

# **Cell cycle regulators in the control of metabolism**

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## **Abstract**

We recently showed that the CDK4-pRB-E2F1 cell cycle regulators directly regulate the expression of *Kir6.2*, which is a key component of the  $K_{ATP}$  channel involved in the regulation of glucose-induced insulin secretion. There is enough evidence to indicate that the CDK4-pRB-E2F1 regulatory pathway is involved in general glucose homeostasis, and metabolism. In this article we discuss which are the metabolic implications of these findings.

## **Introduction**

More than 50 years ago Howard and Pele in 1951 first described the cell cycle and its phases. Cell cycle is controlled by many regulators mechanisms that permit or restrain its progression.<sup>1</sup> The main families of regulatory proteins that play key roles in controlling cell-cycle progression comprise the cyclins (cyc) family, their substrates, the cyclin dependent kinases (cdks), the different families of cdk inhibitors (CKI) and the pocket protein retinoblastoma (pRB) family. This is the essential network of the basic regulatory machinery that catalyses cell cycle transition via modulation of the E2Fs transcription factors family. Cyc/cdk play an important role in the translation of external signaling into transcriptional response, which is the final step of the regulatory cascade. Most of the cyc/cdk complexes have been implicated in the control of cell cycle progression, and ensure the transition through the cell cycle by the appropriate phosphorylation of specific targets, such as the retinoblastoma protein pRB.<sup>2</sup> Members of the E2F family of transcription factors E2F (E2F1-8) are downstream effectors of the cdk pathway and have a pivotal role in controlling cell-cycle progression.<sup>3</sup> E2Fs transcriptional activity is modulated by multiple mechanisms. The best known is the interaction with the pRB protein.<sup>4</sup> This association not only inhibits E2Fs transactivation but also actively represses transcription through the recruitment of chromatin remodeling factors such as histone deacetylases (HDACs) and methyltransferases. The formation of the pRB-E2F complex is dissociated by the phosphorylation of pRB by the cyclins/cdks complexes. Some E2Fs can then activate transcription. E2F activity is essential for proliferation through the transcriptional control of target genes, whose products are implicated in cell proliferation and DNA replication.<sup>5</sup> In addition to the control of proliferation, cell cycle regulators play critical roles in metabolic control, supporting an emerging role of the cell cycle machinery in metabolic processes. This is discussed below.

## **Cell cycle regulators in lipids and adipocytes metabolism**

Lipids metabolism not only consists on lipid synthesis and degradation, but also on lipid signaling, and fatty acid storage in adipose tissue. In this context, participation of cell cycle regulators has been described in adipose tissue development and function. We have previously demonstrated the participation pRB and E2Fs in metabolism. We have shown that E2Fs regulate adipogenesis through modulation of the expression of the nuclear receptor PPAR $\gamma$  which is established as a master regulator of adipogenesis.<sup>6</sup> Opposite to the effects of E2F1 on adipogenesis, we found that PPAR $\gamma$  and RB are part of a repressor complex

containing the histone deacetylase HDAC3, thereby attenuating PPAR $\gamma$ 's capacity to drive gene expression and adipocyte differentiation. Dissociation of the PPAR $\gamma$ -RB-HDAC3 complex by RB phosphorylation or by inhibition of HDAC activity stimulates adipocyte differentiation <sup>7</sup>. Similarly, we have shown that cyclin D3 <sup>8</sup>, cdk4 <sup>9</sup>, and cdk9 <sup>10</sup> are adipogenic factors with strong effects on whole metabolism through modulation of PPAR $\gamma$  activity. These are illustrative examples of how cell cycle regulatory proteins can also modulate metabolic processes. Most interestingly, this is not limited to the control of lipids, and adipocytes metabolism. Cell cycle regulators have been also involved in the control of glucose homeostasis.

### **Cell cycle regulators in glucose homeostasis**

The first member of the cell cycle regulator family to be implicated in the regulation of glucose homeostasis was cdk4. Cdk4  $-/-$  mice have defects on pancreatic cell growth and are diabetic, showing decreased insulin, and increased glucose levels. It was demonstrated that the loss of cdk4 results in the abrogation of insulin production, secondary to the decrease of the islet area by 13-15 fold. In summary, cdk4 $-/-$  mice have selective developmental defect in the endocrine islet compartment.<sup>11</sup> The same group generated mice expressing a mutant cdk4 protein that cannot be inactivated by the cell-cycle inhibitor p16INK4a (cdk4R24C). These mice showed, in contrast to cdk4 $-/-$  mice endocrine islet hyperplasia due to postnatal hyperproliferation of beta cells.<sup>11</sup> Furthermore, mice expressing cdk4R24C only in beta cells showed hyperplasia of the beta cell mass. These mice are more tolerant to glucose due to increased insulin secretion.<sup>12</sup> Similar to cdk4 $-/-$ , E2F1 $-/-$  mice also show impaired glucose homeostasis. E2F1 $-/-$  mice have overall reduction in pancreatic size, as the result of impaired postnatal pancreatic growth, and they present dysfunctional  $\beta$ -cells. Because of the disproportionate small pancreas and dysfunctional islets, E2F1 $-/-$  mice secrete insufficient amounts of insulin in response to a glucose load, resulting in glucose intolerance.<sup>13</sup> The phenotype of E2F1 $-/-$  mice, regarding glucose homeostasis is milder than cdk4 $-/-$  phenotype, likely because compensation by other E2F-family members. Indeed, E2F1/E2F2 double mutant mice develop insulin-deficient diabetes, showing strong reductions in the number and size of pancreatic islets.<sup>14</sup> Finally, cyc D1  $-/-$  and cyc D2  $-/-$  mice show identically decreased beta cell mass concomitant with decreased insulin levels.<sup>15</sup>

All these studies showed that cyclin D1, D2, CDK4 and E2Fs are implicated in glucose homeostasis and more particularly in insulin secretion. This phenotype is the result of

decreased postnatal pancreatic proliferation. Interestingly, E2F1, cdk4, cyclin D1, and RB proteins are, however highly expressed in non-proliferating pancreatic  $\beta$ -cells. This suggested to us that these cell cycle regulators could have an important role, not only in pancreatic development and proliferation, but also in pancreatic  $\beta$ -cell physiology, independent of the control of cell proliferation. This has been the subject of our most recent publication in the august issue of Nature Cell Biol. We show in this study that E2F1 controls insulin secretion of  $\beta$ -cells through transcriptional regulation of Kir6.2 gene expression.<sup>16</sup> We demonstrated that Kir6.2, which plays a major role in the regulation of insulin secretion by controlling membrane polarization, is a direct E2F1 target gene. Moreover, we show that cdk4 is also implicated in the regulation of insulin secretion. Interestingly, pharmacological inhibition of CDK4 results in impaired insulin secretion in response to glucose, secondary to inhibition of Kir6.2 expression. We demonstrated that cdk4 and E2F1 regulate kir6.2 expression in response to increased blood glucose level. High blood glucose levels induce the activation of cdk4, the phosphorylation of pRB, and finally induce the activation of Kir6.2 expression by E2F1. This demonstrates that the cdk4-pRb-E2F1 pathway is a sensor of blood glucose levels, and underscores a dual role for the cdk4-pRb-E2F1 pathway in the control of both cell proliferation and metabolic control. Strikingly, glucose is required for the proliferation of any cell type, which defines a link between both processes.

In addition to the direct participation of cdk4/pRB/E2F1 in lipids physiology (discussed above), and energy metabolism (discussed below), the activation of the cdk4/pRB/E2F1 pathway may have important implications in the context of general metabolism in tissues other than pancreas, secondary to its involvement in insulin secretion (Figure 1). Secreted insulin has pleiotropic effects in peripheral tissues, such as white adipose tissue (WAT), muscle, or liver. In adipose tissue, insulin will shut lipolytic processes, and favor lipogenesis. In muscle and liver, insulin will facilitate glucose disposal and inhibit gluconeogenesis. This is in contrast of some studies showing direct negative effects of cell cycle regulators in glucose utilization, in particular in glycolytic processes. 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. It was demonstrated that E2F1 induces PDK4 transcription and blunts glucose oxidation in muscle.<sup>17</sup> In line with this observation it was shown that cyclin D1, which is the regulatory partner of cdk4 inhibits the activity of the promoter of Hexokinase II (HKII), the enzyme catalyzing the first steps of glycolysis in

epithelial and fibroblastic cells. Furthermore it was shown that transgenic mice expressing antisense cyclin D1 in the mammary gland had increased RNA and protein levels of HKII and pyruvate kinase.<sup>18</sup> Taken together these studies suggest that cell cycle regulators, in addition to the control of insulin secretion are implicated in the regulation of glucose homeostasis via the inhibition of oxidative glycolysis. Further studies are required to shed light on this paradox.

Finally, it is worth to briefly discuss the participation of cell cycle regulators in whole energy homeostasis. Consistent with the anabolic role of insulin in peripheral tissues, cell cycle regulators could contribute, in these tissues, to the channeling of the products of glycolysis towards biosynthetic processes, such as de novo fatty acid synthesis. Inhibition of oxidative phosphorylation could facilitate this specific channeling. Supporting this hypothesis, increases in mitochondrial function were observed in preadipocytes with non-functional pRB.<sup>19</sup> Moreover, the implication of pRB in energy expenditure was demonstrated in mice. Mice with specific deletion of pRB in adipose tissue exhibit a lean phenotype after high-fat feeding. They are protected from excessive weight gain induced by high fat diet because of an increase in energy expenditure.<sup>20</sup> These mice show increased mitochondrial number and increased expression of several genes involved in mitochondrial function. Taken together these studies show that pRB have an important role in the negative regulation of energy expenditure and mitochondrial activity through the modulation of the transcription of genes implicated in these processes.

Currently, proteins such as cyclins, cdk, or E2Fs are being studied in the context of proliferation, cell cycle regulation and cancer. We already demonstrated, however that these factors play crucial roles in the control of metabolism. Our approach not only helps to understand why a cell gives a metabolic, instead of proliferative response when stimulated with a proliferative stimuli, but also contributes to the identification of new pathways and targets for the treatment of metabolic diseases such as diabetes and obesity.

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## Figure legend

**Figure 1. Illustration of the participation of the cdk4/pRB/E2F1 pathway in metabolic processes.** Through regulation of the expression of Kir6.2, E2F1 facilitates insulin secretion. Insulin will signal to peripheral insulin sensitive tissues, such as liver, muscle, or white adipose tissue (WAT). The effects of insulin are described under the cartoon of the tissues. On the other hand, the cdk4/pRB/E2F1 pathway has direct effects on the described metabolic processes in the same tissues.