Master or slave: The complex relationship of RBP2 and pRb

The retinoblastoma protein or its regulators are altered in most human cancers. Although commonly thought of as solely a repressor of E2F-dependent transcription and cell cycle progression, pRb has gained notoriety in recent years as a key actor in cellular differentiation programs. In the June issue of *Molecular Cell*, Benevolenskaya et al. report that a long-known but poorly understood pRb interactor, RBP2, acts as an inhibitor of differentiation contributing to pRb's role as a coordinator of differentiation and cell cycle exit. Loss of pRb may unleash RBP2, maintaining cells in a poorly differentiated progenitor state that is prerequisite to tumor formation.

In the course of normal development, cells differentiate into their specified, terminal state and ultimately exit the cell cycle. The process of differentiation is thus inversely correlated with proliferation. In the past decade, the retinoblastoma protein has emerged from its generic tumor suppressor role as a cell cycle regulator acting solely through the E2F family of transcription factors and is now recognized as a central interpreter of the proliferation-to-differentiation switch in a number of tissues. In osteoblasts, for example, pRb has been shown to directly bind the bone "master" transcription factor Cbfa1/Runx2 and act as a transcriptional coactivator necessary for terminal differentiation (Thomas et al., 2001). Indeed, reintroduction of pRb in the RB1$^{-/-}$ SAOS2 osteosarcoma cell line is sufficient to induce markers of bone differentiation and acquisition of a senescent phenotype (Alexander and Hinds, 2001). Surprisingly, a pRb mutant, RB LP Δ663, which lacks the canonical ability to bind and repress E2F-dependent transcription, is capable of eliciting the same differentiation/senescence response as the wild-type protein (Sellers et al., 1998). This raises the question of what sort of characters pRb might be associating with to accomplish this E2F-independent process.

As reported in the June issue of *Molecular Cell*, Benevolenskaya et al. (2005) used the Δ663 pRb mutant in a yeast 2-hybrid screen to capture pRb-associated factors. What surfaced from this approach was a well-known but largely uncharacterized pRb binding partner, RBP2. Identified as one of the first cellular pRb binding partners, RBP2 had thwarted several attempts at characterization. RBP2 is ubiquitously expressed; has a canonical LXCXE motif; PHD-, ARID-, and Jumonji- domains; and binds to p107 and TATA binding protein, but its function has remained elusive until now.

Remarkably, knockdown of RBP2 in the SAOS2 system was sufficient to block proliferation and elicit the expression of bone differentiation markers. Further, differentiation-associated transcriptional activation via glucocorticoid receptor (GRα) was enhanced upon introduction of wild-type pRb, and this activation effect is similarly seen with RBP2 siRNA transfection. Thus, inactivation of RBP2 is cytostatic and is sufficient for turning on differentiation-associated markers, which ostensibly phenocopies the effect of pRb reintroduction into SAOS-2 cells. Further, in a myelopoiesis model of differentiation, pRb was shown to bind and colocalize with RBP2 correlating with progression from an undifferentiated to a differentiated state, thus implying a mechanism by which pRb binds and attenuates RBP2 repression of differentiation.

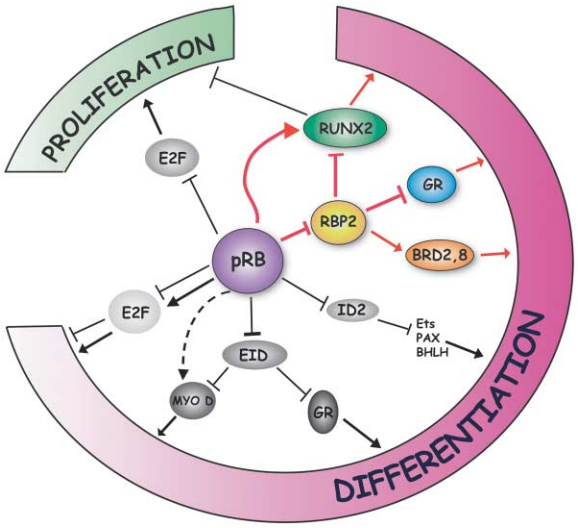

Figure 1. pRb: differentiation and proliferation

Proper tissue development requires a coordinated process of cellular differentiation and cell cycle exit. The tumor suppressor pRb may act as a central decision maker in this process. pRb's best-known function is to block proliferation by binding and inactivating the E2F family of transcription factors. More recently, pRb has been reported to influence the activity of many proteins in order to achieve the switch from proliferation to differentiation. The colored arm of the schematic represents a new pathway of pRb regulation in differentiation described by Benevolenskaya et al. In the absence of a fully functional pRb, RBP2 silencing was sufficient to achieve a proliferative halt and elicit a differentiation program in a variety of cell types in culture. Further, the pRb/RBP2 complex augmented the expression of homeotic genes (BRD2 and -8) that may prove to be a key and fertile area of research. What remains unclear, however, is how does a "differentiation block" work?

In bone, pRb acts to coactivate Runx2-mediated transcription of late markers (Thomas et al., 2001). Benevolenskaya et al. report that like wild-type pRb, Δ663 augments Runx2-dependent transcription in SAOS2 cells, and, remarkably, knockdown of RBP2 showed a similar induction of the bone reporter even in the absence of added pRb. Interestingly, an additive effect on both the GRα and 6OSE2 reporters was observed when wt pRb and RBP2-targeted siRNA where cotransfected. This may be a result of the RBP2 siRNA more effectively eliminating a stoichiometric excess of RBP2 in the cell than is achieved solely with pRb overexpression. Alternatively, pRb may have other roles not dependent on RBP2 interaction, but in any case, induction of Runx2 activity by RBP2 knockdown in the absence of pRb suggests a wholly new model of pRb action in this system. In this model, RBP2 acts to inhibit differentiation-specific genes, and pRb acts at least in part to counteract this repression (Figure 1). Indeed, in chromatin immunoprecipitation (ChIP) experiments, RBP2 was found bound to the osteocalcin promoter, but could be displaced by pRb when introduced. Upon pRb binding of RBP2, the repression is alleviated, and pRb/Runx2 complexes then bind and

activate the promoter as previously proposed.

Benevolenskaya et al. also investigated whether this pRb-RBP2 antagonistic mechanism exists in other cell types that are mesenchymally derived, as reintroduction of pRb in $RB^{-/-}$ cells can also promote differentiation into myoblasts and adipocytes (Classon and Harlow, 2002). Indeed, knockdown of RBP2 caused a proliferation block and differentiation in this system reminiscent of pRb restoration.

The work of Benevolenskaya et al. seems to solidly confirm a role for pRb in differentiation that has been strongly hinted at by other recent studies. The Id family proteins inhibit differentiation and drive proliferation. In particular, Id2 has been shown to bind to pRb and p107. It is proposed that the pRb family proteins act to restrain Id2's antidifferentiative function manifested through inhibition of the activity of transcription factors such Ets, Pax, and bHLH. Of note, $p16^{Ink4a}$ is a target of the Ets transcription factor, and many bHLH proteins play a significant role in differentiation programs (Lasorella et al., 2001).

More recently, it has been shown that the E1A-like inhibitor of differentiation 1 (EID-1) is a pRb binding protein with a classic LXCXE motif that was also identified in a yeast two-hybrid screen (MacLellan et al., 2000). EID-1 overexpression in muscle inhibits differentiation. It has been shown that pRb is necessary for the degradation of the EID-1 complex, which then allows for transcriptional activation of differentiation markers (i.e., GR and MyoD) (Krutzfeldt et al., 2005). This is not a well-understood pathway, but provides another example of how pRb's gene-repressive role is disassociated from its gene-activation role.

When we consider transcription factors within pRb's sphere of influence, it is important to consider the role of the E2F family of transcription factors on differentiation. The activating E2Fs (1-3a) have been shown to turn on genes necessary for S phase progression, while E2F3b-5 enforce cell cycle arrest. However, E2Fs have been implicated in a more direct role in differentiation evidenced by the various tissue-specific defects seen in different E2F knockout animals. Further, in adipocytes, E2Fs have been shown to bind C/EBP and PPAR-γ and modulate differentiation (reviewed in Trimarchi and Lees, 2002). Interestingly, recent reports have shown that direct E2F repression is necessary for keratinocyte differentiation (Wong et al., 2005). Given pRb's role in regulating E2F family function, it is likely that some of pRb's effects on differentiation are mediated by the E2F family.

These multiple functions of pRb in various differentiation systems point out the myriad changes that accompany what now appears to be a pRb-coordinated differentiation and cell cycle exit program. As mentioned earlier, pRb directly binds to Runx2 to turn on genes of late differentiation in bone. Recently, however, Runx2 itself was shown to have independent cytostatic properties. Briefly, ectopically expressed Runx2 caused accumulation of $p27^{Kip1}$, which leads to dephosphorylation of pRb and G1 cell cycle arrest, thereby creating a feedforward loop where cell cycle exit promotes differentiation (Thomas et al., 2004). Interestingly, the proliferation arrest accomplished by RBP2 knockdown experiments in the $RB^{-/-}$ SAOS2 system by Benevolenskaya et al. showed an accumulation of $p27^{Kip1}$, as well $p21^{Cip1}$ and p130. However, not all aspects of the differentiation phenotype (the senescent cell morphology change) could be phenocopied by arresting $RB^{-/-}$ SAOS2 cells via the canonical E2F/pRb pathway, arguing that cell cycle arrest alone is insufficient to potentiate SAOS2 cells for differentiation.

Thus, while compelling in its potential to provide a global, mechanistic explanation for the role of pRb in differentiation and the accompanying cell cycle exit, the work of Benevolenskaya et al. leaves many questions unanswered. How is the cell halting proliferation in the absence of pRb? Is RBP2 inactivation sufficient for turning on a global differentiation cascade, or is it cell-type specific? Is it a complete differentiation program? Is RBP2 redundant to or interactive with the Id, EID-1, or E2F modes of differentiation induction?

To explore the function of RBP2 more fully, the authors looked for other promoters that RBP2 binds by using a ChIP-on-chip (the promoter region of 10,000 human genes) approach in a myelopoiesis differentiation system. They identified the promoters of two homeotic genes, BRD2 and BRD8. Intriguingly, by further characterizing these interactions, they determined that the RBP2/pRb interaction augments transcriptional activation of these genes. Based on the literature, the authors suggest that BRD2 and -8 might be necessary for the maintenance of the active chromosomal architecture of genes necessary for differentiation-homeostasis in postmitotic cells. Taken together, the data presented by Benevolenskaya et al. suggest a biphasic effect of the RBP2/pRb complex: pRb binds RBP2, neutralizing its repression on cell type-specific genes. This RBP2 complex is then free to act as a coactivator for genes that promote stable terminal differentiation (Figure 1).

Much more work will be needed to explore how this growing number pRb interlocutors interact in order to coordinate differentiation with cell cycle exit. Nevertheless, the work of Benevolenskaya et al. highlights a growing view that cancer is not a disorder of proliferation but of cell differentiation (Harris, 2004) and that pRb is likely a central decision maker in this process.

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