Functions of pRb and p53: what's the connection?

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The pRb and p53 proteins have tumour suppressor functions and both are regulators of transcription. Their mechanisms of transcriptional regulation are unrelated in many ways – in contrast, it would appear, to their biological functions. This review highlights their biological connections in the light of recent advances in understanding the mechanisms of pRb and p53 function.

The pRb and p53 proteins play key roles in the regulation of cell proliferation. Inactivation of the genes that express them is often found to accompany tumour development and virally-induced cell transformation. Their biological characteristics are also consistent with the control of cell proliferation: they both induce arrest in the G1 phase. However, this is clearly a simplistic definition of their function since both proteins have other, possibly complementary, roles – pRb is implicated in the induction of differentiation and p53 is involved in apoptosis and DNA repair.

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Functions of the pRb protein

The tumour suppressive functions of pRb are attributed to its ability to regulate transcription. A major target for pRb is the S-phase-inducing transcription factor complex E2F1–DP1 (reviewed in Ref. 1). Binding of pRb to the activation domain of the E2F1 protein silences its function, and in this way pRb is thought to suppress S-phase induction. The 'flip side' of cell proliferation is differentiation, and there is evidence that this is also regulated by pRb. Regulation of differentiation also involves interaction with transcription factors, but this time the target of pRb is a set of differentiation-specific factors, such as myoD and Elf1 (reviewed in Ref. 2).

The pRb protein has a highly mutation-prone pRb 'pocket domain', which is composed of subdomains A and B (Fig. 1). The pocket is required for arrest in G1 phase and it is the site for E2F1 binding. Recently, a sequence C-terminal to the pRb pocket domain was defined as a distinct functional region (C-domain; Fig. 1). Sequences within the C-domain can suppress pRb-induced G1 arrest in a manner that does not involve the removal of pRb from E2F1 (Ref. 3). Interestingly, the C-domain can independently bind two proteins, c-Abl (Ref. 3) and MDM2 (Ref. 4), each of which has been implicated in growth-controlling processes. MDM2 acts as a regulator of transcription: it can bind to and repress the activation domain of p53 (Ref. 5) but can also bind and stimulate the activation domain of E2F1 (Ref. 6). These molecular events may explain the oncogenic capacity of MDM2 and provide an explanation for the MDM2–pRb interaction. However, it is still unclear whether pRb regulates MDM2 activity (by preventing it from stimulating E2F1) or whether MDM2 regulates pRb (by preventing it from repressing E2F1).

A novel mechanism of pRb-induced transcriptional regulation has come to light in the past year. The catalyst for this discovery was the identification of BRG1 (or its relative, hBRM) as a tumour suppressor protein that binds pRb via the pocket domain and cooperates with it to induce G1-phase arrest[7]. A molecular connection between hBRM and pRb was identified when these two proteins were shown to cooperate in stimulating the activity of the glucocorticoid receptor[8,9]. Because a yeast homologue of hBRM (SNF2) is implicated in chromatin remodelling, this raises the interesting possibility that pRb may regulate yet another set of genes by influencing chromatin structure.

The pRb protein has the capacity to bind a variety of DNA-binding transcription factors[2], suggesting that pRb may have many more targets. A recent report highlights a mechanism by which pRb may regulate these proteins[10]. When pRb binds to promoters, either via E2F1 contact or via a heterologous DNA-binding domain, it has the capacity to repress activators on the same promoter. In the case of one activator, PU.1, pRb was shown to displace the TATA-box-binding protein (TBP) from its activation domain. As the pRb pocket has sequence similarity to TBP and TFIIB (Fig. 1; Ref. 11), these data are consistent with the notion that pRb may repress transcription by mimicking basal factors.

The recent demonstration that pRb can also repress RNA polymerase I (polI)-specific transcription[12] suggests that pRb is a more universal regulator than was once thought. The target of pRb in this context is UBF (a polI-specific factor), which has a pRb-binding LXCXE motif within its DNA-binding domain. Since

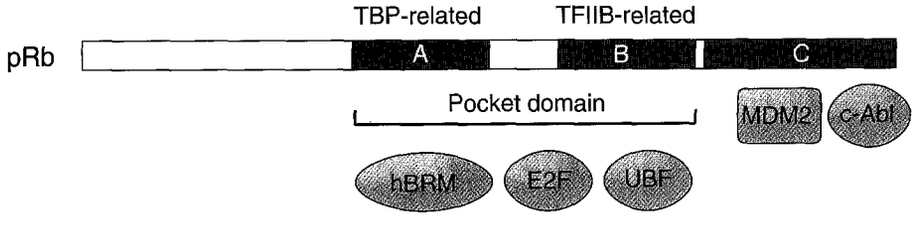

FIGURE 1

pRb has distinct functional domains that bind different proteins. The pocket domain is composed of two subdomains, A and B, which have sequence similarity to TATA-box-binding protein (TBP) and TFIIB, respectively. The pocket domain is essential for the binding of the proteins shown below: E2F (Ref. 1), UBF (Ref. 12) and human BRM (Ref. 7). A distinct domain of pRb, domain C, is sufficient for the binding of MDM2 (Ref. 4) and c-Abl (Ref. 3).

the mutation-prone pRb pocket is required for this polI-specific repression, these results imply that regulation of biosynthetic pathways may be important in the control of cell proliferation.

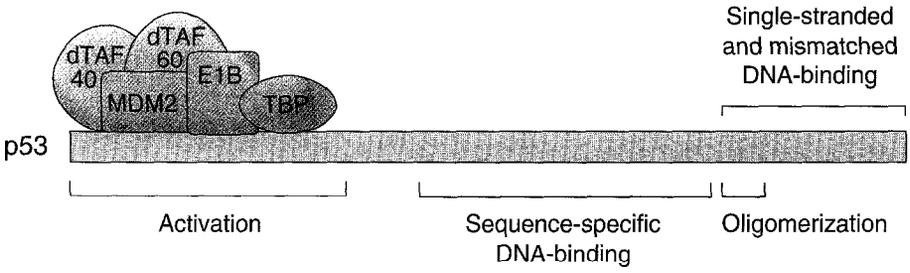

FIGURE 2

p53 contains separate domains required for transcriptional activation, sequence-specific DNA-binding, oligomerization and binding to mismatched DNA. The activation domain binds a number of proteins that regulate its function, including MDM2 (Ref. 5), E1B (Ref. 13), TBP (Ref. 18), *Drosophila* TAF40 (Ref. 14) and *Drosophila* TAF60 (Ref. 14).

Functions of p53

The p53 protein is a tumour suppressor with a specific task, namely the maintenance of DNA integrity. The normally low level of p53 in the cell is increased to high levels in response to DNA damage. At this point, the raised level of p53 protein can, first, stop the cell from proceeding into the DNA replication phase, thus allowing DNA repair, or, second, induce apoptosis, presumably when conditions are such that DNA repair would be futile.

p53 can act as a transcriptional activator. It can bind a specific DNA element by means of a central, 'core' DNA-binding domain, and can activate transcription by using its N-terminal activation domain (Fig. 2). The function of the activation domain is regulated by the binding of proteins. MDM2 and adenovirus E1B protein repress its activity, whereas two proteins, dTAF60 and dTAF40, function as co-activators. MDM2 may repress transcriptional activation by displacing these two TAFs from p53 since mutations in p53 that affect the binding of MDM2 also affect TAF binding[13,14].

A number of genes activated by p53 following DNA damage have now been identified. However, the activation functions of p53 correlate well only with its ability to induce G1 arrest[15]. Other p53 functions, such as apoptosis[16] and suppression of transformation[17], do not seem to require its activation functions.

A second and distinct transcriptional effect of p53 is the repression of transcription[18]. This function is mediated via TATA-box elements when sites for p53 binding are absent in the promoter of the target gene. Although the physiological function of p53-induced repression has not yet been established, there are some indications that it may be linked to p53-mediated apoptosis[19,20].

Recently, the p53 C-terminus was shown to possess a novel DNA-binding activity that mediates binding to mismatched and single-stranded DNA[21,22]. Stable protein–DNA complexes involving tetramers of this p53 domain have been visualized on DNA that contains mismatches[21]. These results strongly implicate p53 in the process of DNA repair, possibly as a primary trigger for the assembly of the repair machinery. In the full-length p53 protein, the C-terminal domain appears to mask the DNA-binding functions of the central domain through a conformational process. Release of core DNA-binding functions can be achieved by phosphorylation of the C-terminus and these functions can also be activated by the binding of the C-terminal domain to single-stranded DNA[22]. These data point to a model whereby binding of p53 to damaged DNA may activate the protein for binding to p53-specific DNA elements (via the core domain) and subsequently lead to activation of transcription.

The p53–pRb connection

The breakthrough in beginning to understand how the functions of these two tumour suppressor proteins are connected came from the study of lens development in pRb- and p53-deficient mice embryos. The results from such experiments (reviewed in Ref. 23) suggest that, in *RB*-null mice, uncontrolled proliferation resulting from the loss of pRb function is kept in check by p53-mediated apoptosis. However, when both p53 and pRb are absent (in *RB*-null *p53*-null mice) p53-induced apoptosis does not occur and uncontrolled cell growth ensues. Turning this around, this means that pRb can protect cells from p53-induced apoptosis. The protective effect of pRb has now been demonstrated in tissue culture cells[24]. In addition, pRb seems to protect against

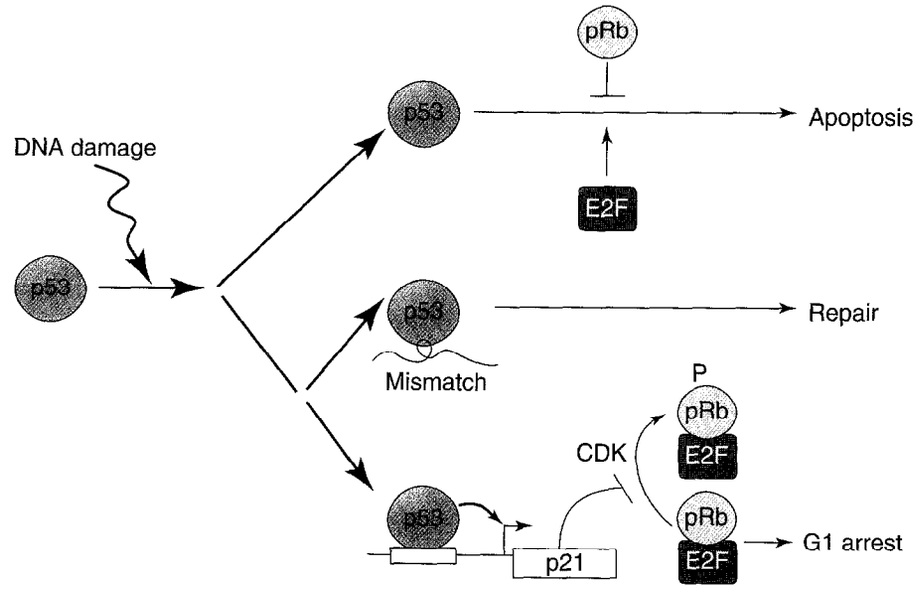

FIGURE 3

Following DNA damage, p53 can either mediate apoptosis or G1 arrest and DNA repair. pRb has been implicated in preventing apoptosis and as being the downstream effector of p53-induced G1 arrest. CDK, cyclin-dependent kinase; P, phosphate.

non-p53-dependent apoptosis[25]. Thus, pRb may now be thought of as an anti-apoptotic device, although this may be a consequence of its function, rather than a primary feature (Fig. 3).

The mechanism by which pRb protects cells from apoptosis remains obscure. A possible clue is the observation that p53 can cooperate with E2F1 to induce apoptosis[26]. This points to a model whereby inappropriate entry into S phase, due to release of the restraining power of pRB, may be an apoptotic trigger (reviewed in Ref. 27). However, there is the alternative argument that high-level expression of E2F1 may induce apoptosis by sequestering a protein required to prevent this process. The recent observation that p53 and E2F1 have homologous activation domains[6], with similar protein-binding sites, highlights this possibility.

Apoptosis is only one side of the p53 coin. G1 arrest is the other and, again, there is evidence for connections between the p53 and pRb pathways. The link was first established when p53 was found to induce the expression of the gene encoding p21 (also known as WAF1, Cip1, Sdi1, p20CAP, Pic1), an inhibitor of cyclin-dependent kinases (CDKs; Refs 28–30). Since the CDKs are responsible for the phosphorylation and removal of pRb from E2F1, the prediction was that expression of the gene encoding p21 would prevent the release of pRb from E2F1, leading to G1 arrest (Fig. 3). Evidence consistent with the model that pRb is a downstream effector of p53-induced G1 arrest has recently been reported[31].

Thus, pRb appears to be involved in both major pathways that p53 levels control – apoptosis and G1 arrest. A possibly parallel pathway to G1 arrest, is p53-induced DNA repair (Fig. 3). The observations that p53 binds mismatched DNA[21] and interacts with the excision-repair protein ERCC3 (Ref. 32) suggest that p53 may be directly involved in mediating the DNA-damage response. This raises the possibility that pRb may also be involved in this p53-mediated process, as it is in apoptosis and G1 arrest. Only time will tell.

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