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Review
Cell cycle and metabolism

Cycling through metabolism

Victor Aguilar[1], *Lluis Fajas*[1]*

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Since the discovery of cyclins, the role of cell cycle regulators in the control of cell proliferation has been extensively studied. It is clear that proliferation requires an adapted metabolic response of the cells; hence the regulation of cell cycle must be linked to metabolic control. While at a much slower pace, the impact that the activities of cell cycle regulators such as cyclins, cyclin dependent kinases or E2F factor, transcription factor have on cell metabolism are also being uncovered. Here we will focus on recent data implicating cell cycle regulators in metabolic control, with particular attention to studies performed using mouse models. Furthermore, we will discuss the possible relevance of these findings in the context of metabolic disorders such as obesity or diabetes.

INTRODUCTION

Cell growth (i.e. increase in cell mass) and proliferation (i.e. increase in cell number) are tightly controlled by growth factors in multicellular organisms. In the presence of excess nutrients, growth factors activate signalling cascades that trigger nutrient uptake and use. Most signalling cascades initiated upon activation of growth factor receptors, including the rat sarcoma oncogene (Ras), myelocytomatosis gene (Myc) or serine/threonine protein kinase B (PI3K/Akt) pathway ultimately converge on activation of cell cycle regulators, notably the cyc/cyclin dependent kinase (CDK)-pRB-E2F pathway (Box 1, Fig 1). In most cells, the entry into S-phase depends on the activation of the G1 cyclins/cdks and the retinoblastoma protein pRB-E2F pathway that controls the G1/S transition of the cell cycle. Cdks are serine/threonine kinases that work in complexes with different types of cyclins to phosphorylate the retinoblastoma family of tumour suppressor proteins (pRB) mediating the commitment of the cells to enter cell cycle in response to external stimuli (reviewed in Ortega et al, 2002). E2F transcription factors are the effectors of this pathway and they control the expression of genes involved in cell cycle progression, apoptosis and deoxyribonucleic acid (DNA) synthesis (for review see Attwooll et al, 2004). E2F activity is the result of the heterodimerization of two proteins belonging to the E2F family (E2F1 to 6) and the dimerization partner (DP) family (DP1 and 2), respectively (Dyson, 1998; Gaubatz et al, 1998). When bound to DNA, this heterodimeric complex exists either as free E2F/DP, or forms a larger complex that contains a member of the retinoblastoma protein family (pRB, p107, p130). E2F complexes can activate (free heterodimers) or repress (large complexes) the transcription of E2F-responsive genes. Such repression is mediated through the recruitment of histone deacetylases, which interact with proteins of the pRB family (reviewed in van den Heuvel & Dyson, 2008). From a canonical point of view the cyc/CDK-pRB-E2F pathway drives cell-cycle progression and division, but as will be discussed here, this pathway is also implicated in the metabolic adaptive response triggered by growth factors.

To support proliferation, the cellular metabolism is directed towards an increased production of energy in order to meet anabolic needs, such as protein, lipids and nucleotide synthesis. In quiescent cells, glucose is primarily metabolized to carbon dioxide by oxidation of glycolytic pyruvate in the mitochondrial tricarboxylic acid cycle. Metabolism in proliferating cells differs from quiescent cell metabolism by higher rates of glycolysis, lactate production and biosynthesis of lipids and other macromolecules (DeBerardinis et al, 2008). Although the shift towards an increased glycolytic flux may appear less efficient in terms of adenosine tri phosphate adenosine tri-phosphate (ATP) production than metabolizing glucose through oxidative phosphorylation, a high glycolytic rate offers several advantages. First, when nutrient availability is not limiting, high glycolytic fluxes can produce more ATP than the oxidative phosphorylation (Guppy et al, 1993). Second, glucose degradation in glycolysis provides intermediates needed for anabolic biosynthesis. Such metabolic adaptation observed in proliferating cells is

IRCM, Institut de Recherche en Cancérologie de Montpellier, INSERM, U896, Université de Montpellier1, CRLC Val d'Aurelle Paul Lamarque, Montpellier, France.

***Corresponding author:** Tel: +33 4 67 61 24 28; Fax: +33 4 67 61 23 33; E-mail: lluis.fajas@inserm.fr

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exacerbated in cancer cells, where high rates of unregulated proliferation take place. Indeed cancer cells have a particular metabolism (reviewed in Vander Heiden et al, 2009) that specifically blocks oxidative glycolysis, resulting in accumulation of pyruvate and further conversion to lactate. This metabolic shift termed Warburg effect is now considered a fundamental hallmark of cancer.

Undoubtedly, there is a cross talk between the cell cycle and metabolic control. Factors such as nutrition, stress or physical exercise are signalled and translated into proliferative stimuli but the cell does not usually proliferate in response to these stimuli. Instead, it activates a metabolic response, such as glucose and fatty acid utilization or insulin secretion. Modulation of the activity of the factors that trigger a metabolic pathway in response to proliferative stimuli might therefore open up new perspectives in the control of metabolic diseases such as type II diabetes, obesity and cancer.

In this review we will discuss recent literature describing how cell cycle regulators control distinct metabolic pathways and diseases (Fig 2). We will focus mostly on results obtained with transgenic, mutant, knock-out or knock-down mice summarized in Table 1.

CELL CYCLE REGULATORS IN THE CONTROL OF METABOLIC PROCESSES

Activation or inactivation of particular metabolic pathways in any cell type is dependent on the energetic status of the cell. We will discuss in this section the participation of cell cycle regulators in whole energy homeostasis. As discussed above, cells require a switch from oxidative to glycolytic metabolism in order to proliferate. While cell cycle regulators play a central role in the control of proliferation of the cells, increasing evidence also points to their role in facilitating this metabolic switch and channelling the products of catabolism (*e.g.* glycolysis) towards biosynthetic processes such as *de novo* fatty acid or protein synthesis (Fig 3). Inhibition of oxidative phosphorylation certainly contributes to this specific channelling. Supporting a role of cell cycle regulators in the modulation of oxidative metabolism is the observation that mitochondrial function is increased in cells with non-functional pRB (Hansen et al, 2004). Moreover, the implication of pRB in energy expenditure was also demonstrated in mice. Mice with specific deletion of pRB in adipose tissue show increased mitochondrial number and increased expression of several genes involved in mitochondrial function in adipocytes, suggesting that pRB represses mitochondrial activity (Dali-Youcef et al, 2007). Consistently E2F1, a transcriptional repressor when complexed to pRB, has also been shown to negatively regulate mitochondrial biogenesis and function. Inhibition of E2F1 activity by ribonucleic acid (RNA) interference (RNAi) in HeLa cells results in increased expression of genes implicated in mitochondrial biogenesis and function, such as mitochondrial topoisomerase I (Goto et al, 2006). Furthermore, recent results in our laboratory show that E2F1 directly modulates the expression of a long list of genes implicated in mitochondrial

Glossary

Adipocyte
Main cell type that forms adipose tissue (fat). Adipocytes store fat in form of triglycerides and release fatty acids during starvation or fasting periods. Adipocytes also secrete hormones and cytokines that signal to distant tissues of the body.

Apoptosis
Programmed cell death characterized by shrinkage of the cell, condensation of chromatin and fragmentation of the cell into membrane-covered bodies that are eliminated by phagocytosis.

β-cells
A type of endocrine cell in the pancreas that secretes insulin.

Cyclin/CDK-pRB-E2F
Cell cycle regulators that regulate the expression of genes implicated in cell cycle during cell division. Cell cycle independent functions have been also demonstrated for these factors.

DNA replication
A process consisting in the copy of the DNA of the cell, using the double-stranded DNA as a template before cell division takes place.

Glucose intolerance
A condition characteristic of insulin resistance, in which insulin is not capable to facilitate glucose uptake and utilization in peripheric tissues, such as muscle, or adipose tissues.

Glycolysis
Conversion of glucose to pyruvate through a metabolic pathway that involves several successive reactions thereby generating ATP and NADH.

Insulin
A hormone secreted by pancreatic β-cells, which is central to regulating energy and glucose metabolism in the body.

Mitosis
Phase of cell division in which the newly replicated DNA, organized in chromosomes, separates in the nuclei into two identical sets.

PPARγ
A hormone nuclear receptor that regulates adipocyte differentiation and the transcriptional response leading to efficient energy storage. PPARγ ligands are insulin sensitizing drugs used to treat type 2 diabetes. PPARγ is also a general transcriptional controller of processes, such as cell cycle carcinogenesis, inflammation, atherosclerosis and immunomodulation.

Quiescent cells
Cells at a postmitotic state in which they do not divide or prepare to divide.

Tricarboxylic acid cycle
Also known as the citric acid cycle or the Krebs cycle, is a series of enzyme-catalyzed chemical reactions of central importance in all living cells that use oxygen for cellular respiration.

Type II diabetes
A metabolic medical condition characterized by insulin resistance and high blood glucose levels.

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BOX 1: Molecular regulation of cell cycle

Over the last few decades, major advances have been made in understanding the machinery controlling cell progression through the cell cycle, in particular identifying cell cycle regulatory proteins (see Fig 1, below). These proteins are divided in families comprising cyclins (cyc), cyclin dependent kinases (CDKs), cyclin dependent kinases inhibitors (CKIs), the pocket protein retinoblastoma family and the E2F family of transcription factors. From a general point of view, quiescent cells enter cell cycle by mitogenic signal induction. The G1 cyclin dependent-kinases transduce then mitogenic signals to the core cell cycle machinery (Sherr & Roberts, 1999). Cell cycle is accompanied by an intensive increase in cell mass and both process (cell growth and cell proliferation) will lead to determination of cell size.

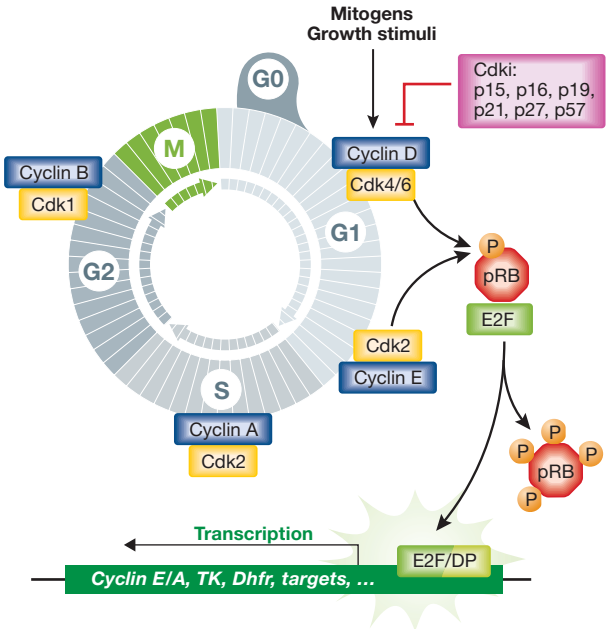

Figure 1. Molecular Regulation of cell cycle. Mitogen-induced signal transduction pathways activates the cyclin D-CDK complex early in G1 phase by: (i) induction of cyclin D transcription, translation, stability, (ii) assembly with CDK partners and (iii) import of the holoenzyme to the nucleus. All these process induce an accumulation of active cyclin D-CDK complex during G1 phase. Activation of these complexes leads to partial inactivation by phosphorylation of the pocket proteins, comprising Rb, p107 and p130. Inactivated pocket proteins will release E2Fs transcription factor activity and thus, allow the expression of E-type cyclins, necessary for G1/S transition. CDKs are further activated during cell cycle by A-type cyclins to drive transition form S phase to mitosis, a period known as G2 phase. The activities and functions of cyclin/Cdk complexes are regulated by CKIs both under normal as well as extreme conditions, such as stress, DNA damage and others. There are two families of CKIs. The first includes the INK4 proteins that specifically bind and inhibit the catalytic subunits of CDK4 and CDK6. The INK4 family includes four members p16INK4a, p15INK4b, p18INK4c and p19INK4d. The locus that encodes p16INK4a also directs the expression of a second unrelated protein designated as p19ARF. The second family of CKIs is termed Cip/Kip and has a broad action of inhibition including the activities of both cyclins and CDKs. This class includes p21Cip1, p27Kip1 and p57Kip2.

function (Fajas, unpublished work). These studies imply that the pRB-E2F1 complex negatively regulates energy expenditure and mitochondrial activity through the modulation of the transcription of genes implicated in these processes. Paradoxically, studies using cyclin D1 antisense transgenic mice show that, similar to pRB and E2F1, cyclin D1 inhibits mitochondrial activity through the repression of genes governing glycolysis and mitochondrial action (Sakamaki et al, 2006). This is at odds with the classical view of cyclin function, which is promoting pRB phosphorylation. If this was the case, then cyclin D1 inhibition should result in hypophosphorylated pRB, and therefore sustained repression of genes implicated in mitochondrial function. Instead, cyclin D1 inhibition results, not in repression, but in the activation of these mitochondrial genes. This apparent paradox can however be explained by E2F1-pRB-independent cyclin D1 effects. Indeed, cyclin D1 represses the activity of nuclear respiratory factor-1 (NRF-1), one of the master genes that drive the expression of several mitochondrial genes. This repression is dependent on the cdk activity, but independent of pRB (Wang et al, 2006).

In addition to mitochondrial activity, E2F1 has also been implicated in the control of glycolysis. Strikingly, increased aerobic glycolysis is a hallmark of highly proliferating cells, which have augmented E2F activity. E2F1 loss in mice improves muscle glucose oxidation, as a result of decreased pyruvate dehydrogenase kinase 4 (PDK4) expression. PDK4 is a critical nutrient sensor and inhibitor of glucose oxidation, through phosphorylation of pyruvate dehydrogenase (Sugden & Holness, 2006). Induction of PDK4 transcription by E2F1 then blunts glucose oxidation (Hsieh et al, 2008). In line with this observation, cyclin D1 inhibits the activity of the promoter of Hexokinase II (HKII), the enzyme catalyzing the first steps of glycolysis in epithelial and fibroblastic cells (Sakamaki et al, 2006). Furthermore, transgenic mice expressing antisense cyclin D1 in the mammary gland showed increased RNA and protein levels of HKII and pyruvate kinase in this tissue (Sakamaki et al, 2006). Taken together these studies demonstrate that cell cycle regulators are implicated in the regulation of glucose homeostasis via the inhibition of oxidative glycolysis. Participation of cell cycle regulators in the control of energy homeostasis is represented in Fig 4.

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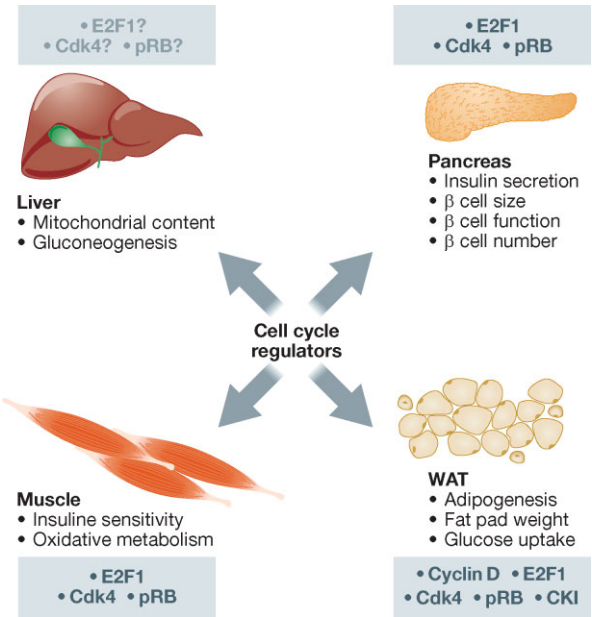

Figure 2. Schematic representation of the participation of cell cycle regulators in the function of four main metabolic tissues. In pancreas, E2F1 regulates the expression of genes, such as Kir6.2 implicated in insulin secretion, as described in the text. In addition, E2F1, cdk4 and pRB participate in the control of β-cell growth and replication. Impairment of the function of these cell cycle regulators in pancreas often results in diabetes in mice. White adipose tissue (WAT) is another important metabolic tissue that controls whole body lipids and glucose homeostasis. Cyclins D, cdk4, E2F1 and pRB, as well as CKI have been directly implicated in adipose tissue differentiation and function. This is described in the text in the section concerning obesity. Finally, recent literature implicates E2F1, cdk4 and pRB in the oxidative metabolism of muscle. Participation of these factors in liver is likely, although not yet demonstrated.

CELL CYCLE REGULATORS IN THE CONTROL OF METABOLIC DISEASES

The roles that cell cycle regulators play in both cellular proliferation and metabolism imply that they are also relevant in the development of pathologies associated with such processes. The involvement of cell cycle regulators in uncontrolled proliferation and cancer development is obvious but as discussed previously, cancer development is also associated with major alterations of the metabolism of the cells. Enhanced glycolysis and de novo fatty acids synthesis are indeed characteristic features of cancer. On the other hand, metabolic abnormalities are at the origin of several physiological dysfunctions or pathologies such as obesity and diabetes, major morbidity and mortality factors in western societies. Obesity is characterized by abnormal fat accumulation, and most obese individuals become insulin resistant and type II diabetics. Type 2 diabetes is characterized by hyperglycemia which is contributed to by both insulin resistance and islet β-cell dysfunction. While insulin resistance may be present well before the development of any clinical symptoms, it is the decline in β-cell function that is responsible for the transition of an individual from impaired glucose tolerance to diabetes (Kahn et al, 2006).

Diabetes

Many studies in flies, mice, worms and plants have suggested that cyclin D/Cdk4 complexes function not only as cell cycle regulators but also as metabolic drivers. A first observation supporting this hypothesis came from phenotypic characterization of mice lacking Cyclin D1 or Cdk4 that were substantially smaller than wild type littermates (Fantl et al, 1995; Rane et al, 1999). Cyclin D knock out mice models revealed an intricate situation where different members of the family can partially compensate for each other depending on tissue expression and roles. The study of the participation of cyclins in cell growth and proliferation is thus complicated by the potential functional redundancy of the three D type cyclins. Some individual D cyclins show, however marked metabolic phenotypes, first demonstrated for cyclin D2 in pancreatic tissue. Proliferation of β-cells is a key mechanism to maintain postnatal β-cell mass

Table 1. Major metabolic phenotypes of cell cycle regulators mutant mice

Gene	Metabolic phenotype	References
Cyclin D1	Small body size	Fantl et al (1995); Sicinski et al (1995)
Cyclin D2	Abnormal development of pancreatic β-islets. Glucose intolerance	Georgia & Bhushan (2004)
Cyclin D3	Impaired adipogenesis. Insulin sensitivity	Sarruf et al (2005)
CDK4	Small body size. Abnormal development of pancreatic β-islets and adipose. Diabetes	Abella et al (2005); Rane et al (1999)
CDK5	Decreased insulin secretion. Increased glucose uptake	Lalioti et al (2009); Okada et al (2008); Wei et al (2005)
CDK6	Small body size (females)	Malumbres et al (2004)
CDK2	Small body size	Ortega et al (2003)
CDK9	Adipogenic factor	Iankova et al (2006)
E2F1	Small pancreatic β-cell. Decreased insulin secretion. Impaired adipogenesis	Annicotte et al (2009); Fajas et al (2004)
pRb	Adipogenic factor. Control of energy expenditure and mitochondrial content	Fajas et al (2002a)
p107	Small body size. Impaired adipogenesis. Increased mitochondrial content	LeCouter et al (1998a); Scime et al (2005)
P18INK4c	Increased body size and overgrowth of some organs	Franklin et al (1998); Latres et al (2000)
P27Kip1	Increased body size. Increased adipose tissue, muscle and β-cell mass	Lin et al (2003); Missero et al (1996); Naaz et al (2004); Uchida et al (2005)
P21	Increased adipose tissue mass	Naaz et al (2004)

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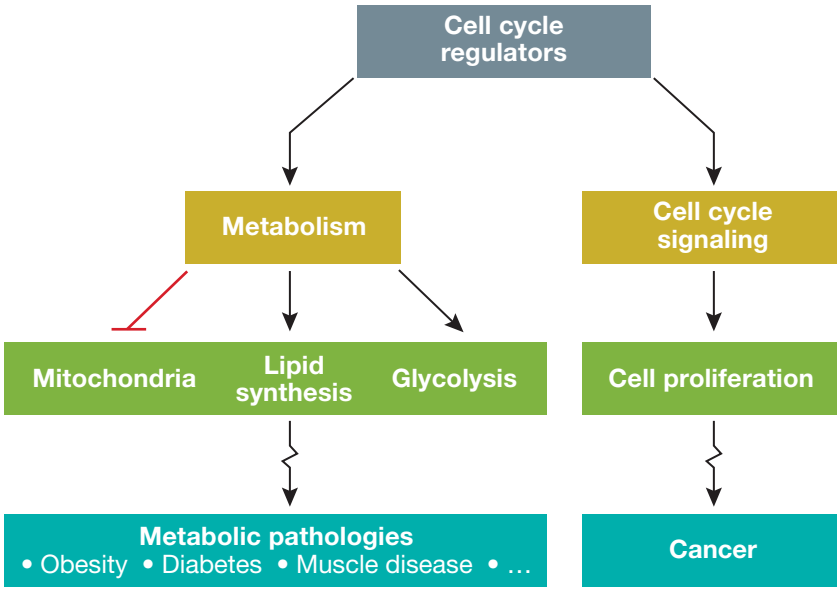

Figure 3. General overview of the dual role of the cdk-pRB-E2F complex in the regulation of both metabolism and cancer. Under the proper stimuli cell cycle regulators trigger proliferation of the cells. This is accompanied by an adapted metabolic response that includes inhibition of oxidative metabolism, increased glycolysis and lipid synthesis. This is particularly relevant for cancer cells, which require these precise changes in metabolism. Under particular conditions, however, when cells are not 'primed' to proliferate, cell cycle regulators are pure metabolic regulators, for instance in response to fasting or hormonal induction. Deregulation of this system can therefore result in the development or progression of metabolic pathologies such as obesity or diabetes.

(Kassem et al, 2000; Meier et al, 2008), and it is the primary mechanism for β-cell regeneration (Dor et al, 2004; Georgia & Bhushan, 2004; Teta et al, 2007). It is now well established that pancreatic β-cells are able to replicate, albeit the origin of newly formed islets remains controversial. Cyclin D2$^{-/-}$ mice showed decreased postnatal β-cell mass and deregulated glucose homeostasis, glucose intolerance as well as diabetes (Georgia & Bhushan, 2004). Although cyclin D1$^{+/-}$ mice were normal, life-threatening diabetes developed in 3-month-old cyclin D1$^{+/-}$ D2$^{-/-}$ mice as β-cell mass decreased after birth (Kushner et al, 2005). Hence, cyclins D2 and D1 are essential for β-cell expansion in adult mice. A deeper analysis of the data presented in one of these studies (Georgia & Bhushan, 2004) allows further interpretations. Cyclin D2$^{-/-}$ mice have fasting insulin levels similar to cyclin D2$^{+/+}$ mice, however upon glucose stimulation, the levels of insulin do not increase in cyclin D2 whereas they double in cyclin D2$^{+/+}$ mice (Georgia & Bhushan, 2004). This suggests that in addition to the control of β-cell mass, cyclin D2 also participates in the control of β-cell function and is fully consistent with the function of E2F1 in these cells, as will be discussed later.

Since CDK4 is the major partner of D-type cyclins and in light of this cyclin's role (described above), it is not surprising that the most marked phenotype of mice lacking CDK4 is reduced body size and insulin-deficient diabetes due to a severe decrease in pancreatic β-cell growth (Rane et al, 1999; Tsutsui et al, 1999). Re-expression of CDK4 in β-cells of the Cdk4$^{-/-}$ mice restores cell proliferation and normoglycemic condition. However, re-expression of Cdk4 does not rescue small body size suggesting that this phenotype is not due to endocrine defects, secondary to decreased insulin levels, but rather cell autonomous in peripheric tissues. As regulation of cell growth is dependent of cell metabolism, this phenotype suggests that cdk4 participates in metabolic control. These results are summarized in Fig 5.

Cdk6 is another partner of cyclin D but cdk6 knockout mice do not display any metabolic phenotype, except that female mice are smaller (Malumbres et al, 2004). The mild phenotype of this knockout mice suggest that the functions of Cdk6 are mainly compensated by Cdk4, or by Cdk2 which can interact with D-type cyclins in a Cdk4/Cdk6 double knockout background (Malumbres et al, 2004).

Cdk5, an atypical CDK family member with no known cyclin partner has also been implicated in the regulation of insulin secretion. Mice lacking p35, a CDK5 activator, have increased insulin secretion in response to elevated glucose (Wei et al, 2005). Chemical inhibition of cdk5, or p35 deficiency resulted in increased insulin secretion in isolated β-cells. The effects of p35 deficiency were mediated by cdk5, since inhibition of cdk5 had no effect on insulin secretion in p35$^{-/-}$ β-cells. The closure of K_{ATP} channels in response to glucose stimulation of pancreatic β-cells is followed by Ca^{++} influx through the L-VDCC channels, a required event in the insulin secretion process. Interestingly, it was shown in this study that the L-VDCC channel was not inhibited in p35$^{-/-}$ or cdk5-inhibited β-cells (Wei et al, 2005). Furthermore, the authors concluded that the α1C subunit of L-VDCC was a phosphorylation inactivating target of cdk5. Two other reports have implicated CDK5 in the regulation of glucose homeostasis in adipocytes. Okada et al reported that CDK5 phosphorylation of TC10alpha (a Rho family GTPase) increases GLUT4 translocation and hence glucose import in adipocytes (Okada et al, 2008). GLUT4 is a glucose transporter whose activity is markedly regulated by insulin in muscle and adipose tissue cells (Huang & Czech, 2007). The translocation of GLUT4 was also increased by CDK5 phosphorylation of E-Syt1 (a 5C2-domain protein related to synaptotagmins). Phosphorylation of E-Syt1 leads to its increased association with GLUT4 and increased glucose uptake (Lalioti et al, 2009).

Since cdk activity is regulated by cdk inhibitors, it is not surprising that these proteins also have a role in the control of

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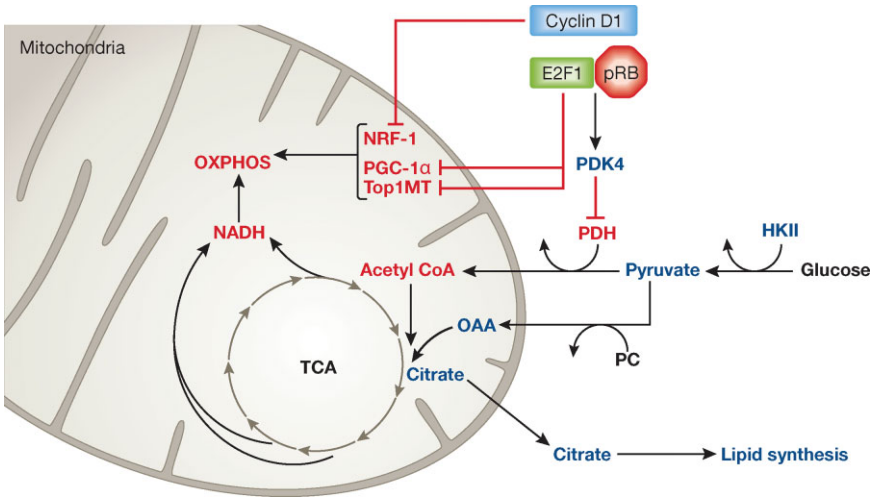

Figure 4. Participation of cell cycle regulators in energy homeostasis. E2F1 has two distinct roles. First, it regulates the expression of PDK4, which inhibits the activity of PDH, and therefore blocks the conversion of pyruvate in to acetyl CoA. Second, associated to pRB E2F1 modulates the expression of key genes implicated in mitochondrial biogenesis or oxidative phosphorylation (OXPHOS), such as Top1MT or PGC-1α. Overall, E2F1 negatively regulates mitochondrial function. Cyclin D1 has an E2F1-independent role in the control of oxidative metabolism. It directly modulates the activity of the transcription factor NRF-1 thereby inhibiting OXPHOS. In addition cyclin D1 facilitates the expression of HKII facilitating glycolysis. Concomitant increase in glycolysis and blockade of OXPHOS may result in the accumulation of TCA intermediates that leave the TCA cycle and the mitochondria in order to provide substrates for biosynthetic processes, such as lipid synthesis. In red and blue color are indicated the factors or processes that are, respectively, inhibited or activated by cell cycle regulators. NRF-1, nuclear respiratory factor-1; PGC-1α, PPAR gamma coactivator-1, Top1MT, mitochondrial topoisomerase 1; PDK4, pyruvate dehydrogenase kinase-4, PDH, pyruvate dehydrogenase; HKII, hexokinase II; TCA, tricarboxylic cycle.

glucose homeostasis. Consistently, insulin sensitivity and secretion was not changed in p21$^{-/-}$ mice under chow diet, but these mice displayed improved insulin resistance under high fat high sucrose (HFHS) diet when compared to WT mice (Inoue et al, 2008). The authors of this study postulated an improvement of insulin resistance in peripheral tissue, as β-cell function was normal in these mice. In contrast, p27 was shown to participate in β-cell mass determination (Uchida et al, 2005). Deletion of this gene increased islet mass and insulin secretion and prevented hyperglycemia in diabetic mice models. Since the metabolic effects of p21 and p27 are most likely mediated by the inhibition of cdks, differences in cdk activity in these mice could explain the distinct phenotypes.

Well-established E2F target genes include effectors of DNA replication, mitosis, DNA repair, apoptosis, differentiation and development (Dimova & Dyson, 2005). In addition, disruption of E2f1 also highlighted its role in the regulation of glucose homeostasis. *E2f1*$^{-/-}$ mice have decreased pancreatic size, as a result of impaired postnatal pancreatic growth. On the other hand, E2F1 is also highly expressed in non-proliferating pancreatic β-cells, which suggests that besides controlling β-cell number, the protein has a role in pancreatic β-cell function (Fajas et al, 2004). Indeed, we recently demonstrated both *in vitro* and *in vivo* that E2F1 directly regulates the expression of *Kir6.2*, a key component of the K_{ATP} channel involved in the regulation of glucose-induced insulin secretion in pancreatic β-cells (Fig 5). Expression of *Kir6.2* is lost in pancreas of *E2f1*$^{-/-}$ mice, resulting in insulin secretion defects in these mice. E2F1 transcriptional activity is regulated by glucose and insulin through the CDK4-dependent inactivation of the pRB protein (Annicotte et al, 2009). Interestingly, E2F1$^{-/-}$ mice are not diabetic. They have dramatically increased insulin sensitivity, secondary to decreased white adipose tissue. These effects are specific for E2F1, whereas the expansion of β-cells can be compensated by E2F2. Consistently, E2f1/E2f2 double mutant mice display insulin deficient diabetes (Iglesias et al, 2004; Li et al, 2003).

Both the proliferative and metabolic effects of insulin on β-cells appear to be mediated by an increase of CDK4 activity and subsequent E2F1 transcriptional activity (Fig 5). This further suggests that both cell proliferation and metabolic responses are intimately linked, and regulated by the same upstream factors. In agreement with this hypothesis, caAKT^{Tg} transgenic mice that specifically overexpress a constitutively active form of Akt in β-cells show higher β-cell mass and proliferation rate with increased β-cell size. Interestingly, these effects were abrogated when mice were bred in a cdk4$^{-/-}$ genetic background, demonstrating that AKT induces β-cell proliferation in a CDK4-dependent manner (Fatrai et al, 2006). The pathway that leads to cdk4-RB-E2F1 activation is however still unknown. The mTOR pathway is central to transduction of nutrient availability signals (Wullschleger et al, 2006). Strikingly, the metabolic phenotype of E2F1$^{-/-}$ mice is reminiscent of the phenotype of mice carrying inactivating mutations in the mTOR substrate S6K1 (Aguilar et al, 2007; Um et al, 2004). Both E2F1$^{-/-}$ and S6K1$^{-/-}$ mice show impaired metabolism in pancreatic β-cells, adipose tissue, muscle and likely in other tissues with metabolic functions, such as liver or brown adipose

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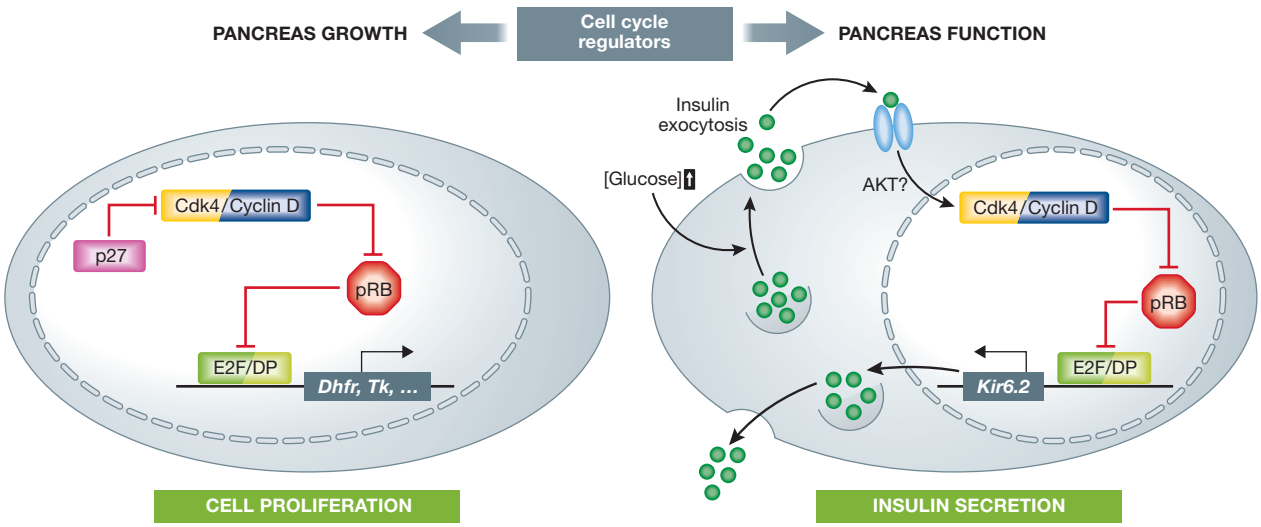

Figure 5. Schematic representation of the dual role of cell cycle regulators in pancreatic growth and function. Proliferation of β-cells or precursors is regulated by the classical cdk-pRB-E2F pathway. In this way, proliferative stimuli activates cdk4 that phosphorylates the retinoblastoma protein pRB, thereby releasing E2F activity which regulates the expression of genes, such as TK or DHFR implicated in cell division. In this context, CKIs, such as p27 inhibit cdk4 action and have a negative role in β-cell expansion. In addition to the control of β-cell proliferation cell cycle regulators participate in the insulin secretion process of β-cells. Under glucose-stimulated insulin signalling the cyclin D/cdk holoenzyme phosphorylates pRB and facilitates the expression of Kir6.2 gene under the control of E2F1. Expression of the ATP-dependent Kir6.2 potassium channel is required for insulin secretion.

tissue. This suggests a cross talk between the mTOR-S6K and cdk4-RB-E2F1 pathways and we speculate that the mTOR-S6K pathway controls metabolic processes, at least in part through regulation of the cdk4-pRB-E2F1 activity. The transcriptional program controlled by the CDK4-pRB-E2F1 pathway in the endocrine pancreas remains unknown too as do the signals and molecular mechanisms that underlie the particular contribution of E2F1, CDK4 and pRB in the metabolic *versus* proliferative response.

Obesity

Genetic, nutritional and environmental factors are key determinants of obesity, which is characterized by increased adipose tissue mass. Growth of adipose tissue is the result of both hypertrophy (increase in size) and hyperplasia (increase in number) of adipocytes. It is likely that hypertrophy is the initial event that occurs during the development of obesity. However, adipocytes cannot grow and accumulate lipids indefinitely. Increasing adipocyte number accounts, therefore for the adipose tissue expansion observed in obesity. Hyperplasia is a direct result of the generation of new adipocytes from precursor cells, a process that we call adipogenesis or adipocyte differentiation.

Adipogenesis is a particular system, which involves two major events: preadipocyte proliferation, and adipocyte differentiation (reviewed in Fajas, 2003). Both processes are tightly regulated and the cross talk that exists between them determines the final adipocyte phenotype of the cell. Although *in vivo* studies elucidating the molecular mechanisms taking place during adipogenesis are still limited, cellular *in vitro* models, such as the 3T3-L1 preadipocyte cell line have been instrumental to study this process. Re-entry into cell cycle of growth arrested preadipocytes following hormonal induction is a required initial event occuring during adipogenesis (Fig 6). After several rounds of clonal expansion, cells arrest proliferation again and undergo terminal adipocyte differentiation. Increased E2F activity has been observed during the initial steps of this process (Richon et al, 1997). Consequently, expression of classical E2F1 target genes implicated in cell proliferation, such as cyclin D1, cyclin E or DHFR is increased in the early stages of adipogenesis (Reichert and Eick, 1999). Interestingly, blocking cell cycle re-entry with a DNA synthesis inhibitor, prevents adipocyte differentiation, suggesting that an active cell cycle machinery is required for the differentiation process (Richon et al, 1997). Similar results were obtained when degradation of p27 was prevented using a protease inhibitor (Patel & Lane, 2000). As a consequence of p27 protein acumulation, cell cycle re-entry was blocked and thus, differentiation of preadipocytes was inhibited (Patel & Lane, 2000). In addition to regulate cell cycle re-entry in the clonal expansion phase, we demonstrated an independent role of E2F1 in adipogenesis through regulation of the expression of peroxisome proliferator-activated receptor-γ (PPARγ), which is the master regulator of adipocyte differentiation (Fajas et al, 2002b). Using a combination of *in vivo* experiments, with knock-out and chimeric animals, and *in vitro* experiments we demonstrated that the absence of E2F1 impairs, whereas its overexpression stimulates adipogenesis. E2Fs represent hence the link between proliferative signalling pathways, triggering clonal expansion and terminal adipocyte differentiation through regulation of PPARγ expression.

pRB is a major regulator of E2F1 activity, and its participation in adipogenesis could be therefore considered as obvious. The role of the pRB protein family in adipose tissue development is,

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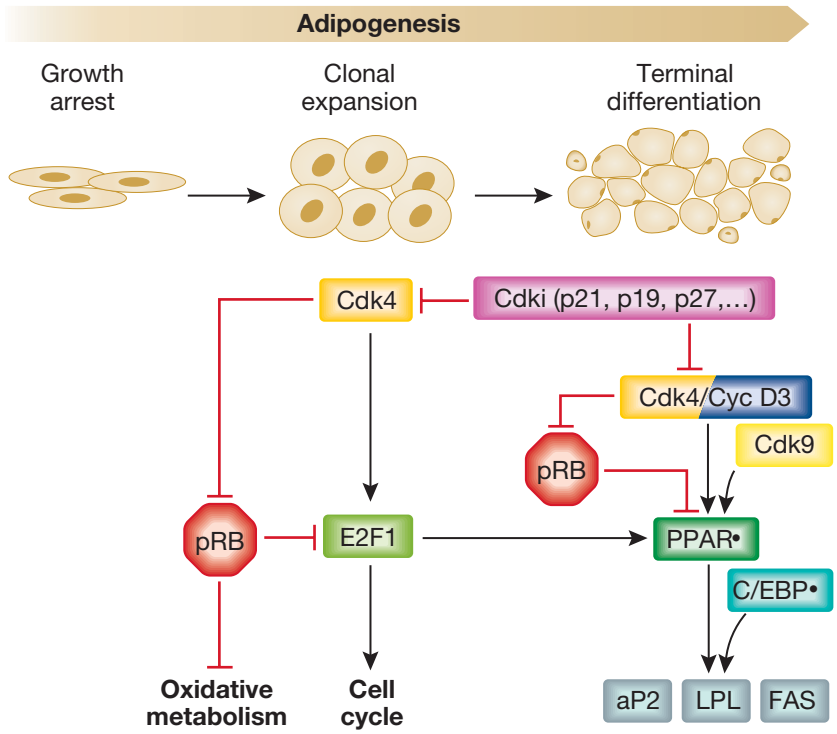

Figure 6. Participation of cell cycle regulators in the different stages of adipogenesis. During the clonal expansion phase of adipocyte differentiation E2F1 regulates the expression of genes implicated in the entry of the cells into cell cycle. In addition, E2F1 regulates, at this stage the expression of the master regulator of adipogenesis, PPARγ. pRB represses E2F1 activity, whereas cdk4 represses pRB, and therefore activates E2F1. In terminal differentiation cyclins and cyclin-dependent kinases still participate in the biology of adipocytes through regulation of the activity of PPARγ in an E2F-independent manner. pRB can also directly repress PPARγ activity at this stage.

however more complex than expected. $pRb^{-/-}$ mice die at embryonic days 13–14 due to extensive apoptosis in central and peripheral central nervous system, lack of differentiation of skeletal muscle, lens and cells of the erythropoietic lineage (Lee et al, 1992). Most of the metabolic functions of pRB were thus inferred from conditional knockout mice. We established that pRB has an inhibitory role at early stages of adipocyte differentiation, through the formation of a complex including HDAC3 that inhibits PPARγ-dependent gene expression and adipocyte differentiation (Fajas et al, 2002a). In addition, mice with a conditional deletion of pRB in adipose tissue have increased mitochondrial activity resulting in an increased energy expenditure, which protects them from diet-induced obesity (Dali-Youcef et al, 2007). These apparently opposite roles of pRB in adipogenesis (pRb acts to inhibit PPARγ and adipogenesis but $pRb^{-/-}$ mice have decreased adipose tissue mass) can be reconciled as during early stages of adipocyte differentiation, cells need to exit cell cycle. In this withdrawal stage pRB plays a major role, and positively regulates adipogenesis (Richon et al, 1997) in a PPARγ-independent manner. Later on during differentiation, pRB represses PPARγ activity (Fajas et al, 2002a) but the net result is still decreased fat mass in the absence of pRB (Dali-Youcef et al, 2007). A similar phenotype was observed in mice lacking p107, another member of the pocket proteins family. These mice are leaner than $p107^{+/+}$ littermates and have a decreased fat pad mass with an increased mitochondrial mass (LeCouter et al, 1998b; Scime et al, 2005).

Other cell cycle players that impact on metabolism and obesity are the cyclin/cdks proteins. Cyclin D3 disruption in mice results in protection from diet-induced obesity, reduced adipocyte size and increased sensitivity to insulin (Sarruf et al, 2005). Interestingly, the effects of cyclin D3 are independent of the control of proliferation or E2F activity. Cyclin D3 regulates adipose tissue mass through its direct interaction with PPARγ (Sarruf et al, 2005). On the other hand, cyclin D1 appears to have a negative role on adipogenesis as Fu et al reported that this cyclin negatively regulates PPARγ-mediated adipocyte differentiation through recruitment of HDACs to the promoter region of PPARγ target genes (Fu et al, 2005). In addition, cdk4 also participates in adipose tissue biology, not only through the control of the clonal expansion phase of adipogenesis but also through direct regulation of PPARγ activity (Abella et al, 2005). This is also the case for CDK9 that phosphorylates and activates PPARγ, consequently increasing adipogenesis (Iankova et al, 2006).

Pending issues

- Expression studies of cell cycle regulators, including cdk4, CKIs or E2Fs comparing normal subjects with pathological conditions, such as obesity or diabetes.
- Analysis of genetic mutations or polymorphisms of genes implicated in cell cycle control in obese or diabetic patients.
- Studies directed to the use of cdk4 inhibitors for the treatment of obesity.
- Study of the participation of other cyclins, such as cyclin E or cyclin A and cdks in metabolic processes.

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As the name implies, CKIs such as p21, p27 or p19 mediate the exit of the cell cycle through inhibition of cdk activity. Therefore, it was reasonable to expect an involvement of these proteins in adipose tissue development and function. Indeed, both p27 and p21 are important regulators of adipogenesis and loss of either of these CKIs in mice induces adipocyte hyperplasia (Naaz et al, 2004). Combined deletion of p27 and p21 in a double knockout mice induces an increase in adipocyte number, fat pad weights and obesity that exceeds those induced by the deletion of each single CKI, indicating that their roles are not redundant. There are however several discrepancies in the literature in what concerns the metabolic roles of these two CKIs. One study showed that there was no difference in fat depot weights of $p27^{-/-}$ *versus* $^{+/-}$ mice (Lin et al, 2003). Another study (Inoue et al, 2008), reported that the targeted disruption of p21 induced no difference in fat pad weight under normal diet, but that $p21^{-/-}$ mice fed a HFHS diet had a decreased fat weight. Other studies have shown, although not directly, the participation of p27 in fat development. Uchida et al showed that deletion of p27 results in increased fat mass in leptin receptor KO mice (Uchida et al, 2005). Since the highest differences in weight are observed in $p27^{-/-}$ mice and in the double $p21^{-/-}$ $p27^{-/-}$ mice, it is possible that p27 can partly compensate for the p21 deficiency in the single $p21^{-/-}$ mice and could underlie the observed discrepancies between the different models.

Cancer

One of the biochemical hallmarks of cancer cells are changes in metabolism. Warburg observed that tumour cells have a higher rate of glucose metabolism than their normal counterparts and preferentially use glycolysis instead of OXPHOS even under appropriate oxygen concentrations (Warburg, 1930, 1956a,b). Since this first observation, the 'aerobic' glycolysis switch has been detected in many tumour types and the evidence for a global metabolic reorganization concomitant to cancer progression is indisputable. Most tumour cells are characterized by higher rates of glycolysis, lactate production and macromolecules and lipids biosynthesis (Kroemer & Pouyssegur, 2008; Vander Heiden et al, 2009).

The cdk-pRB-E2F activity is commonly elevated in numerous human cancers, including glioblastoma, lung, ovarian, breast, stomach and colon cancers (Chen et al, 2009). It is clear that an increase in E2F activity contributes to uncontrolled proliferation of cancer cells but we argue that a specific role for this pathway in cancer cell metabolism cannot be ruled out. In fact, as discussed above the cdk-pRB-E2F pathway positively regulates lipid biosynthesis and glycolysis while it negatively regulates oxidative phosphorylation—the exact metabolic features of cancer cells. This strongly suggests that the cdk-pRB-E2F axis is a key regulator of cell metabolism in cancer cells, however additional studies are needed to prove this hypothesis.

CONCLUDING REMARKS

While the role of cell cycle regulators in the control of cell growth, proliferation and apoptosis has been extensively studied, there is less data on the metabolic effects of such factors. This is in spite of the fact that in a very large number of cases, mice deficient in such cell cycle regulators, present a metabolic defect rather than or in addition to a proliferation one. Cell cycle regulators coordinately regulate cell physiology, beyond their regulatory role in cell cycle. The fact that factors, such as E2F1, trigger both cell cycle progression and metabolic responses provides direct support for the hypothesis that these factors play a central role in coordinating the transition between cell proliferation and metabolism. Modulation of the activity of these factors might open up new perspectives in the control of metabolic diseases. Interestingly, some studies have already shown the potential interest of these analyses. Variants in the CDK4 and in the CDKN1C locus have been associated to type II diabetes, obesity and glucose metabolism in humans (Meenakshisundaram & Gragnoli, 2009; Meenakshisundaram et al, 2009; Nielsen et al, 2005). Larger populations, and a more systematic study using all cell cycle regulators are definitely required.

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