and correctly differentiated cells (1, 2) and sufficient self-

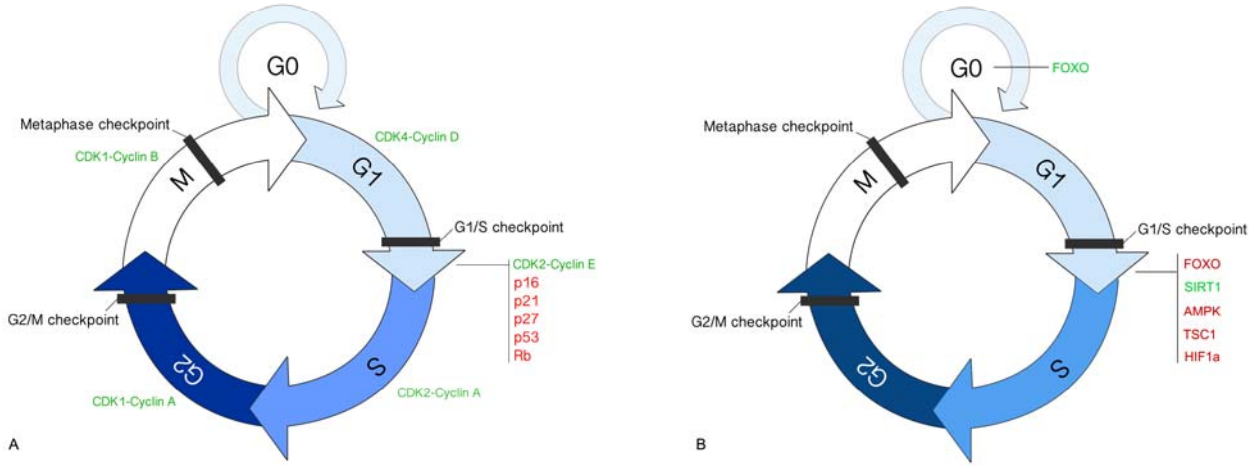

Figure 1. Highly simplified version of the eukaryotic cell cycle. The cell cycle can be divided in four segments: Mitosis (M), Gap 1 (G1), synthesis (S) and Gap 2 (G2). The cell cycle checkpoints are depicted in black bars; G1/S checkpoint, during which the cell controls if cellular and extra-cellular conditions are favorable to continue through the cell cycle. At the G2/M checkpoint, cells check their size and for faithful DNA replication. The last checkpoint (metaphase) controls the appropriate connection of mitotic spindle to the chromatids. The G1/S checkpoint is the most prominent one, since most cells that overcome this checkpoint will complete their cell cycle. A) Positive regulators of the cell cycle being the cyclins connected to cyclin-dependent kinases (CDKs) are shown in green. Some important negative regulators of cell cycle progression are shown in red. B) The main metabolic regulators mentioned in this review and their positive (green) or negative (red) influence on the cell cycle are depicted.

renewal is crucial to prevent depletion of the stem cell pool (3).

How stem cell self-renewal is orchestrated is not fully understood, but molecular mechanisms that play important roles in this process are increasingly being revealed. Recently, it has become evident that nutrient availability at the cellular as well as at the systemic level plays a crucial role in the decision of stem cells to (re) enter the cell cycle, which will be further discussed in this review.

Cell-based assays and *in vitro* manipulation of stem cells have contributed greatly to our knowledge of stem cell behavior and impact of metabolic regulation on their homeostasis. However, control of energy metabolism involves communication between different organs and tissues, invokes systemic responses such as hormone release and the operation of positive and negative feedback loops between signaling cascades. For these reasons, *in vivo* studies in animal models are necessary to dissect the impact of fluctuations of energy availability on organismal physiology.

The metabolic regulation of stem cell behavior has been described in invertebrates such as the fruit fly (*Drosophila melanogaster)* (4-8) and the worm (*Caenorhabditis elegans*) (9, 10), as well as *in vitro* in mammalian Embryonic Stem Cells and Induced Pluripotent Stem Cells (iPSC) (11). In vertebrates, the mouse is the most prominently used model for these studies, but in recent years the zebrafish (*Danio rerio*) has emerged as a valid model for studies on metabolic regulation of stem cell behavior. Major metabolic processes, such as gluconeogenesis and lipid metabolism, are conserved between zebrafish and mammals (12). Furthermore, induction of gluconeogenesis in response to interruption of maternal nutrient supply is conserved in fish (13) and zebrafish models of metabolic disease are being generated (14). Moreover, the small size, high fecundity and well characterized hematopoietic stem cells, in particular of the hematopoietic system (15), make the zebrafish an attractive model for studies on stem cell physiology.

In this review, we discuss the role of molecular energy-sensing pathways in stem cell physiology with a particular emphasis on cell-cycle regulation by those pathways. We focus mainly on hematopoietic and neural stem cells, as those are the most intensively studied in the context of influence of metabolism on cell cycle regulation.

3. EFFECTS OF NUTRIENT AVAILABILITY ON CELL CYCLE AND STEM CELL-MAINTENANCE

3.1. The eukaryotic cell cycle

The cell cycle of eukaryotic cells can be divided into the mitotic phase, during which the cell divides into two cells, and the interphase which can be subdivided into G1 phase, S phase and G2 phase (Figure 1). During interphase, cells produce extra proteins and cytoplasmic organelles and duplicate their DNA (S phase).

There are three major checkpoints at which the cell cycle can be halted, namely the G1/S, G2/M and metaphase checkpoint. The main molecules that drive cell cycle progression are cyclin-dependent kinases (CDKs), which as the name implies, depend on the presence of cyclin(s) for their activity. The activity of these CDKs fluctuates according to the levels of cyclin in the cell (16). The G1/S phase is the most studied checkpoint and has recently been reviewed (17). Most cells that overcome this checkpoint will complete their cell cycle. There are various

proteins that can influence the transition through these checkpoints, either in a positive or negative way. For instance, activation of p53 when DNA damage is detected results in inhibition of DNA synthesis and cell cycle arrest at the G1/S checkpoint (18). This allows the cells to repair the damage before continuation of the cell cycle. Another potent G1-S checkpoint regulator is the retinoblastoma protein (Rb). This protein prevents cell cycle progression via inhibition of E2F (19). The function of Rb is often impaired in cancer (20), which facilitates cancer growth via unrestricted cell division, but also via other mechanisms (21).

Most cells in an adult organism are differentiated and remain in a quiescent state (G0). Adult stem cells can remain quiescent for extended periods but maintain the ability to divide when needed either for replenishment of the stem cell pool, to give rise to differentiated cell types, or to repair damaged tissue. For instance, stem cells in rapidly regenerating organs such as the blood, skin, and intestine divide frequently, whereas liver, or brain stem cells remain quiescent for most of their lifetime. Minor differences have been reported in the way that stem cells progress through the cell cycle. Mouse embryonic stem cells (mESCs) were shown to cycle in a different manner than tissue stem cells, in the sense that their G1 phase is very short (22) and the cells rapidly enter S phase (23). Interestingly, when the mESCs differentiated into multipotent tissue specific stem cells, the G1 phase lengthened, and diverse proteins such as CDKs needed to be activated for cell cycle progression (24). This suggests that the cell cycle of pluripotent stem cells is less tightly regulated than that of multipotent stem cells.

3.2. Metabolic pathways in the cell

Cells require energy for cellular activity. Various metabolic pathways that break down complex macromolecules into intermediate metabolites during the process of catabolism, lead to the mobilization of stored energy resources to generate ATP. The major catabolic pathway in cells is the breakdown of glucose and other organic molecules in the presence of oxygen called 'cellular respiration' or aerobic metabolism; a process that in animal cells takes place mostly in the mitochondria.. The other main pathway is glycolysis, a process during which less oxygen is required and results in lower energetic yield than cellular respiration (for a comprehensive review of these pathways see (25)). Most differentiated cells use oxidative phosphorylation to generate energy, since this pathway generates the most ATP molecules per molecule of glucose (36-38 ATPs/glucose molecule). In brief, each glucose molecule is converted into pyruvate, which is transported into the mitochondria where it is further broken down via the citric acid cycle and most ATP molecules are generated via the electron transport chain. This process can only occur when molecular oxygen is present in the cells and has a side effect of generating reactive oxygen species (ROS). Anaerobic glycolysis on the other hand, does not require oxygen and converts the pyruvate into lactate. Only 2 ATP molecules are generated via this process. Despite the fact that the overall ATP yield per glucose molecule is drastically lower through glycolysis than through oxidative phosphorylation, it is well demonstrated that anaerobic glycolysis is an efficient pathway to provide energy and carbon building blocks for rapidly proliferating cells (25). Proliferative cells, as well as certain stem cells use this pathway to meet their energetic demands with the added benefit that it generates lower amounts of ROS in the cell.

3.3. Effects of nutrient availability at the cellular level

Cells can adapt their metabolic activity according to the presence of "energy units" in the cell. For instance, the level of ATP and its ratio to ADP or AMP is monitored via different metabolic enzymes, with AMP-activated protein kinase (AMPK) being the major energy sensor. When AMP/ATP or ADP/ATP ratios increase, AMPK regulates the energy balance via the activation of catabolic pathways and down-regulation of anabolic pathways (26). During cellular respiration, byproducts like Reactive Oxygen Species (ROS) are produced, which could be harmful for cellular integrity, but, as we discuss later, also induce cellular pathways that are necessary for appropriate functioning of stem cells (27). It has been recognized that some stem cells exhibit distinct metabolic properties than other cell types, namely a preference for glycolysis and a better adaptation to hypoxic environments. Interactions between the stem cells and the respective niche can also shape the metabolic requirements and characteristics of a stem cell population, as for instance was shown for the role of Paneth cells that respond to the organismal nutritional state to regulate intestinal stem cell function (28).

3.4. Effects of nutrient availability at the systemic level

At the systemic level, the presence of nutrients such as glucose in the blood elicits physiological reactions that influence the whole body. Cells and organs respond to the fluctuating levels of nutrients in the blood in order for organismal homeostasis to be maintained. These responses impact the physiology of stem cells as well. For instance, hematopoietic stem cells (HSC) have been shown to be sensitive to systemic levels of glucose to proliferate during embryogenesis (27), and the correct coordination of glucose levels with proliferation or differentiation decisions is essential. Exposure of zebrafish embryos to high glucose levels resulted in enhanced hematopoietic stem cell formation and function. Exposure to high glucose levels *in utero* associated with higher birth weight in humans, has been shown to enhance the risk for acute lymphoblastic leukemia (ALL) and acute myelogenous leukemia (AML) (29, 30). Furthermore, children who suffered from diabetes type I exhibited a higher risk to develop ALL (31) and in diabetic patients, mobilization of HSC progenitors from the bone marrow is compromised (32). Thus, deregulation of metabolic pathways, as for instance in patients that suffer from diabetes, challenges the ability of stem cells to maintain the balance of self-renewal versus differentiation and may result in depletion of the stem cell pool, premature aging, aberrant differentiation and even cancer.

4. KEY PLAYERS IN THE COORDINATION OF METABOLIC STATE WITH STEM CELL HOMEOSTASIS

4.1. FoxOs

The Forkhead box O (FOXO) family is a family of transcription factors with diverse cellular functions.

FOXOs are phosphorylated by PI3K, which is activated by insulin or other growth factor pathways. The phosphorylation prevents the nuclear localization of FOXOs thereby inhibiting their function (33, 34). Upon cellular stress (through ROS, energetic or genotoxic stress) or when growth-factor signaling is decreased, FOXOs are dephosphorylated and translocated into the nucleus where they increase transcriptional activity of target genes (35). In addition, the deacetylase Sirtuin 1 (SIRT1) can deacetylate FOXOs, which also promotes FOXO-mediated gene transcription (36). FOXOs-mediated signaling has important roles in stem cell maintenance and stem cell self renewal and has been linked with ROS-regulated processes and hypoxia-induced cellular responses (37).

Genetic deletion of FOXO family members (FOXO1, 3a and 4) in the mouse was shown to lead to the depletion of the stem cell pool in both neural stem cells (NSCs) and hematopoietic stem cells (HSCs) (1, 3, 38). Similar results were shown *in vitro*, where FOXO3 was shown to be essential for the maintenance and homeostasis of HSCs (39, 40) and in human embryonic stem cells, where FOXO1 was shown to regulate the expression of OCT4 and SOX2, which are two transcription factors that play a critical role in the stem cell self-renewal (41).

Specifically with respect to cell cycle regulation, FOXOs act to maintain stem cell quiescence (by negatively regulating G0 exit), which is a fundamental property of stem cells. FOXOs have been proposed to promote cellular quiescence by promoting the expression of proteins involved in cell cycle arrest such as $p27^{KIP1}$, $p53^{KIP2}$ and Cyclin G2 (3, 38). FOXO-null NSCs showed increased expression of cyclins and cyclin-dependent kinases and a decrease in cyclin-dependent kinase inhibitors ($p53^{KIP2}$). As a result, the NSCs exhibited hyper proliferation that led to depletion of the stem cell pool (3). In mice, deletion of FOXO members 1, 3 and 4 in bipotential progenitors of osteoblasts and adipocytes resulted in increased proliferation of osteoprogenitor cells, which was attributed to the upregulation of Wnt/beta-catenin signaling and Cyclin D1 expression (42). Given the critical role of Wnt signaling in stem cell biology (43), it is important to note the association of FOXO1 with beta-catenin a key effector of Wnt signaling in oxidative stress signaling (44).

As regards the interplay between FOXO signaling and other metabolic pathways, FOXO signaling has been linked to resistance to oxidative stress and the inhibition of premature differentiation (45, 46). The activity of FOXO3 as a transcriptional activator has been linked to target genes such as SODs, antioxidant enzymes that play a role in lowering of cellular ROS levels (38, 47) and it was shown that the loss of FOXO3a in NSCs resulted not only in the depletion of the NSC pool but also in the inability to generate appropriate neural lineages (1). Another interesting study showed that FOXO1 is dephosphorylated and translocated to the nucleus during prolonged fasting in mice, due to decreased insulin signaling. This resulted in the transcriptional activation of genes involved in gluconeogenesis (48). In addition, AMPK activation resulting from low cellular ATP levels, led to the activation of gluconeogenesis via phosphorylation of FOXO3 (49). Furthermore, FOXO1 was shown to physically and functionally interact with Notch signaling (50). The FOXO1-Notch cooperation integrates environmental cues via Notch, with metabolic cues via FOXO1 during muscle differentiation. The role of FOXO in this context was shown to be independent of its transcriptional function.

Taken together, the effects of FOXOs on stem cell maintenance and homeostasis are striking and diverse, and the underlying mechanisms are currently being unraveled. The main effects of FOXOs on stem cell homeostasis appear to be mediated by the transcriptional activation of genes that are involved in the promotion of cellular quiescence. Furthermore, FOXO signaling is implicated in the resistance to oxidative stress, the response to low oxygen levels and the activation of the gluconeogenic pathway, thereby integrating energy-metabolism and ROS responses with stem cell maintenance and correct differentiation.

4.2. Sirtuins

Sirtuins belong to a family of proteins that deacetylate histones and other protein targets (51). The family consists of seven members that vary in cellular localization and targets and have been implicated in the control of cellular metabolism (52). Sirtuins are activated when the ratio of [NAD+] to [NADH] is elevated, a state that is associated with low energy and oxidative stress (reviewed in (52)). In particular, sirtuin 1 (SIRT1) has been demonstrated as a crucial regulator of cellular and organismal metabolism in mammals, including essential functions in mediating the effects of caloric restriction (48, 52, 53). Furthermore, activators of sirtuins have been proposed for the treatment of type 2 diabetes and neurodegenerative diseases (54). SIRT1 activation elicits pleiotropic effects that include the downregulation of glycolysis, upregulation of the protective response to ROS and activation of mitochondrial biogenesis. These effects are mediated by activation of PGC1alpha, PGC1alpha/FOXO and inhibition of CRTC2/CREB and HIF1a (52). SIRT1 can also deacetylate p53 (55), thereby inhibiting its function resulting in the delay of p53-mediated cellular senescence and promoting cell survival (56, 57).

In HSC maintenance, SIRT1 is proposed to maintain the stem cell pool through ROS elimination, FOXO activation, and p53 inhibition resulting in the suppression of differentiation of hematopoietic stem cells (57). Deletion or knockdown of SIRT1 in NSCs led to enhanced differentiation into astrocytes at the expense of neurons (58). Overexpression on the other hand resulted in increased neuronal differentiation (59). This could be explained by the role of SIRT1 in silencing of Notch target genes (60) that has also been shown in endothelial cells (61). Several studies on the role of sirtuins and in particular SIRT1 in stem cell biology have revealed that they promote maintenance of the stem cell pool by protecting genome integrity, as well as regulating the nuclear translocation of FoxO and p53. However, it has emerged that the functions

of SIRT1 are not the same in all stem cell types and that they are also age- and context-dependent (11, 52).

4.3. AMPK

AMPK is regarded as the key energy sensor in eukaryotic cells. AMPK activation initiates a cascade of downstream events with the purpose to preserve energy (26). AMPK activation leads to activation of catabolic processes such as fatty acid oxidation, glucose uptake and glycolysis and simultaneous inhibition of catabolic processes. Attenuation of catabolism includes inhibition of fatty acid and sterol synthesis as well as, inhibition of ribosome biogenesis and translation through negative regulation of mTOR signaling (26). It has been reported that increased mTOR activity leads to abnormal proliferation of HSCs (2) and AMPK deletion has been shown to cause similar effects (62). In contrast, activation of AMPK in ESCs with AICAR resulted in a decrease of cellular proliferation and loss of pluripotency markers (63). AMPK is also involved in the up-regulation of genes involved in resistance to oxidative stress and oxidative metabolism, through the regulation of transcription factors such as FOXO family members (64) and DAF16 (65).

Most relevant for this review, is the implication of AMPK in cell-cycle control.. The influence of nutrient concentrations in cell cycle progression is illustrated by the glucose-dependent checkpoint that occurs at the G1/S phase. Activation of AMPK results in cell cycle arrest in the G1/S phase via the phosphorylation of p53, cyclin-dependent kinase inhibitor 1B (CDKN1B or $p27^{kip1}$) (66) and the up-regulation of CDKN1A ($P21^{WAF1}$) (67). AMPK phosphorylation enables the survival of cells during glucose deprivation via a p53–dependent cell cycle arrest. Constitutive AMPK activation on the other hand leads to accelerated p53-dependent cellular senescence (68) implying that there is adaptation to fluctuating glucose levels in the body, as well as a point of "no return". Finally, an elegant chemical genetic screen for (novel) AMPK substrates identified several proteins involved in mitosis (69). These data demonstrated that an important function of AMPK is to coordinate nutrient status with mitosis completion, a process that is important for the organism's response to low nutrients during development, as well as in adult stem cell homeostasis.

4.4. LKB1

The tumor suppressor gene LKB1 is a serine-threonine kinase that activates AMPK and AMPK-related kinases (70, 71). The discovery that LKB1 is the major upstream activator of AMPK established one of the first links between energy-metabolism control and tumor suppression. LKB1 inactivation in the mouse led to lethality at an early embryonic stage (72), whereas heterozygous *lkb1*/+ mice developed polyps (73). The increased proliferation of different tissues was attributed at least in part to aberrant activation of the mTOR pathway (74).

Interestingly, conditional inactivation of LKB1 in mouse HSCs resulted in increased HSC division and depletion of the stem cell pool (62, 75, 76). Initially HSCs increased in number, which is associated with enhanced proliferation, however, metabolically compromised *Lkb1*-deficient HSCs could not be maintained leading to the depletion of the stem cell pool (62, 75, 76). This finding indicated that LKB1 is implicated in the negative regulation of stem cell division, as well as the maintenance of the stem cell pool. These functions could be partly explained via the LKB1-AMPK pathway, but it was shown that LKB1 also regulates maintenance of the HSC population by AMPK-independent mechanisms. One of the mechanisms unraveled, was the reduction in mitochondrial membrane potential, which was observed in LKB1-deficient but not AMPK-deficient HSCs. Although HSCs deficient for either AMPK or LKB1 showed similar phenotypic traits, AMPK was not required for stem cell maintenance, whereas LKB1 was.

Another study focused on the effect of LKB1-deletion on chromosome stability. LKB1-deficient HSC showed an increase in the amount of centrosomes and aberrant mitotic spindles, resulting in aneuploidy of offspring cells, a phenotype not found in AMPK deficient HSCs (62). This was intriguing since it provided a new perspective on how stem cell physiology and chromosome stability can be dramatically influenced by energy metabolism, independently of the most well studied mTOR and ROS pathways. These phenotypes could be attributed to either different (perhaps as yet not identified) LKB1 substrates, or by deregulation of pathways that are critically dependent on the energetic status of the cell.

LKB1 inactivation in zebrafish led to a whole-organism inability to cope with energetic stress but with only modest deregulation of mTOR signaling (77). In light of recent findings that HSC-potency is influenced by glucose levels (27), it would be interesting to investigate HSC physiology in the context of deregulated metabolism.

4.5. mTOR

The mechanistic target of rapamycin (mTOR), is a well described integrator of nutrient availability with cellular growth (78). When nutrient concentrations rise in the bloodstream, the mTOR complex 1 (mTORC1) and 2 (mTORC2) are activated, resulting in the activation of translation, cell proliferation and the inhibition of autophagy and glycogen synthesis. During fasting, when blood glucose levels decrease, mTORC activity is inhibited mainly by activation of AMPK and TSC1/2, ensuing to adaptation to the shortage of nutrients. An excess of nutrients can lead to chronic activation of mTORC1, which can result in resistance to insulin signaling due to the over-activation of a negative feedback loop via S6 kinase 1 (S6K1) (63). Deletion of the negative regulator of mTOR signaling TSC1 in HSCs resulted in a shift from quiescent state to rapid cell cycling via an increased expression of cyclin-dependent kinase inhibitors p16, p19 and p21, combined with increased levels of mitochondrial biogenesis and ROS production (63). Treatment with ROS antagonists could restore HSC balance, indicating that the TSC-mTOR pathway mediated HSC maintenance via the inhibition of ROS production. However, it could also be that the detrimental effects of TSC1 deletion occurred due to the

increased ROS levels, which was rescued with the ROS-antagonists.

Rapamycin is a drug that as the name implies targets the mTOR pathway and has been used to investigate the effects of mTOR inhibition in various models. Adult mice in which mTOR signaling was genetically increased displayed similar effects as the TSC1 deletion mentioned above and treatment of these mice with rapamycin increased the lifespan of adult mice (79). Most relevantly for this review, HSC self-renewal and hematopoiesis were also restored upon rapamycin treatment. Specifically, upon TSC1 deletion that led to overactivation of mTORC1 signaling in aging HSCs, the upregulation of cyclin-dependent kinases resulted in exhaustion of the stem cell pool (79). Positive effects of mTORC1 inhibition on stem cell maintenance *in vivo* have also been described elsewhere (2, 80).

The mTOR pathway is also a negative regulator of autophagy, a process in which the cell is "digesting" its own materials in order to recycle cellular products and eliminate damaged organelles and proteins. Under starvation, autophagy is activated as a survival mechanism. The effects of rapamycin treatment or TSC deletion on stem cell fitness could also be linked with the regulation of autophagy. The mTOR pathway inhibits autophagy, which could lead to accumulation of aberrantly folded proteins and damaged organelles resulting in diminished cellular health. It was shown that autophagy plays a protective role in stem cells biology under conditions of starvation. Activation of AMPK led to the inhibition of mTOR, thereby promoting autophagy culminating in enhanced survival of the stem cells during times of starvation (81). .

Caloric restriction in mice resulted in preservation of the intestinal stem cell pool *in vivo* through promoting stem cell renewal, while decreasing differentiation (28). Interestingly, these effects were mediated by surrounding Paneth cells and not by the intestinal stem cells. Caloric restriction led to a decrease in mTORC1 activation in Paneth cells and reactivation of mTORC1 in these cells was sufficient to circumvent the effects on surrounding intestinal stem cells. This highlights the importance of the surrounding niche, whether it being nutrients or cells, on stem cell behavior.

4.6. Reactive oxygen species (ROS)

ROS are a byproduct of cellular metabolism. The three main sources of ROS are: the mitochondrial electron-transport chain (ETC), the membrane-bound NADPH oxidase (NOX) complex and the endoplasmic reticulum (82). The effects of ROS on the homeostasis of stem cells have been discussed in a recent review (83) and it is clear that a shift in the "dogma" that ROS are always harmful for cellular integrity is emerging, with the notion of 'pathological' versus 'homeostatic' levels of ROS. High levels of ROS result in cellular responses like differentiation and apoptosis (84), which are not favorable for the maintenance of the stem cell population. It is believed that stem cells have low intracellular levels of ROS, which is accomplished via three different mechanisms. The first is via the expression of the mitochondrial uncoupling protein 2 (UCP2) and antioxidant enzymes like SOD that actively lower ROS (69, 70). The second mechanism involves the expression of transcription factors such as FOXOs and nuclear factor erythroid-2-related factor 2 (Nrf2) (85) that result in the transcriptional activation of antioxidant enzymes and finally, through the expression of redox regulator genes such as apurinic/apyrimidinic (AP) endonuclease1/redox factor-1 (APE1/Ref-1) (86) and ataxia telangiectasia mutated (ATM) (87).

The notion of "homeostatic" versus "pathological" ROS is illustrated by results that the presence of 'basal' levels of ROS stimulated HSC production and efficiency during embryogenesis (27). The positive effect of glucose on the HSC formation during embryogenesis was attributed to the ROS that originated from metabolism of glucose at the mitochondria. This finding was confirmed by exposing zebrafish embryos to exogenous ROS (H_2O_2) and ROS inhibitors (N-acetylcysteine, Vitamin C). The effects of ROS exposure at the time of HSC emergence during embryogenesis were attributed to the stabilization of HIF1a by ROS (27), which in turn activated pathways involved in cell cycle progression and metabolism that stimulate HSC formation. ROS as by-product of cellular metabolism are always present in living cells. The homeostatic levels are necessary for correct differentiation for instance, during embryonic expansion of the stem cell pool, but also for correct differentiation. However, too high levels of ROS appear detrimental to self-renewal and to appropriate differentiation. The cells respond to high ROS levels by upregulating the expression of genes involved in ROS elimination.

4.7. Hypoxia inducible factors

Intracellular oxygen levels appear to have a big impact on stem cells that ranges from switching metabolic pathways to altering cell proliferation status. The effects are largely mediated by hypoxia inducible factors (HIFs) and in particular, HIF1a that is stabilized under hypoxic conditions. Activation of HIF1a leads to many downstream processes, which are orchestrated either via direct interaction of HIF1a with other proteins, or by activation of proteins that are involved in transcriptional regulation.
Pluripotency of human embryonic stem cells (hESCs) is enhanced by exposure to hypoxic conditions (30), which had been shown in other stem cell types (71). Under these hypoxic conditions, the cells consumed low levels of oxygen, high levels of glucose and produced high levels of lactate, typical hallmarks of glycolysis metabolism. HSCs were also shown to utilize mainly glycolysis to generate energy (43, 72). Employing glycolysis as the major energy-producing pathway appears to provide two benefits: (1) it enables the stem cells to survive under hypoxic conditions, but also (2) limits the amount of ROS produced by oxidative phosphorylation thereby safeguarding the integrity of the stem cell pool. Interestingly, in order for differentiation to occur, it has been suggested that proliferative, stem and progenitor cells switch from glycolysis to a combination of glycolysis and

Table 1. Summary of key players in stem cell maintenance and cell cycle progression

	Effect on stem cell pool	**Effect on cell cycle**
FoxOs	Deletion of FoxO 1, 3 and 4 led to depletion of the stem cell pool in NSCs and HSCs (28-30, 76) FoxOs have shown to be essential for maintenance and homeostasis of HSCs (39, 40)	Induction of cell cycle arrest via $p27^{KIP1}$, $p53^{KIP2}$ and Cyclin G2 (28, 30) FoxOs negatively regulated G0 exit in NSCs (3)
Sirtuins	Deletion of SIRT1 resulted in impaired self-renewal of HSCs (57) SIRT1 maintained the HSC pool via suppression of differentiation (57) Deletion or knockdown of SIRT1 in NSCs led to increased differentiation into astrocytes at the expense of neurons (58) Over-expression on the other hand resulted in increased neuronal differentiation (59)	SIRT1 can deacetylate p53, thereby inhibiting its function (55)
AMPK	Deletion of AMPK resulted in abnormal proliferation in HSCs (2) Constitutively active AMPK resulted in accelerated p53-dependent cellular senescence in HSCs (68) Activation of AMPK by AICAR resulted in decreased proliferation and loss of pluripotency markers in ESCs (63)	Activation of AMPK resulted in cell cycle arrest in G1/S phase, via phosphorylation of p53 (67)
LKB1	Conditional inactivation of LKB1 in mouse HSCs resulted in increased HSC division and depletion of the stem cell pool (62, 75, 76) LKB1 deficiency in HSCs resulted in aneuploidy of offspring cells (62)	
mTOR	Deletion of TSC1 in HSCs resulted in reduced hematopoiesis and self renewal (94) Treatment of HSCs with rapamycin restored HSC self renewal and hematopoiesis (79) Inhibition of mTOR by AMPK activation results in upregulation of autophagy, which facilitates survival of stem cells during times of starvation (81) Caloric restriction resulted in decreased mTORC1 activation in Paneth cells, which promoted stem cell self renewal and reduced proliferation into differentiated cells (28)	Deletion of TSC1 in HSCs resulted in a shift from quiescent to rapid cell cycling state via increased expression of CDK inhibitors p16, p19 and p21 (48, 95)
ROS	'Normal' levels of ROS stimulate HSC production and efficiency during embryogenesis (27)	
HIFs	Pluripotency of hESCs is enhanced by hypoxic conditions (30)	HIF1a activation leads to inhibition of MYC, resulting in inhibition of cell proliferation (96)

oxidative phosphorylation (88), such as during differentiation of HSC into myeloid cells (89). The same findings have been observed during normal tissue development in the frog (*Xenopus laevis*) retina (90).

Furthermore, HIF1a stabilization results in FOXO3a activation that leads among other effects to the down-regulation of the expression of mitochondrial genes and the production of ROS-eliminating enzymes. In addition to these effects in cellular metabolism, HIF1a activation also leads to inhibition of MYC, which results in inhibition of cell proliferation, thereby contributing to the quiescent phenotype of stem cells (39). Another link to the cell cycle progression, is that HIF1a stabilization led to activation of genes such as p53, p21, Bcl-2 in embryonic stem cells, thereby decreasing proliferation (73).

Finally, HIF1a was shown to interact with the intracellular domain of the Notch receptor, potentiating the transcription of Notch target genes that promote an undifferentiated state of neural stem cells and other progenitor cells (91). Moreover, HIF1a was also shown to interact with beta-catenin leading to the down-regulation of a subset of Wnt-target genes, causing cell growth arrest (92). However, it must be noted that this conclusion was made based on experiments using cancer cell lines, so it needs to be validated in adult tissue stem cells before conclusions regarding healthy stem cells can be drawn.

Overall, it has become clear that HIF1a orchestrates various cellular processes that maintain the stem cell population by either regulation of metabolic pathways or by the inhibition of inappropriate cell differentiation. The roles of the key players in cell-cycle progression and stem cell maintenance are summarized in Table 1.

5. SUMMARY AND PERSPECTIVE

All stem cells exhibit two essential characteristics: the ability to self-renew and the ability to give rise to differentiated cells, a characteristic called potency. It has been recognized that the balance between these two fates of stem cells is intricately regulated and even slight disturbances in this equilibrium can have catastrophic consequences for the organism. Emerging evidence suggests that certain stem cells display metabolic properties that are distinct from their surrounding cells. For instance, stem cells, particularly those residing in hypoxic niches, utilize mainly anaerobic glycolysis and thrive in hypoxic environments. This may be an adaptation to the specific microenvironment where stem cells reside, but it also has important implications for the homeostasis of stem cells, such as to protect the stem cells from potentially harmful situations like low oxygen and nutrient levels and increased ROS levels. The employment of glycolysis as the main energy production pathway by stem cells located in a hypoxic niche, serves to minimize harmful ROS production and ensures DNA integrity. In addition, HIFa orchestrates many other cascades to ensure cellular quiescence. For appropriate differentiation to occur on the other hand, it is suggested that stem cells need to switch to cellular respiration, which results in higher levels of ROS as a by-product. These ROS assume a signaling role inducing other cell intrinsic pathways that stimulate appropriate differentiation.

At the molecular level, since the balance between self-renewal and potency of stem cells is so delicate, several genes and signaling cascades are implicated in its regulation. Energy sensing pathways perform signaling

roles and major signaling pathways impinge on metabolism regulation. Furthermore, there are extensive interactions and complex feedback loops between energy-sensing signaling pathways. Namely, the concerted activity of AMPK and SIRT1 regulate the stability of HIF1a, HIF1a stabilization activates FOXO3 that evokes ROS elimination processes. HIF1a activates glycolysis that minimizes ROS production as discussed above and HIF1a promotes stem cell quiescence via interactions with the Notch and Wnt signaling cascades. These interactions and crosstalk result under normal healthy circumstances in a sufficiently replenished stem cell pool and accurate differentiation to downstream lineages. While it is appreciated that stem cells exhibit distinct metabolic properties than differentiated progeny, these are not as yet fully characterized. It is a challenge for the future of regenerative medicine to harness the unique metabolic properties of stem cells with a view to ultimately selectively direct the system to self-renew or differentiate by manipulating (also) their metabolic state. Another challenge for future research is the clarification of the impact of organismal metabolism on stem cell fate choices. Most of the energy-responsive signaling pathways described above are implicated in modulating whole-organism metabolism, which in turn can influence stem cell homeostasis via cell autonomous and non-cell autonomous mechanisms.

The metabolic state of a whole organism can be modified not only by food consumption but also from intrinsic signals such as circulating hormones and is likewise affected by metabolic disorders. In this field of research, the zebrafish can prove a valuable model system. For instance, the possibility to assess the effects of glucose, or compounds on whole-organism metabolism including stem cell physiology has already offered insights into regulation of stem cell behavior by environmental factors (13, 27). An additional layer of complexity is the realization that the downstream effectors of the metabolic signaling pathways impinge on regulation of the cell–cycle to exert their effects. Recently, studies using zebrafish genetic models and employing cell-cycle genetic indicators have unraveled the plasticity of differentiated tissues in response to nutrients (93). An integrated analysis of the molecular mechanisms that link the systemic metabolism with cell-cycle regulation and stem cell fate is required for further advancements in regenerative medicine.

5. ACKNOWLEDGEMENTS

Work in the laboratory is funded by a Dutch Cancer Society (Koningin Wilhelmina Fonds, KWF) grant to A-P.G.H.

6. REFERENCES

1. V M Renault, V A Rafalski, A A Morgan, D A M Salih, J O Brett, A E Webb, S A Villeda, P U Thekkat, C Guillerey, N C Denko, T D Palmer, A J Butte and A Brunet: FoxO3 Regulates Neural Stem Cell Homeostasis. *Cell Stem Cell*, 5(5), 527-539 (2009)

2. O H Yilmaz, R Valdez, B K Theisen, W Guo, D O Ferguson, H Wu and S J Morrison: Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. *Nature*, 441(7092), 475-82 (2006)

3. J-h Paik, Z Ding, R Narurkar, S Ramkissoon, F Muller, W S Kamoun, S-S Chae, H Zheng, H Ying, J Mahoney, D Hiller, S Jiang, A Protopopov, W H Wong, L Chin, K L Ligon and R A DePinho: FoxOs Cooperatively Regulate Diverse Pathways Governing Neural Stem Cell Homeostasis. *Cell Stem Cell*, 5(5), 540-553 (2009)

4. C J McLeod, L Wang, C Wong and D L Jones: Stem cell dynamics in response to nutrient availability. *Curr Biol*, 20(23), 2100-5 (2010)

5. L E O'Brien, S S Soliman, X Li and D Bilder: Altered modes of stem cell division drive adaptive intestinal growth. *Cell*, 147(3), 603-14 (2011)

6. J M Chell and A H Brand: Nutrition-responsive glia control exit of neural stem cells from quiescence. *Cell*, 143(7), 1161-73 (2010)

7. R Sousa-Nunes, L L Yee and A P Gould: Fat cells reactivate quiescent neuroblasts via TOR and glial insulin relays in Drosophila. *Nature*, 471(7339), 508-12 (2011)

8. J Shim, T Mukherjee and U Banerjee: Direct sensing of systemic and nutritional signals by haematopoietic progenitors in Drosophila. *Nat Cell Biol*, 14(4), 394-400 (2012)

9. M Fukuyama, K Sakuma, R Park, H Kasuga, R Nagaya, Y Atsumi, Y Shimomura, S Takahashi, H Kajiho, A Rougvie, K Kontani and T Katada: C elegans AMPKs promote survival and arrest germline development during nutrient stress. *Biol Open*, 1(10), 929-36 (2012)

10. H Jasper and D L Jones: Metabolic regulation of stem cell behavior and implications for aging. *Cell Metab*, 12(6), 561-5 (2010)

11. V A Rafalski, E Mancini and A Brunet: Energy metabolism and energy-sensing pathways in mammalian embryonic and adult stem cell fate. *J Cell Sci*, 125(Pt 23), 5597-608 (2012)

12. A Schlegel and D Y Stainier: Lessons from "lower" organisms: what worms, flies, and zebrafish can teach us about human energy metabolism. *PLoS Genet*, 3(11), e199 (2007)

13. P Gut, B Baeza-Raja, O Andersson, L Hasenkamp, J Hsiao, D Hesselson, K Akassoglou, E Verdin, M D Hirschey and D Y Stainier: Whole-organism screening for gluconeogenesis identifies activators of fasting metabolism. *Nat Chem Biol*, 9(2), 97-104 (2013)

14. A Seth, D L Stemple and I Barroso: The emerging use of zebrafish to model metabolic disease. *Dis Model Mech*, 6(5), 1080-8 (2013)

15. L I Zon: Intrinsic and extrinsic control of haematopoietic stem-cell self-renewal. *Nature*, 453(7193), 306-13 (2008)

16. T Evans, E T Rosenthal, J Youngblom, D Distel and T Hunt: Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell*, 33(2), 389-96 (1983)

17. C Bertoli, J M Skotheim and R A de Bruin: Control of cell cycle transcription during G1 and S phases. *Nat Rev Mol Cell Biol*, 14(8), 518-28 (2013)

18. M B Kastan, O Onyekwere, D Sidransky, B Vogelstein and R W Craig: Participation of p53 protein in the cellular response to DNA damage. *Cancer Res*, 51(23 Pt 1), 6304-11 (1991)

19. R A Weinberg: The retinoblastoma protein and cell cycle control. *Cell*, 81(3), 323-30 (1995)

20. E S Knudsen and K E Knudsen: Tailoring to RB: tumour suppressor status and therapeutic response. *Nat Rev Cancer*, 8(9), 714-24 (2008)

21. F A Dick and S M Rubin: Molecular mechanisms underlying RB protein function. *Nat Rev Mol Cell Biol*, 14(5), 297-306 (2013)

22. P Savatier, S Huang, L Szekely, K G Wiman and J Samarut: Contrasting patterns of retinoblastoma protein expression in mouse embryonic stem cells and embryonic fibroblasts. *Oncogene*, 9(3), 809-18 (1994)

23. E Stead, J White, R Faast, S Conn, S Goldstone, J Rathjen, U Dhingra, P Rathjen, D Walker and S Dalton: Pluripotent cell division cycles are driven by ectopic Cdk2, cyclin A/E and E2F activities. *Oncogene*, 21(54), 8320-33 (2002)

24. P Savatier, H Lapillonne, L A van Grunsven, B B Rudkin and J Samarut: Withdrawal of differentiation inhibitory activity/leukemia inhibitory factor up-regulates D-type cyclins and cyclin-dependent kinase inhibitors in mouse embryonic stem cells. *Oncogene*, 12(2), 309-22 (1996)

25. M G Vander Heiden, L C Cantley and C B Thompson: Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*, 324(5930), 1029-33 (2009)

26. D G Hardie, F A Ross and S A Hawley: AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nature Reviews Molecular Cell Biology*, 13(4), 251-262 (2012)

27. J M Harris, V Esain, G M Frechette, L J Harris, A G Cox, M Cortes, M K Garnaas, K J Carroll, C C Cutting, T Khan, P M Elks, S A Renshaw, B C Dickinson, C J Chang, M P Murphy, B H Paw, M G Vander Heiden, W Goessling and T E North: Glucose metabolism impacts the spatiotemporal onset and magnitude of HSC induction *in vivo*. *Blood*, 121(13), 2483-93 (2013)

28. O H Yilmaz, P Katajisto, D W Lamming, Y Gultekin, K E Bauer-Rowe, S Sengupta, K Birsoy, A Dursun, V O Yilmaz, M Selig, G P Nielsen, M Mino-Kenudson, L R Zukerberg, A K Bhan, V Deshpande and D M Sabatini: mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake. *Nature*, 486(7404), 490-5 (2012)

29. L L Hjalgrim, T Westergaard, K Rostgaard, K Schmiegelow, M Melbye, H Hjalgrim and E A Engels: Birth weight as a risk factor for childhood leukemia: a meta-analysis of 18 epidemiologic studies. *Am J Epidemiol*, 158(8), 724-35 (2003)

30. L L Hjalgrim, K Rostgaard, H Hjalgrim, T Westergaard, H Thomassen, E Forestier, G Gustafsson, J Kristinsson, M Melbye and K Schmiegelow: Birth weight and risk for childhood leukemia in Denmark, Sweden, Norway, and Iceland. *J Natl Cancer Inst*, 96(20), 1549-56 (2004)

31. R G Feltbower, P A McKinney, M F Greaves, R C Parslow and H J Bodansky: International parallels in leukaemia and diabetes epidemiology. *Arch Dis Child*, 89(1), 54-6 (2004)

32. F Ferraro, S Lymperi, S Mendez-Ferrer, B Saez, J A Spencer, B Y Yeap, E Masselli, G Graiani, L Prezioso, E L Rizzini, M Mangoni, V Rizzoli, S M Sykes, C P Lin, P S Frenette, F Quaini and D T Scadden: Diabetes impairs hematopoietic stem cell mobilization by altering niche function. *Sci Transl Med*, 3(104), 104ra101 (2011)

33. W H Biggs, 3rd, J Meisenhelder, T Hunter, W K Cavenee and K C Arden: Protein kinase B/Akt-mediated phosphorylation promotes nuclear exclusion of the winged helix transcription factor FKHR1. *Proc Natl Acad Sci U S A*, 96(13), 7421-6 (1999)

34. E L Greer and A Brunet: FOXO transcription factors at the interface between longevity and tumor suppression. *Oncogene*, 24(50), 7410-25 (2005)

35. A Eijkelenboom and B M Burgering: FOXOs: signalling integrators for homeostasis maintenance. *Nat Rev Mol Cell Biol*, 14(2), 83-97 (2013)

36. A Brunet, L B Sweeney, J F Sturgill, K F Chua, P L Greer, Y Lin, H Tran, S E Ross, R Mostoslavsky, H Y Cohen, L S Hu, H L Cheng, M P Jedrychowski, S P Gygi, D A Sinclair, F W Alt and M E Greenberg: Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science*, 303(5666), 2011-5 (2004)

37. P Storz: Forkhead homeobox type O transcription factors in the responses to oxidative stress. *Antioxid Redox Signal*, 14(4), 593-605 (2011)

38. Z Tothova and D G Gilliland: FoxO Transcription Factors and Stem Cell Homeostasis: Insights from the Hematopoietic System. *Cell Stem Cell*, 1(2), 140-152 (2007)

39. K Miyamoto, K Y Araki, K Naka, F Arai, K Takubo, S Yamazaki, S Matsuoka, T Miyamoto, K Ito, M Ohmura, C Chen, K Hosokawa, H Nakauchi, K Nakayama, K I Nakayama, M Harada, N Motoyama, T Suda and A Hirao: Foxo3a is essential for maintenance of the hematopoietic stem cell pool. *Cell Stem Cell*, 1(1), 101-12 (2007)

40. K Miyamoto, T Miyamoto, R Kato, A Yoshimura, N Motoyama and T Suda: FoxO3a regulates hematopoietic homeostasis through a negative feedback pathway in conditions of stress or aging. *Blood*, 112(12), 4485-93 (2008)

41. X Zhang, S Yalcin, D F Lee, T Y Yeh, S M Lee, J Su, S K Mungamuri, P Rimmele, M Kennedy, R Sellers, M Landthaler, T Tuschl, N W Chi, I Lemischka, G Keller and S Ghaffari: FOXO1 is an essential regulator of pluripotency in human embryonic stem cells. *Nat Cell Biol*, 13(9), 1092-9 (2011)

42. S Iyer, E Ambrogini, S M Bartell, L Han, P K Roberson, R de Cabo, R L Jilka, R S Weinstein, C A O'Brien, S C Manolagas and M Almeida: FOXOs attenuate bone formation by suppressing Wnt signaling. *J Clin Invest*, 123(8), 3409-19 (2013)

43. T Reya and H Clevers: Wnt signalling in stem cells and cancer. *Nature*, 434(7035), 843-50 (2005)

44. M A Essers, L M de Vries-Smits, N Barker, P E Polderman, B M Burgering and H C Korswagen: Functional interaction between beta-catenin and FOXO in oxidative stress signaling. *Science*, 308(5725), 1181-4 (2005)

45. T B Dansen and B M Burgering: Unravelling the tumor-suppressive functions of FOXO proteins. *Trends Cell Biol*, 18(9), 421-9 (2008)

46. T B Dansen: Forkhead Box O transcription factors: key players in redox signaling. *Antioxid Redox Signal*, 14(4), 559-61 (2011)

47. S Yalcin, X Zhang, J P Luciano, S K Mungamuri, D Marinkovic, C Vercherat, A Sarkar, M Grisotto, R Taneja and S Ghaffari: Foxo3 is essential for the regulation of ataxia telangiectasia mutated and oxidative stress-mediated homeostasis of hematopoietic stem cells. *J Biol Chem*, 283(37), 25692-705 (2008)

48. D Chen, J Bruno, E Easlon, S J Lin, H L Cheng, F W Alt and L Guarente: Tissue-specific regulation of SIRT1 by calorie restriction. *Genes Dev*, 22(13), 1753-7 (2008)

49. F Wang, C B Marshall, K Yamamoto, G Y Li, G M Gasmi-Seabrook, H Okada, T W Mak and M Ikura: Structures of KIX domain of CBP in complex with two FOXO3a transactivation domains reveal promiscuity and plasticity in coactivator recruitment. *Proc Natl Acad Sci U S A*, 109(16), 6078-83 (2012)

50. T Kitamura, Y I Kitamura, Y Funahashi, C J Shawber, D H Castrillon, R Kollipara, R A DePinho, J Kitajewski and D Accili: A Foxo/Notch pathway controls myogenic differentiation and fiber type specification. *J Clin Invest*, 117(9), 2477-85 (2007)

51. J N Feige and J Auwerx: Transcriptional targets of sirtuins in the coordination of mammalian physiology. *Curr Opin Cell Biol*, 20(3), 303-9 (2008)

52. R H Houtkooper, E Pirinen and J Auwerx: Sirtuins as regulators of metabolism and healthspan. *Nat Rev Mol Cell Biol*, 13(4), 225-38 (2012)

53. Y Kanfi, R Shalman, V Peshti, S N Pilosof, Y M Gozlan, K J Pearson, B Lerrer, D Moazed, J C Marine, R de Cabo and H Y Cohen: Regulation of SIRT6 protein levels by nutrient availabilit.y *FEBS Lett*, 582(5), 543-8 (2008)

54. A Balcerczyk and L Pirola: Therapeutic potential of activators and inhibitors of sirtuins. *Biofactors*, 36(5), 383-93 (2010)

55. H Vaziri, S K Dessain, E Ng Eaton, S I Imai, R A Frye, T K Pandita, L Guarente and R A Weinberg: hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. *Cell*, 107(2), 149-59 (2001)

56. J Luo, A Y Nikolaev, S Imai, D Chen, F Su, A Shiloh, L Guarente and W Gu: Negative control of p53 by Sir2alpha promotes cell survival under stress. *Cell*, 107(2), 137-48 (2001)

57. K Matsui, S Ezoe, K Oritani, M Shibata, M Tokunaga, N Fujita, A Tanimura, T Sudo, H Tanaka, M W McBurney, I Matsumura and Y Kanakura: NAD-dependent histone deacetylase, SIRT1, plays essential roles in the maintenance of hematopoietic stem cells. *Biochemical and Biophysical Research Communications*, 418(4), 811-817 (2012)

58. T Prozorovski, U Schulze-Topphoff, R Glumm, J Baumgart, F Schroter, O Ninnemann, E Siegert, I Bendix, O Brustle, R Nitsch, F Zipp and O Aktas: Sirt1 contributes critically to the redox-dependent fate of neural progenitors. *Nat Cell Biol*, 10(4), 385-94 (2008)

59. S Hisahara, S Chiba, H Matsumoto, M Tanno, H Yagi, S Shimohama, M Sato and Y Horio: Histone deacetylase SIRT1 modulates neuronal differentiation by its nuclear translocation. *Proc Natl Acad Sci U S A*, 105(40), 15599-604 (2008)

60. L Tiberi, J van den Ameele, J Dimidschstein, J Piccirilli, D Gall, A Herpoel, A Bilheu, J Bonnefont, M Iacovino, M Kyba, T Bouschet and P Vanderhaeghen: BCL6 controls neurogenesis through Sirt1-dependent epigenetic repression of selective Notch targets. *Nat Neurosci*, 15(12), 1627-35 (2012)

61. V Guarani, G Deflorian, C A Franco, M Kruger, L K Phng, K Bentley, L Toussaint, F Dequiedt, R Mostoslavsky, M H Schmidt, B Zimmermann, R P Brandes, M Mione, C H Westphal, T Braun, A M Zeiher, H Gerhardt, S Dimmeler and M Potente: Acetylation-dependent regulation of endothelial Notch signalling by the SIRT1 deacetylase. *Nature*, 473(7346), 234-8 (2011)

62. D Nakada, T L Saunders and S J Morrison: Lkb1 regulates cell cycle and energy metabolism in haematopoietic stem cells. *Nature*, 468(7324), 653-658 (2010)

63. H D Chae, M R Lee and H E Broxmeyer: 5-Aminoimidazole-4-carboxyamide ribonucleoside induces G(1)/S arrest and Nanog downregulation via p53 and enhances erythroid differentiation. *Stem Cells*, 30(2), 140-9 (2012)

64. E L Greer, D Dowlatshahi, M R Banko, J Villen, K Hoang, D Blanchard, S P Gygi and A Brunet: An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in C elegans *Curr Biol*, 17(19), 1646-56 (2007)

65. E L Greer, P R Oskoui, M R Banko, J M Maniar, M P Gygi, S P Gygi and A Brunet: The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. *J Biol Chem*, 282(41), 30107-19 (2007)

66. J Liang, S H Shao, Z X Xu, B Hennessy, Z Ding, M Larrea, S Kondo, D J Dumont, J U Gutterman, C L Walker, J M Slingerland and G B Mills: The energy sensing LKB1-AMPK pathway regulates p27(kip1) phosphorylation mediating the decision to enter autophagy or apoptosis. *Nat Cell Biol*, 9(2), 218-24 (2007)

67. K Imamura, T Ogura, A Kishimoto, M Kaminishi and H Esumi: Cell cycle regulation via p53 phosphorylation by a 5'-AMP activated protein kinase activator, 5-aminoimidazole- 4-carboxamide-1-beta-D-ribofuranoside, in a human hepatocellular carcinoma cell line. *Biochem Biophys Res Commun*, 287(2), 562-7 (2001)

68. R G Jones, D R Plas, S Kubek, M Buzzai, J Mu, Y Xu, M J Birnbaum and C B Thompson: AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. *Mol Cell*, 18(3), 283-93 (2005)

69. M R Banko, J J Allen, B E Schaffer, E W Wilker, P Tsou, J L White, J Villen, B Wang, S R Kim, K Sakamoto, S P Gygi, L C Cantley, M B Yaffe, K M Shokat and A Brunet: Chemical genetic screen for AMPKalpha2 substrates uncovers a network of proteins involved in mitosis. *Mol Cell*, 44(6), 878-92 (2011)

70. D R Alessi, K Sakamoto and J R Bayascas: LKB1-dependent signaling pathways. *Annu Rev Biochem*, 75, 137-63 (2006)

71. D B Shackelford and R J Shaw: The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. *Nat Rev Cancer*, 9(8), 563-75 (2009)

72. A Ylikorkala, D J Rossi, N Korsisaari, K Luukko, K Alitalo, M Henkemeyer and T P Makela: Vascular abnormalities and deregulation of VEGF in Lkb1-deficient mice. *Science*, 293(5533), 1323-6 (2001)

73. N Bardeesy, M Sinha, A F Hezel, S Signoretti, N A Hathaway, N E Sharpless, M Loda, D R Carrasco and R A DePinho: Loss of the Lkb1 tumour suppressor provokes intestinal polyposis but resistance to transformation. *Nature*, 419(6903), 162-7 (2002)

74. R J Shaw, N Bardeesy, B D Manning, L Lopez, M Kosmatka, R A DePinho and L C Cantley: The LKB1 tumor suppressor negatively regulates mTOR signaling. *Cancer Cell*, 6(1), 91-9 (2004)

75. B Gan, J Hu, S Jiang, Y Liu, E Sahin, L Zhuang, E Fletcher-Sananikone, S Colla, Y A Wang, L Chin and R A Depinho: Lkb1 regulates quiescence and metabolic homeostasis of haematopoietic stem cells. *Nature*, 468(7324), 701-4 (2010)

76. S Gurumurthy, S Z Xie, B Alagesan, J Kim, R Z Yusuf, B Saez, A Tzatsos, F Ozsolak, P Milos, F Ferrari, P J Park, O S Shirihai, D T Scadden and N Bardeesy: The Lkb1 metabolic sensor maintains haematopoietic stem cell survival. *Nature*, 468(7324), 659-63 (2010)

77. Y U van der Velden, L Wang, J Zevenhoven, E van Rooijen, M van Lohuizen, R H Giles, H Clevers and A P Haramis: The serine-threonine kinase LKB1 is essential for survival under energetic stress in zebrafish. *Proc Natl Acad Sci U S A*, 108(11), 4358-63 (2011)

78. M Laplante and David M Sabatini: mTOR Signaling in Growth Control and Disease. *Cell*, 149(2), 274-293 (2012)

79. C Chen, Y Liu and P Zheng: mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells. *Sci Signal*, 2(98), ra75 (2009)

80. R M Castilho, C H Squarize, L A Chodosh, B O Williams and J S Gutkind: mTOR mediates Wnt-induced epidermal stem cell exhaustion and aging. *Cell Stem Cell*, 5(3), 279-89 (2009)

81. Q Zhang, Y J Yang, H Wang, Q T Dong, T J Wang, H Y Qian and H Xu: Autophagy activation: a novel mechanism of atorvastatin to protect mesenchymal stem cells from hypoxia and serum deprivation via AMP-activated protein kinase/mammalian target of rapamycin pathway. *Stem Cells Dev*, 21(8), 1321-32 (2012)

82. B C Dickinson and C J Chang: Chemistry and biology of reactive oxygen species in signaling or stress responses. *Nat Chem Biol*, 7(8), 504-11 (2011)

83. K Wang, T Zhang, Q Dong, E C Nice, C Huang and Y Wei: Redox homeostasis: the linchpin in stem cell self-renewal and differentiation. *Cell Death Dis*, 4, e537 (2013)

84. L S Haneline: Redox regulation of stem and progenitor cells. *Antioxid Redox Signal*, 10(11), 1849-52 (2008)

85. Q Ma: Role of nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol*, 53, 401-26 (2013)

86. P Angkeow, S S Deshpande, B Qi, Y X Liu, Y C Park, B H Jeon, M Ozaki and K Irani: Redox factor-1: an extra-nuclear role in the regulation of endothelial oxidative stress and apoptosis. *Cell Death Differ*, 9(7), 717-25 (2002)

87. Z Guo, R Deshpande and T T Paull: ATM activation in the presence of oxidative stress. *Cell Cycle*, 9(24), 4805-11 (2010)

88. N Shyh-Chang, G Q Daley and L C Cantley: Stem cell metabolism in tissue development and aging. *Development*, 140(12), 2535-47 (2013)

89. W M Yu, X Liu, J Shen, O Jovanovic, E E Pohl, S L Gerson, T Finkel, H E Broxmeyer and C K Qu: Metabolic regulation by the mitochondrial phosphatase PTPMT1 is required for hematopoietic stem cell differentiation. *Cell Stem Cell*, 12(1), 62-74 (2013)

90. M Agathocleous, N K Love, O Randlett, J J Harris, J Liu, A J Murray and W A Harris: Metabolic differentiation in the embryonic retina. *Nat Cell Biol*, 14(8), 859-64 (2012)

91. M V Gustafsson, X Zheng, T Pereira, K Gradin, S Jin, J Lundkvist, J L Ruas, L Poellinger, U Lendahl and M Bondesson: Hypoxia requires notch signaling to maintain the undifferentiated cell state. *Dev Cell*, 9(5), 617-28 (2005)

92. A Kaidi, A C Williams and C Paraskeva: Interaction between beta-catenin and HIF-1 promotes cellular adaptation to hypoxia *Nat Cell Biol*, 9(2), 210-7 (2007)

93. N Ninov, D Hesselson, P Gut, A Zhou, K Fidelin and D Y Stainier: Metabolic regulation of cellular plasticity in the pancreas. *Curr Biol*, 23(13), 1242-50 (2013)

94. C Chen, Y Liu, R Liu, T Ikenoue, K L Guan and P Zheng: TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. *J Exp Med*, 205(10), 2397-408 (2008)

95. C Chen, Y Liu and P Zheng: The axis of mTOR-mitochondria-ROS and stemness of the hematopoietic stem cells. *Cell Cycle*, 8(8), 1158-60 (2009)

96. P Carmeliet, Y Dor, J M Herbert, D Fukumura, K Brusselmans, M Dewerchin, M Neeman, F Bono, R Abramovitch, P Maxwell, C J Koch, P Ratcliffe, L Moons, R K Jain, D Collen and E Keshert: Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature*, 394(6692), 485-90 (1998)

Abbreviations: AMPK: AMP-activated Kinase, ESC: Embryonic Stem Cell HSC: Hematopoietic Stem Cell, HIF: Hypoxia-Inducible Factor, NSC: Neural stem cell, ROS: Reactive Oxygen Species, SIRT: Sirtuin.

Key Words: Metabolism, Stem Cells, Cell Cycle, Self-Renewal, Review

Send correspondence to: Anna-Pavlina Haramis, Institute of Biology (IBL), Leiden University, Sylvius Laboratory, Sylviusweg 72, 2333 BE Leiden, The Netherlands, Tel: 31715274782, Fax: 31715275088, E-mail: a.haramis@biology.leidenuniv.nl