Published in final edited form as:

Curr Opin Cell Biol. 2013 December ; 25(6): . doi:10.1016/j.ceb.2013.07.012.

The multiple connections between pRB and cell metabolism

Brandon N. Nicolay and Nicholas J. Dyson

Laboratory of Molecular Oncology, Massachusetts General Hospital Cancer Center, Building 149, 13th Street, Charlestown, Massachusetts, 02129, USA

Abstract

The pRB tumor suppressor is traditionally seen as an important regulator of the cell cycle. pRB represses the transcriptional activation of a diverse set of genes by the E2F transcription factors and prevents inappropriate S-phase entry. Advances in our understanding of pRB have documented roles that extend beyond the cell cycle and this review summarizes recent studies that link pRB to the control of cell metabolism. pRB has been shown to regulate glucose tolerance, mitogenesis, glutathione synthesis, and the expression of genes involved in central carbon metabolism. Several studies have demonstrated that pRB directly targets a set of genes that are crucial for nucleotide metabolism, and this seems likely to represent one of the ways by which pRB influences the G1/S-phase transition and S-phase progression.

Keywords

RBF; RB; *Drosophila*; metabolism; glutamine; glucose; cancer

Introduction

The mechanisms that enable the Retinoblastoma tumor suppressor (*RB1*) and pRB-related proteins (p107, and p130) to regulate cell cycle progression have been the focus of a great deal of research. From this body of work we know that pRB becomes phosphorylated by cyclin dependent kinases (CDK's) as cells progress through G1 towards S-phase and that this suppresses the ability of pRB to antagonize E2F, a transcription factor that drives expression of genes that promote S-phase entry and cell division. This key role of pRB is compromised in many human cancers.

Much of the RB literature has focused on how E2F-dependent changes in cell cycle control contribute to tumorigenesis. It is now well accepted that the inactivation of RB not only disrupts the regulation of the G1/S-phase transition, but also alters many other cellular processes. Processes that are affected by *RB1* loss include chromatin organization [1-3], differentiation pathways [4-7], mitotic progression [1, 3], apoptosis [8-10], autophagy [11, 12], red/ox balance [13, 14], and metabolism [15-18]. Because the functions of pRB-regulated genes are highly interconnected, tumor cells lacking *RB1* cannot be said to have a single deregulated pathway, but are in an altered state in which the status and regulation of many processes has changed. This “network” view of pRB function is illustrated by the series of papers that have described links between pRB and cell metabolism.

© 2013 Elsevier Ltd. All rights reserved.

Corresponding authors: Brandon N Nicolay; bnicolay@partners.org, Nicholas J Dyson; dyson@helix.mgh.harvard.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Metabolic adaptation is key for any cell to survive in less than optimal environments. This is especially important in cancer cells that often must divide under low nutrient/low oxygen conditions. Recent studies have underscored the evidence that the reprogramming of mitochondria and glycolysis are important events in tumor cell development (reviewed in: [19, 20]). Though the current information is far from complete, there is a growing body of evidence showing that the loss of *RB1* alters the metabolic state of the cell. This review will summarize this body of literature. A theme that runs through these studies is the idea that pRB is needed for cells to maintain a normal metabolic balance, and that the loss of pRB leads to reprogramming of specific pathways. Metabolic genes are not the most famous of pRB's targets but it seems highly likely that the effects of pRB on metabolic processes have a significant impact on pRB-dependent regulation of the G1/S phase transition, and contribute to the altered proliferative properties of *RB1*-deficient cells.

pRB function impacts glucose tolerance

Under normal conditions most cells utilize glucose as the major carbon source for oxidation. Glucose uptake has been shown to promote the phosphorylation of pRB, thereby promoting E2F activity, (i.e. E2F-dependent transcriptional activation) [15, 21]. In contrast, glucose withdrawal from the cell culture medium promotes the accumulation of hypophosphorylated pRB [15].

These experiments suggest that pRB's activity is regulated in response to changes in glucose levels. Interestingly several genetic studies have also shown that changes in the levels of pRB/E2F1 proteins impacts glucose tolerance (ie the ability of an animal to control glucose levels after feeding). *E2f1*^{-/-} mice are resistant to diet-induced obesity [5]. pRB has been shown to inhibit the production of insulin in pancreatic beta cells in response to glucose [15]. Recently, Lu et al., found that a local deletion of the *Rb1* gene in the mouse hypothalamus leads to glucose intolerance and obesity [16]. Furthermore, this phenotype was largely reversed when *Rb1* and *E2f1* were simultaneously deleted [16]. These effects may be, at least in part, a consequence of the increased expression of E2F1 target genes. In addition to its cell cycle targets, E2F1 has been shown to directly regulate the expression of several genes that have established roles in glucose regulation [13, 15, 22-24]. For example, E2F1 drives expression of the PDK4 enzyme, a known modulator of glucose tolerance [25]. *PDK4*^{-/-} mice were shown to have improved glucose tolerance compared to their littermate controls [26].

Diabetic mice were found to have high levels of phosphorylated RB [21] and, collectively, the findings described above suggest that pRB-dependent regulation of glucose homeostasis may help to prevent glucose intolerance, a prerequisite for diabetes. Somewhat paradoxically, one study has found that mice haploinsufficient for *Rb1* have better glucose tolerance and reduced obesity [27]. It is not yet known how the links between pRB/E2F1 and glucose regulation impact pRB's tumor suppressor activities, and the answer to this question is likely to be complex. For example, sustained glucose uptake by tumor cells may help to promote the inactivation of pRB. Another intriguing possibility is that some tumor cells may find it beneficial to retain a basal level of pRB function to prevent glucose intolerance, and this may be one of the reasons why the *RB1* gene is not mutated more frequently in human tumors. To date, just one study has examined the effects of diet on tumor formation in *Rb1*^{+/-} and control animals and the small differences observed did not reach statistical significance [28]. Undoubtedly, this is a subject that needs to be examined further.

pRB activity regulates oxidative metabolism

The acute deletion of *Rb1* in mice has been shown to result in reduced expression of numerous genes with functions in oxidative metabolism [29]. Reduced expression of oxidative metabolism genes was coupled to decreased mitochondrial biogenesis following acute deletion of *Rb1* mice erythrocytes [29]; As well as erythrocytes, decreased mitochondrial biogenesis was also seen following *RB1* loss in human myocytes [11], and in MEFs that lack all three of the pRB family proteins (pRB, p107, p130) [18]. In the light of these observations, one might predict that *RB1*-deficient cells would have trouble maintaining ATP production. However, one study found that loss of *RB1* reduced cellular ATP levels [11], while another study found that MEFs lacking pRB family proteins have increased ATP [18], suggesting that any effects may be context dependent.

The idea that pRB/E2F1 proteins regulate the expression of genes with functions in oxidative metabolism is strongly supported by analysis of *E2f1*^{-/-} mice. When studied in the context of cell cycle progression, pRB generally antagonizes E2F1. Unexpectedly, characterization of *E2f1*^{-/-} mice revealed that the loss of E2F1 increased the expression of a set of genes involved in oxidative metabolism [13]. Molecular studies suggested that pRB represses the expression of these genes via an interaction with E2F1 at the promoter. Importantly, the authors found that conditions that elevated CDK activity and inactivated pRB enabled both adipose and skeletal tissue to sustain a high level of oxidative metabolism during cycles of fasting-refeeding [13]. Interestingly, pRB-dependent differentiation of white adipose tissue requires that pRB mediate downregulation of the brown adipose tissue specific expression of uncoupling protein 1 (*UCP1*) through direct repression of the transcription factor PGC1 [30, 31]. Consistently, increased UCP1 activity is known to increase oxidative metabolism [32]. Thus, pRB can both directly and indirectly impact oxidative metabolism through regulation of distinct suites of genes.

The functions and regulation of pRB/E2F1 proteins are highly conserved between flies and mammals and, similar to mammalian cells, the *Drosophila* pRB ortholog (RBF1) is inactivated by phosphorylation by CDKs [33]. Recently, the co-overexpression of the fly *cyclin D/cdk4* genes was shown to increase mitochondrial biogenesis [34], suggesting that the coordination of pRB phosphorylation and mitogenesis has been conserved during evolution.

pRB and the TCA cycle

Cells maintain ATP production through oxidative phosphorylation coupled to the TCA cycle (under normoxic conditions) or using glycolysis (under low oxygen conditions). A recurrent theme in the literature is the notion that activation of oncogenes, or loss of tumor suppressors, yields cells that are addicted to glutamine [19, 20]. The general implication is that non-transformed cells typically oxidize glucose-derived carbon in the TCA cycle, while many transformed cells primarily oxidize glutamine-derived carbon. Potentially the inactivation of pRB might affect the balance between the two main processes of carbon oxidation, or the type of carbon source that is oxidized. Recent studies using isotopic tracer experiments discovered changes in the utilization of glutamine in pRB-deficient cells that are evident in both mammals and *Drosophila* [17, 18]. To date, however, no isotopic tracing study has been published that specifically compares the fate of glucose in *RB1*^{+/+} and *RB1*^{-/-} cells.

The catabolism of glucose and glutamine is not only important for ATP synthesis but is also needed to produce metabolic precursors that are required by dividing cell, such as dNTPs. An example of this is aspartate. Aspartate is derived primarily from the TCA metabolite malate; it provides the carbon backbone for pyrimidine synthesis, and contributes an amino

group to purine bases [35]. Interestingly, an increased flux of glutamine carbon to aspartate has been demonstrated in cells lacking all three pRB-family of proteins [18], in cells depleted of pRB [17], and in *rbf1* mutant larvae [17]. At first glance these findings are consistent with the hypothesis that the loss of pRB/RBF promotes dNTP production through increased glutamine catabolism. However, in experiments where this was examined, there was little to no detectable increase in glutamine-derived carbon in pyrimidine nucleotide precursors, such as UMP [17]. Currently, it is clear that the inactivation pRB/RBF1 increases glutamine flux to aspartate, but the precise fate of this aspartate is less certain.

In both mammalian cells and *Drosophila* the increased flux of glutamine to aspartate seen in cells lacking pRB-family proteins was accompanied by a second change that increased the flux of glutamine to glutathione [17, 18]. Glutathione is important for cells to maintain redox balance; additionally, glutathione production impacts nucleotide synthesis because the activity of the ribonucleotide reductase enzyme complex (discussed below) is dependent on protein thiol-disulfide exchange reactions. *rbf1* mutant larvae and RBF1-depleted cells have a heightened sensitivity to fasting and to reactive oxygen species. These sensitivities were specifically suppressed by feeding mutant larvae with glutamine, or by addition of N-acetylcysteine, a potent antioxidant [17]. In the future it will be important to determine whether there is a specific pattern of glutamine flux that is uniquely regulated by pRB, and whether this can be exploited to reduce the viability of *RBI*-mutant tumor cells.

pRB and dNTP synthesis

The notion that *RBI*-loss alters the metabolic flux into nucleotide synthesis is appealing because genes involved in nucleotide synthesis, such as dihydrofolate reductase (*DHFR*), thymidylate synthase (*TS*), ribonucleotide reductase (*RNR*), and thymidine kinase (*Tk*) were among the first E2F-regulated genes to be described and are known to be induced by E2F1 [36-39]. The transcription of each of these genes increases in the absence of pRB, leading to elevated protein levels [40]. The potential significance of the E2F/pRB-dependent regulation of nucleotide synthesis is underscored by the fact that E2F-regulation of these targets is conserved during evolution [17, 41, 42].

These studies raise the possibility that pRB-mediated control of nucleotide synthesis is one of the ways by which pRB regulates the G1/S-phase transition. Several studies have described data that is consistent with this model. Expression of a constitutively active pRB protein in asynchronously cycling rat cells led to a coordinated increase in the G1 population of cells, decreased levels of DHFR, TS, and RNR, and a concomitant reduction in dNTP pools [40]. Studies of oncogene-induced senescence (OIS) suggest that this regulation is important for pRB function. pRB, but not p107 or p130, has been found to repress the two RNR complex genes (*RRM2*, *RRM1*) and the *DHFR* gene in cells that have undergone RAS-induced senescence [43]. Importantly, targeted knockdown of pRB in these cells reversed these changes in gene expression and allowed cells to reenter the cell cycle [43]. Using a similar experimental system, Aird et al have recently demonstrated that RAS-induced senescence leads to depletion of dNTP pools that is dependent on the downregulation of *RRM2* [44]. The repression of *RRM2* correlated with a decreased affinity of E2F1 for the *RRM2* promoter. Furthermore, the overexpression of *RRM2* was shown to prevent RAS OIS and to enable senescent cells to reenter the cell cycle [44].

A notable gap in the evidence for this model of pRB function is that, to date, there is no published study that has directly measured changes in nucleotide levels or synthesis following the specific loss of pRB. Perhaps the strongest data in support of this model comes from studies in *Drosophila*. Gene ontology (GO) enrichment demonstrated that genes involved in nucleotide synthesis are directly bound by RBF1 and are upregulated in *rbf1*-/-

larvae [42, 45]. Moreover, metabolic profiling experiments demonstrate that depletion of RBF1 not only increases the expression of *rnr* genes but also elevates dNTP pools [17]. Thus, in this experimental system, the loss of RBF1-mediated repression appears to have direct consequences on nucleotide metabolism.

The RNR enzyme complex establishes a dNTP pool that is necessary for S-phase entry. In addition, RNR must continuously synthesize dNTPs during S-phase for normal cell cycle progression [46]. Curiously, the expression of HPV E6 and E7 proteins decreases dNTP pools and leads to replication stress [47]. Depletion of dNTPs was apparent despite increased expression of *RRM2* and this finding is the opposite of what would be predicted from the studies described above. From the published data it is unclear whether the effects of the HPV proteins are mediated primarily via E7's ability to bind to pRB and to activate E2F, or whether other viral targets are involved. Expression of the viral proteins appears to increase the number of origins that initiate DNA replication and it is possible that the pools of dNTP's are more rapidly depleted upon S-phase entry than in control cells. Clearly, more studies are needed to determine how the specific inactivation of pRB impacts both the levels of dNTP's, and the rate of dNTP synthesis.

Final thoughts

Reviews of the literature on pRB-mediated control of the cell cycle often focus on pRB's effects on the levels or activity of cyclins, cdk's and cdk-inhibitors. This emphasis is logical because fluctuations in cdk activity control cell cycle transitions, and are particularly important at the G1/S boundary. This review highlights the fact that, in addition to these effects, studies of pRB and E2F1 have uncovered a series of direct links to enzymes involved in nucleotide metabolism [17, 36, 37, 39, 40, 42, 43], glycolysis [22-25], the TCA cycle [13, 42, 43], and the electron transport chain [13]. These connections are especially interesting given the rapidly growing evidence that mutations that drive tumorigenesis often reprogram central carbon metabolism. Metabolic reprogramming is thought to enhance the ability of cancer cells to sustain the intermediates needed for synthesis of proteins, lipids, and DNA. Currently, it is unclear which of the metabolic changes in pRB deficient cells are required for cell survival and which are necessary to sustain cell proliferation. The work summarized here suggests a simple model in which pRB functions to coordinate DNA replication with the energetic demands of a cell. Presumably, this co-ordination helps the cell ensure that the decision of whether or not to progress into S-phase is coupled with other anabolic and catabolic demands. However, this model remains to be tested. In addition to giving us a more complete appreciation of pRB function, such insights may identify vulnerabilities of tumor cells that can be exploited.

Acknowledgments

We apologize to our colleagues whose work we failed to include due to space limitations. BNN is supported by the NIH NRSA fellowship F32CA165856. N.J.D. is the James and Shirley Curvey MGH Research Scholar. This work was supported by the MGH Federal Share of Program Income under C06 CA059267.

Literature Cited

*- of special interest

** - of outstanding interest

1. Coschi CH, et al. Mitotic chromosome condensation mediated by the retinoblastoma protein is tumor-suppressive. *Genes Dev.* 2010; 24(13):1351–63. [PubMed: 20551166]

2. Longworth MS, et al. RBF1 promotes chromatin condensation through a conserved interaction with the Condensin II protein dCAP-D3. *Genes Dev.* 2008; 22(8):1011–24. [PubMed: 18367646]
3. Manning AL, Longworth MS, Dyson NJ. Loss of pRB causes centromere dysfunction and chromosomal instability. *Genes Dev.* 2010; 24(13):1364–76. [PubMed: 20551165]
4. Calo E, et al. Rb regulates fate choice and lineage commitment in vivo. *Nature.* 2010; 466(7310):1110–4. [PubMed: 20686481]
5. Fajas L, et al. E2Fs regulate adipocyte differentiation. *Dev Cell.* 2002; 3(1):39–49. [PubMed: 12110166]
6. Gu W, et al. Interaction of myogenic factors and the retinoblastoma protein mediates muscle cell commitment and differentiation. *Cell.* 1993; 72(3):309–24. [PubMed: 8381715]
7. Lipinski MM, Jacks T. The retinoblastoma gene family in differentiation and development. *Oncogene.* 1999; 18(55):7873–82. [PubMed: 10630640]
8. Chau BN, Wang JY. Coordinated regulation of life and death by RB. *Nat Rev Cancer.* 2003; 3(2):130–8. [PubMed: 12563312]
9. Hilgendorf KI, et al. The retinoblastoma protein induces apoptosis directly at the mitochondria. *Genes Dev.* 2013; 27(9):1003–15. [PubMed: 23618872]
10. MacPherson D, et al. Cell type-specific effects of Rb deletion in the murine retina. *Genes Dev.* 2004; 18(14):1681–94. [PubMed: 15231717]
11. Ciavarrà G, Zacksenhaus E. Rescue of myogenic defects in Rb-deficient cells by inhibition of autophagy or by hypoxia-induced glycolytic shift. *J Cell Biol.* 2010; 191(2):291–301. [PubMed: 20937698]
12. Jiang H, et al. The RB-E2F1 pathway regulates autophagy. *Cancer Res.* 2010; 70(20):7882–93. [PubMed: 20807803]
- 13**. Blanchet E, et al. E2F transcription factor-1 regulates oxidative metabolism. *Nat Cell Biol.* 2011; 13(9):1146–52. This study shows that RB/E2F complexes regulate the expression of important oxidative metabolism genes during metabolic stress. Loss of E2f1 leads to sustained expression of oxidative metabolism genes during fasting-refeeding. [PubMed: 21841792]
14. Li B, et al. Specific killing of Rb mutant cancer cells by inactivating TSC2. *Cancer Cell.* 2010; 17(5):469–80. [PubMed: 20478529]
15. Annicotte JS, et al. The CDK4-pRB-E2F1 pathway controls insulin secretion. *Nat Cell Biol.* 2009; 11(8):1017–23. [PubMed: 19597485]
- 16**. Lu Z, et al. pRb is an obesity suppressor in hypothalamus and high-fat diet inhibits pRb in this location. *EMBO J.* 2013; 32(6):844–57. This paper demonstrates that loss of RB in the mouse hypothalamus, and the deregulation of E2F, leads to glucose intolerance and obesity. [PubMed: 23403926]
- 17**. Nicolay BN, et al. Loss of RBF1 changes glutamine catabolism. *Genes Dev.* 2013; 27(2):182–96. Loss of RBF1 causes a sensitivity to fasting that is rescued by glutamine supplementation or treatment with N-acetylcysteine. Isotopic tracer analysis determined the carbon fate of glutamine in RBF1-deficient flies and RB-depleted human cells and found increased flux to glutathione synthesis. [PubMed: 23322302]
- 18**. Reynolds MR, et al. Control of glutamine metabolism by the tumor suppressor Rb. *Oncogene.* 2013 Rb1^{-/-}, p107^{-/-}, p130^{-/-} MEFs were found to have increased levels of ATP, increased ROS, and increased carbon flux of glutamine to glutathione synthesis when compared to cell lines derived from littermate controls.
19. Dang CV. Links between metabolism and cancer. *Genes Dev.* 2012; 26(9):877–90. [PubMed: 22549953]
20. Metallo CM, Vander Heiden MG. Understanding metabolic regulation and its influence on cell physiology. *Mol Cell.* 2013; 49(3):388–98. [PubMed: 23395269]
21. Feliars D, Frank MA, Riley DJ. Activation of cyclin D1-Cdk4 and Cdk4-directed phosphorylation of RB protein in diabetic mesangial hypertrophy. *Diabetes.* 2002; 51(11):3290–9. [PubMed: 12401721]
22. Darville MI, et al. An E2F-dependent late-serum-response promoter in a gene that controls glycolysis. *Oncogene.* 1995; 11(8):1509–17. [PubMed: 7478575]

23. Darville MI, Rousseau GG. E2F-dependent mitogenic stimulation of the splicing of transcripts from an S phase-regulated gene. *Nucleic Acids Res.* 1997; 25(14):2759–65. [PubMed: 9207022]
24. Fernandez de Mattos S, Lam EW, Tauler A. An E2F-binding site mediates the activation of the proliferative isoform of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase by phosphatidylinositol 3-kinase. *Biochem J.* 2002; 368(Pt 1):283–91. [PubMed: 12139485]
25. Hsieh MC, et al. Regulation of the PDK4 isozyme by the Rb-E2F1 complex. *J Biol Chem.* 2008; 283(41):27410–7. [PubMed: 18667418]
26. Hwang B, Jeoung NH, Harris RA. Pyruvate dehydrogenase kinase isoenzyme 4 (PDHK4) deficiency attenuates the long-term negative effects of a high-saturated fat diet. *Biochem J.* 2009; 423(2):243–52. [PubMed: 19627255]
27. Mercader J, et al. Haploinsufficiency of the retinoblastoma protein gene reduces diet-induced obesity, insulin resistance, and hepatosteatosis in mice. *Am J Physiol Endocrinol Metab.* 2009; 297(1):E184–93. [PubMed: 19417128]
28. Sharp ZD, et al. Minimal effects of dietary restriction on neuroendocrine carcinogenesis in Rb +/- mice. *Carcinogenesis.* 2003; 24(2):179–83. [PubMed: 12584165]
29. Sankaran VG, Orkin SH, Walkley CR. Rb intrinsically promotes erythropoiesis by coupling cell cycle exit with mitochondrial biogenesis. *Genes Dev.* 2008; 22(4):463–75. [PubMed: 18258751]
30. Hansen JB, et al. Retinoblastoma protein functions as a molecular switch determining white versus brown adipocyte differentiation. *Proc Natl Acad Sci U S A.* 2004; 101(12):4112–7. [PubMed: 15024128]
31. Scime A, et al. Rb and p107 regulate preadipocyte differentiation into white versus brown fat through repression of PGC-1alpha. *Cell Metab.* 2005; 2(5):283–95. [PubMed: 16271529]
32. Rousset S, et al. The biology of mitochondrial uncoupling proteins. *Diabetes.* 2004; 53(1):S130–5. [PubMed: 14749278]
33. Xin S, et al. The role of RBF in developmentally regulated cell proliferation in the eye disc and in Cyclin D/Cdk4 induced cellular growth. *Development.* 2002; 129(6):1345–56. [PubMed: 11880344]
34. Icreverzi A, et al. Drosophila cyclin D/Cdk4 regulates mitochondrial biogenesis and aging and sensitizes animals to hypoxic stress. *Cell Cycle.* 2012; 11(3):554–68. [PubMed: 22293404]
35. Voet, DV.; J, G. *Biochemistry.* Wiley: 2004. p. 1520
36. DeGregori J, Kowalik T, Nevins JR. Cellular targets for activation by the E2F1 transcription factor include DNA synthesis- and G1/S-regulatory genes. *Mol Cell Biol.* 1995; 15(8):4215–24. [PubMed: 7623816]
37. Dou QP, et al. G1/S-regulated E2F-containing protein complexes bind to the mouse thymidine kinase gene promoter. *J Biol Chem.* 1994; 269(2):1306–13. [PubMed: 8288595]
38. Good L, Chen J, Chen KY. Analysis of sequence-specific binding activity of cis-elements in human thymidine kinase gene promoter during G1/S phase transition. *J Cell Physiol.* 1995; 163(3):636–44. [PubMed: 7775606]
39. Good L, et al. Regulation of dihydrofolate reductase gene expression and E2F components in human diploid fibroblasts during growth and senescence. *J Cell Physiol.* 1996; 168(3):580–8. [PubMed: 8816912]
40. Angus SP, et al. Retinoblastoma tumor suppressor targets dNTP metabolism to regulate DNA replication. *J Biol Chem.* 2002; 277(46):44376–84. [PubMed: 12221087]
41. Duronio RJ, et al. The transcription factor E2F is required for S phase during Drosophila embryogenesis. *Genes Dev.* 1995; 9(12):1445–55. [PubMed: 7601349]
42. Korenjak M, et al. RBF binding to both canonical E2F targets and noncanonical targets depends on functional dE2F/dDP complexes. *Mol Cell Biol.* 2012; 32(21):4375–87. [PubMed: 22927638]
- 43*. Chicas A, et al. Dissecting the unique role of the retinoblastoma tumor suppressor during cellular senescence. *Cancer Cell.* 2010; 17(4):376–87. This paper distinguishes the role of RB from the RB-related proteins p107 and p130, and describes a unique role for RB in the repression of key nucleotide synthesis genes during oncogene induced senescence. [PubMed: 20385362]
- 44*. Aird KM, et al. Suppression of nucleotide metabolism underlies the establishment and maintenance of oncogene-induced senescence. *Cell Rep.* 2013; 3(4):1252–65. The authors propose that oncogene induced senescence is mediated by depletion of dNTP pools. They show

that senescence can be bypassed through expression of the ribonucleotide reductase complex or nucleotide supplementation. [PubMed: 23562156]

45. Longworth MS, et al. A shared role for RBF1 and dCAP-D3 in the regulation of transcription with consequences for innate immunity. *PLoS Genet.* 2012; 8(4):e1002618. [PubMed: 22496667]
46. Kumar D, et al. Highly mutagenic and severely imbalanced dNTP pools can escape detection by the S-phase checkpoint. *Nucleic Acids Res.* 2010; 38(12):3975–83. [PubMed: 20215435]
- 47*. Bester AC, et al. Nucleotide deficiency promotes genomic instability in early stages of cancer development. *Cell.* 2011; 145(3):435–46. This paper reports that the expression of HPV E6 and E7 in primary cells causes a depletion of dNTP pools, replication stress and DNA damage. These effects can be rescued through nucleotide supplementation or increased expression of nucleotide synthesis genes. [PubMed: 21529715]

Highlights

- pRB directly regulates glucose tolerance
- pRB inactivation directly reduces mitogenesis and alters oxidative metabolism
- Loss of pRB alters glutamine catabolism in fly and mammalian cells
- Direct pRB regulation of nucleotide metabolism genes appears important for cell cycle inhibition

Figure 1. A summary of the links between pRB/E2F and cell metabolism

A) When pRB is inactivated during cell cycle progression the liberated E2F does not simply promote the expression of cyclins, cdks and replication proteins that drive cells into S-phase. In addition, pRB/E2F proteins control the expression of genes in several metabolic pathways that are key for cells to replicate DNA and sustain cell proliferation. Effects of pRB/E2F on genes involved in nucleotide synthesis, glutathione synthesis, glycolysis and oxidative metabolism are described in the text. There is also evidence that the phosphorylation state of pRB is influenced by glucose levels.

B) The metabolic pathways that are impacted by pRB (nucleotide synthesis, glutathione synthesis, and glycolysis and oxidative metabolism) are all interconnected. The effects of pRB/E2F proteins on these processes is likely to impact the synthesis of nucleotides, the production of ATP and the extent of oxidative stress. These changes may all contribute to the altered patterns of DNA replication and cell proliferation seen in *RB*-mutant tissue.