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## Transcription factors that interact with p53 and Mdm2

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### Abstract

The tumor suppressor p53 is activated upon cellular stresses such as DNA damage, oncogene activation, hypoxia, which transactivates sets of genes that induce DNA repair, cell cycle arrest, apoptosis, or autophagy, playing crucial roles in the prevention of tumor formation. The central regulator of the p53 pathway is Mdm2 which inhibits transcriptional activity, nuclear localization, and protein stability. More than 30 cellular p53-binding proteins have been isolated and characterized including Mdm2, Mdm4, p300, BRCA1/2, ATM, ABL, and 53BP-1/2. Most of them are nuclear proteins; however, not much is known about p53-binding transcription factors. In this review, we focus on transcription factors that directly interact with p53/Mdm2 through direct binding including Dmp1, E2F1, YB-1, and YY1. Dmp1 and YB-1 bind only to p53 while E2F1 and YY1 bind to both p53 and Mdm2. Dmp1 has been shown to bind to p53 and block all the known functions for Mdm2 on p53 inhibition, providing a secondary mechanism for tumor suppression in *Arf*-null cells. Although E2F1-p53 binding provides a checkpoint mechanism to silence hyperactive E2F1, YB-1 or YY1 interaction with p53 subverts the activity of p53, contributing to cell cycle progression and tumorigenesis. Thus, the modes and consequences for each protein-protein interaction vary from the viewpoint of tumor development and suppression.

### Keywords

transcription factor; Dmp1 (Dmtf1); p53; Mdm2; E2F1; YB-1; YY1; Arf; polycomb; apoptosis; cancer

### Introduction

The tumor suppressor p53 is activated upon cellular stresses such as DNA damage, oncogene activation, or hypoxia; and initiates a series of transcriptional programs that induces DNA repair, cell cycle arrest, apoptosis, or autophagy (1, 2). A central regulator of the p53 pathway is the Mdm2 protein (Hdm2 in humans) that inhibits transcriptional activity, nuclear localization, and protein stability of p53 (for reviews, 3–5; Fig. 1). The *Mdm2* gene is transcriptionally activated by p53 through its direct binding to the p53-responsive elements located within the P2 promoter (4). Mutations in *TP53* that disrupt p53 function occur in nearly 50% of human cancers (6, 7), and the alteration of regulators of p53

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occurs in most of the remainder, and thus p53 is functionally inactivated in nearly all cancer cells.

The activity of Mdm2 is negatively regulated by p19<sup>Arf</sup> (p14<sup>ARF</sup> in humans) in response to oncogenic stress (8–12). p19<sup>Arf</sup> directly binds to Mdm2, and thereby stabilizes and activates p53. The Arf induction by potentially harmful growth-promoting signals forces early-stage cancer cells to undergo p53-dependent and p53-independent cell cycle arrest, apoptosis, or autophagy, thus providing a powerful mode of tumor suppression (8–12). The *Arf* promoter monitors early stage oncogenic signals *in vivo*, and hence *Arf*-null mice are highly prone to spontaneous tumor development (13, 14). The *Arf* promoter is directly activated by E2Fs and Dmp1 (cyclin D binding myb-like protein 1; Fig. 2A) (15–18) while it is repressed by polycomb repressive complex 1 (PRC1) proteins: BMI1, PCGF1, PCGF2/MEL18, CBX2/7/8 RING1B; PRC2 proteins: EED, SUZ12, EZH2, and long non-coding RNA *ANRIL* (19).

E2Fs are a group of transcription factors (TFs) that regulate cell cycle, DNA repair, replication, and mitochondrial function through making heterodimeric complexes with dimerization partners, DPs (for reviews, 20–22; Fig. 2B). The E2F family proteins are generally grouped by function into two categories: transcription activators and repressors. Activators such as E2F1, E2F2, and E2F3a promote and help cell cycle progression while repressors (E2F3b, E2F4-8) inhibit the cell cycle. Pocket proteins such as pRB and related proteins p107 and p130, can bind to E2F when hypophosphorylated (Fig. 2B). Cyclin D/Cdk4(6) and cyclin E/cdk2 phosphorylate pRB, p107, and p130 allowing them to dissociate from E2F/DP. The inactivating function of cyclin/cyclin-dependent kinases is opposed by the Ser/Thr protein phosphatases PP2A and PP1 (23). In activators, E2F binding with pRB has been shown to mask the transactivation domain responsible for transcription activation (20, 21). E2F activator levels are cyclic, with maximal expression during G1/S. In contrast, E2F repressors stay constant, and they are often expressed in quiescent cells. Activator E2Fs transactivate S phase-promoting genes, such as *DNA polymerase  $\alpha$  dihydrofolate reductase*, and *thymidine kinase* (20, 21). E2F1, the prototype of activator E2Fs, was found to be a repressor of *hTERT* transcription by directly binding to its promoter, thereby shortening telomere length by inhibiting hTERT protein expression (24). E2F1 also represses the transcription of tumor suppressor genes such as *Dmp1* and *ARH1* (25). Among E2F proteins, only E2F1-3a has a nuclear localization signal and interacts with p53.

The Y-box-binding protein YB-1, also known as nuclease-sensitive protein 1 (NSEP1), is a member of the cold shock family of proteins that contain a highly conserved nucleic acid-binding motif (26) named Y-box to which all the family members bind (Fig. 3A), and is very similar to the common CCAAT box. Y-box proteins contain a cold-shock domain, which function as RNA chaperones, RNA splicing, translation, DNA repair, and transcription (26; Fig 3A). YB-1 directly binds to p53, and the interaction of YB-1 with p53 affects the sequence-specific DNA-binding of p53 to its consensus sequences (27).

YY1 (Yin Yang 1) is a nuclear protein that is ubiquitously expressed in all tissues and highly conserved from *Xenopus* to human (28). The name “Yin Yang” represents its two opposite functions as a TF to act as either a repressor or an activator (28, 29). YY1 is a TF

belonging to the GLI-Krüppel class of zinc finger proteins (Fig. 3B). It has fundamental roles in embryogenesis, cell proliferation, and differentiation (28, 29). YY1 exerts its biological effects through its ability to transactivate or repress gene expression, depending upon the genes to which it binds. Recent studies show that YY1 indirectly activates or represses gene expression without DNA-binding, through interaction with histone modifiers and chromatin remodeling proteins (28, 29). In addition to directly binding to p53, YY1 also binds to Arf and Mdm2 and enhances p53 ubiquitination and degradation, thus YY1 is a negative regulator of p53 (30).

This review will summarize the molecular basis and biological consequences of p53 protein-protein interactions with these TFs to demonstrate the differences in how these TFs might function to promote or prevent tumorigenesis. The manipulation of these p53 protein interactions represents a promising therapeutic avenue to promote tumor cell death in cancers that have intact *p53* genes, but nevertheless p53 is functionally inactive.

## Dmp1

We have identified the *Arf* regulating transcription factor Dmp1 (cyclin **D** binding **myb**-like protein **1**; also named Dmtf1; refs. 15–18, 31–42) as a direct interacting molecule for p53 (Fig. 2A; 31). The original form for Dmp1 (Dmp1 $\alpha$ ) has been isolated in a yeast two-hybrid screen of a murine T cell library with cyclin D2 bait (15). We recently detected endogenous hDMP1-cyclin D1 complex in the lysate of human breast cancer (40). When *Dmp1* was cloned, it was expected to be a regulator of the Rb pathway, but later studies have shown that Dmp1 is a critical transcriptional activator for *Arf* (17, 18). The gene product, p19<sup>Arf</sup> (or p14<sup>ARF</sup>) stabilizes nucleoplasmic p53 by binding to Mdm2, sequesters it in the nucleolus, and directly inhibits the ubiquitin ligase activity of Mdm2 (8–12). *DMP1* is a tumor suppressor deleted in ~35% of human non-small-cell lung carcinomas and 42% of breast cancer (32–34). Mitogenic signals from oncogenic Ras (35) and HER2/neu (36) have been shown to activate the *Dmp1* promoter, while physiological mitogens (25) as well as genotoxic stimuli mediated by NF- $\kappa$ B (37) cause repression. *E $\mu$ -Myc*, *K-ras<sup>LA</sup>*, and *HER2/neu*-driven tumor development was significantly accelerated in both *Dmp1*<sup>+/-</sup> and *Dmp1*<sup>-/-</sup> mice with no significant differences in the survival between the two cohorts, suggesting that Dmp1 is haplo-insufficient for tumor suppression (18, 32, 36, 38–40). We recently showed that Dmp1 $\alpha$  has the ability to activate both p53 and Rb pathways and prevent tumor development in *MMTV-neu* mammary tumor model (41). In *E $\mu$ -Myc* lymphomas, the combined frequencies of p53 mutation and *Arf* deletion in mice of *Dmp1*<sup>+/-</sup> or *Dmp1*<sup>-/-</sup> background were much lower than that in *Dmp1*<sup>+/+</sup> littermates, indicating that Dmp1 is a physiological regulator of the Arf-p53 pathway *in vivo* (39). Of note, the frequency of p53 mutation (~40%) was significantly decreased in both *Dmp1*<sup>+/-</sup> and *Dmp1*<sup>-/-</sup> backgrounds (<10%) even in *K-ras<sup>LA</sup>* lung cancer where the *Ink4a/Arf* involvement was rare (32). This suggested an *Arf*-independent mechanism of p53 regulation by Dmp1 in epithelial tissues. Dmp1 $\alpha$  regulates genes other than *Arf* (e.g. *Tsp-1*, *JunB*, and *Egr1*) and contributes to tumor suppression (42).

In our recent study (31), we searched for binding partners for the Dmp1 $\alpha$  protein to explain the *Arf*-independent function of Dmp1 in tumor suppression. We found that Dmp1 $\alpha$

physically bound to p53, but not to Mdm2, Arf, c-Myc, or c-Myb (31). Dmp1 $\alpha$  binding to p53 neutralized the activity of Hdm2 on p53's ubiquitination in H1299 cells with *ARF* deletion. Conversely, DMP1 $\beta/\gamma$  lack most of the amino acid sequence for the p53 interaction, and thus will not directly affect the activity of the p53 pathway (43). We also showed that Dmp1 $\alpha$  neutralized the activity of Hdm2 using recombinant proteins prepared in bacteria (Mdm2, ubiquitin) and Sf9 cells (Dmp1) (31). Dmp1 antagonized nuclear export of p53 by Hdm2 in transfection studies conducted in H1299 cells, which was confirmed in *ARF*-null MCF7 cells by depleting endogenous *hDMP1* using shRNA (31). We mapped the Dmp1-binding domain at the carboxyl-terminus of p53 (Fig. 1A; 31), suggesting that Dmp1-p53 binding does not interfere with p53 binding to target genes or physical interaction between Mdm2 and p53. Conversely, the p53-binding domain was mapped to the DNA-binding domain of Dmp1 (Fig. 2A), and thus binding of Dmp1 to p53 and DNA are mutually exclusive (31).

To study the significance of Dmp1-p53 interaction *in vivo*, doxorubicin was injected in wild-type and *Dmp1*-null mice, and binding of p53 to target gene (*p21<sup>Cip1</sup>* and *bbc3*) promoters was studied by chromatin immunoprecipitation in thymus (31). Significant binding of p53 to the *p21<sup>Cip1</sup>* and *bbc3* promoters was found in wild-type, but not in *Dmp1*-null thymus, suggesting that Dmp1 is indispensable for p53-binding to target genes (31). When injected with doxorubicin, both *p21<sup>Cip1</sup>* and *bbc3* increase was more significantly subverted in *Dmp1<sup>-/-</sup>* mice than in *Arf<sup>-/-</sup>* mice; thus our data indicate that Dmp1 activates p53 target genes independent of *Arf* in response to the genotoxic drug (31). Together, our data indicate that (i) expression of Dmp1 $\alpha$  interferes with the known activities of Hdm2 on the ubiquitination and nuclear localization of p53 in an *Arf*-independent fashion and (ii) p53-binding to target genes and their inductions are *Dmp1*-dependent. Interestingly, the most representative molecule that neutralizes all the activities of Mdm2 on p53 is p19<sup>Arf</sup>, which is a direct target of Dmp1 $\alpha$ . Hence Dmp1 $\alpha$  is an opponent regulator for Mdm2 by directly transactivating the *Arf* transcription as well as by direct binding to p53 in *Arf*-null cells (31).

## E2F1

During the cell cycle, the expression of E2F1-3a and cyclin A are both precisely regulated: the levels of these E2Fs are high at the G<sub>1</sub>/S transition, whereas cyclin A is high at mid-S to G<sub>2</sub>/M (44). Thus G<sub>1</sub>/S transition is the time when E2F1-3a are important in cell cycle regulation because cyclin A/CDK2 binds directly to E2F1 and inhibits its DNA-binding by phosphorylation (45). Like pRb, p53 only binds to E2F1, 2, and 3a (Fig. 2B) through the conserved domain in the N-terminus, which is not present in E2F3b, 4–8 (46). The p53-binding domain was mapped in or around the cyclin A-binding domain of E2F1 since 1–119 24 mutant did not show any p53-binding (Fig. 2B; 47). Since the p53-binding domain in E2F1-3a also binds to cyclin A, cyclin A and p53 potentially compete for binding to E2Fs.

Nipp et al. found that the transactivation domain for E2F1 was dispensable for p53 induction by E2F (47); therefore it was expected that E2F1-p53 complex does not bind to E2F-consensus sequences, just like Dmp1-p53. Fogal et al. reported that the amino terminal domain in E2F1 (a.a. 1–108) binds to amino acid residues 347–370 of p53 that overlaps its

C-terminal nuclear export sequence (NES), enhancing nuclear retention of Ser315-phosphorylated p53, and thus inducing p21<sup>Cip1</sup>, PIG3, and bax (46). Phosphorylation of p53 at Ser315 destabilizes the p53 tetramer by exposing its NES and increases the likelihood for p53 to be exported into the cytoplasm (46). It should be noted that Ser315 is the only site in p53 that is phosphorylated by cyclin-dependent and aurora kinases that regulate cell cycle progression (46). E2Fs' preferential binding to Ser315-phosphorylated p53 through protein-protein interaction is cell cycle-dependent and interferes with the degradation of p53 in the cytoplasm, leading to activation of p53 (Fig. 4B). E2F1-p53 interaction thus provides a checkpoint function to provide a feedback loop to silence the activities for E2F1 (46). Zhou et al. recently showed that p53 interacts with E2F1 to form p53-E2F1-DNA complex suppressing E2F1-dependent *PLK1* expression promoting apoptosis in response to DNA-damage (48). In this case, the protein complex binds to the E2F target, not the p53 target, thus the mode and significance of p53-E2F1 binding is different dependent on the biological context. Although E2F1 specifically binds to p53 and stimulates its DNA-binding, transactivation, and apoptotic functions of the protein, it does not bind to p63 or p73 (for p63 and p73 review, see ref. 49) showing the specificity of E2F1-p53 interaction.

E2F1 physically interacts with Mdm2 through a.a. 180–298 (50; Fig. 2B). Since E2F1 uses the DP-binding domain for the physical interaction with Mdm2, it is speculated that the E2F1-Mdm2 complex does not bind to DPs. Mdm2 prolongs the half-life of the E2F1 protein by inhibiting its ubiquitination. Mdm2 displaces the E2F1 E3 ligase SCF (SKP2) through direct binding independent of pRB or ARF (50). Thus stabilization of the E2F1 protein is one p53-independent mechanism of Mdm2-mediated tumorigenesis (50). Physical interaction of E2F1 and Mdm2 in the nucleus contributes to TAp73 transcriptional activity, suggesting a novel regulation pathway of TAp73 (51).

## YB-1

YB-1 acts as a regular TF through its property of binding to double-stranded DNA called the Y-box, reverse CCAAT sequences. YB-1 must translocate from the cytoplasm to nucleus for any activities in the nucleus since the majority of the YB-1 protein is in the cytoplasm, much like the mechanism for NF- $\kappa$ B activation (52). Nuclear translocation of YB-1 and transactivation of target genes occur in response to a variety of stresses that include UV exposure, DNA-damaging agents, hyperthermia, and cytokines (53–55). YB-1 must first be cleaved (Fig. 3a) and then only the N-terminal portion translocates to the nucleus, which occurs exclusively at the G<sub>1</sub>/S phase boundary (55, 56). Nuclear accumulation of YB-1 is associated with transactivation of the *cyclin A/B1* genes and a subsequent increase in the cyclin A/B1 proteins (56). This process is dependent on binding to the splicing factor SRp30c, and requires Akt-dependent phosphorylation of YB-1 (57).

It has been hypothesized that YB-1 has a role in promoting cell proliferation and tumorigenesis since Y-box elements are present in the promoters of several genes whose activity is associated with cell division, e.g. *epidermal growth factor receptor (EGFR)*, *c-ErbB2*, *proliferating cell nuclear antigen (PCNA)*, *DNA polymerase  $\alpha$* , *thymidine kinase*, and *topoisomerase IIa*; among these, *PCNA*, *DNA polymerase  $\alpha$* , *thymidine kinase* are classical E2F targets as well (21). Indeed, Berquin et al. screened a cDNA expression library

for genes that mediate EGF-independent proliferation of human mammary epithelial cells (HMECs) (58), and isolated the YB-1 cDNA, which conferred growth factor independence to HMECs.

In addition to transactivation for genes essential for cell proliferation, YB-1 represses the transcription of cell death-promoting genes, such as *FAS* and *p53* by binding possible Y-box sequences (59). YB-1 expression in tumor specimens thus correlates with a progressed clinical stage and poor prognosis of patients in a variety of human cancers, including malignancies of the lung, breast, colon, and prostate (60). Overexpression of YB-1 is frequently observed in drug-resistant tumors since its expression is closely associated with that of P-glycoprotein encoded by the *multidrug resistance 1* gene and *mitogen-activated protein kinase-interacting serine/threonine kinase 1* gene responsible for impairing trastuzumab resistance (60, 61). The oncogenic activity of YB-1 has been demonstrated *in vivo* using a mouse model for breast cancer (62).

YB-1 directly binds to p53 both *in vitro* and *in vivo* and the interaction of YB-1 with p53 modifies the sequence-specific DNA-binding of p53 to its consensus sequences (27). It has been shown that three independent domains of YB-1 interact with p53 (27; Fig. 3A; a.a. 1–51, 129–205, and 272–324). Conversely, a 14 amino acid sequence at the C-terminal of p53 (a.a. 363–376) is required for its interaction with YB-1 (27; Fig. 1A). YB-1 and p53 also interact with a common transcription factor AP2 to co-regulate the *gelatinase A* gene that facilitate neo-angiogenesis and distal metastases (63). Importantly, YB-1 prevents p53 from inducing apoptosis by inhibiting p53 from transactivating pro-apoptotic target genes such as *APAF-1*, *Noxa*, and *Bax* (59, 64). The apoptosis stimulating p53-binding proteins, ASPP1 and ASPP2, are the first two common activators of the p53 protein family that selectively enable the latter to regulate specific apoptotic target genes, such as *Bax* and *Pig3*. YB-1 thus acts in an opposite manner to ASPP1/2 that binds to p53. Transcriptionally active p53 is required for nuclear localization of YB-1 suggesting that p53 transactivates effector(s) for YB-1 nuclear translocation (64).

Although YB-1 inhibits the ability of p53 to cause cell death, YB-1 does not interfere with the ability of p53 to transactivate *p21<sup>Cip1</sup>* nor *Mdm2* (Fig. 4C). In contrast to E2F1-p53 binding, YB-1-p53 interaction does not provide a checkpoint mechanism to quench hyperactive YB-1 signaling in cell cycle progression/tumor development (Fig. 4C). Selective alteration of the p53 activity by nuclear YB-1 provides an explanation for the correlation of YB-1 with drug resistance and poor tumor prognosis. Since inhibition of YB-1 upregulates p21<sup>Cip1</sup> expression with p53-dependent apoptosis in human cancer cell lines (59), the YB-1-p53 interaction is an ideal molecular target for drug screenings for future cancer therapy.

## YY1

The domain structure for YY1 has been extensively studied (30; Fig. 3B). The viral oncogene product Adenovirus E1A regulates the transcriptional activity of YY1 by converting it from a repressor to an activator, suggesting an important role of YY1 in cell proliferation (28, 29). YY1 plays essential roles in cell proliferation and differentiation by

interacting with numerous proto-onco- (e.g. c-Myc, c-Fos, EGFR, c-ErbB2, and Mdm2) and tumor-suppressor genes/proteins (e.g. ARF, p53, p73, and Rb; refs. 28, 29). YY1 contributes to breast cancer development by accelerating p27<sup>Kip1</sup> ubiquitination through direct binding (65). The *YY1* gene expression itself has been shown to be regulated by prohibitin through an E2F1-binding element (66). YY1 expression is associated with chemo- and immune-resistance in cancer therapy (28, 29), and thus is a novel therapeutic target.

Constitutional ablation of *yy1* in mice results in peri-implantation lethality and hence phenotypic analysis of the whole animal is not possible (67). The same group later created conditional knockout mice bearing an *yy1<sup>fllox</sup>* hypomorphic allele (68). Genetic crosses of *yy1<sup>fllox</sup>* mice with *yy1<sup>+/-</sup>* mice allowed them to generate mutant mice expressing 75%, 50%, and 25% of the normal *yy1* level. Consistent with the data from tissue culture studies, phenotypic analysis of these mice and corresponding embryonic fibroblasts revealed a critical, dosage-dependent requirement for *yy1* in late embryonic development and cell proliferation (68). Specifically, *p21<sup>Cip1</sup>*, *p27<sup>Kip1</sup>*, *p57<sup>Kip2</sup>*, *Btg2*, *Gadd45 $\gamma$* , *Dnmt3a*, and *TGF $\beta$ 2* genes were upregulated in *yy1<sup>fllox/-</sup>* and/or *yy1<sup>-/-</sup>* murine embryonic fibroblasts while *Suv39h2*, *Polr1a*, *cyclin B1/B2*, *Plk1*, and *Cdc6* were downregulated, confirming that *yy1* regulates various genes involved in cell cycle progression, cytokinesis, and differentiation as well as apoptosis, oncogenic transformation, and DNA repair (68). Although YY1 has been suggested to play significant roles in oncogenesis, no transgenic studies have been conducted for *in vivo* tumor development and would therefore be an interesting topic for future studies.

YY1 physically interacts with p53, Mdm2, and ARF. Sui *et al.* reported that YY1 depletion resulted in p53 accumulation due to a reduction of p53 ubiquitination in cells (30). YY1 directly binds to both Hdm2 and p53 and enhances p53 ubiquitination and degradation, thus YY1 is a negative regulator of p53 (30). GST pull-down assay showed that YY1 binds to C-terminal regulatory region of p53 through two different regions on YY1 (a.a. 142–224, 331–414 [zinc finger domain]; Fig. 3B). Because the p53-binding domain is different from the Hdm2 interaction domain (a.a. 201–295, mainly 226–295, Fig. 3B), and because YY1 and Hdm2 interact with p53 through different regions (a.a. 300–393 for YY1 and a.a. 1–42 for Hdm2; Fig. 1A), it was speculated that YY1, Hdm2, and p53 make a ternary complex, which was proved by glycerol gradient centrifugation (30; Fig. 4D). YY1 promotes p53 polyubiquitination through Hdm2 since the YY1 mutant defective for Hdm2-binding failed to augment the physical interaction between Hdm2 and p53. It was also speculated that the YY1-p53 complex recognizes p53 targets rather than YY1 targets since p53 occupies the DBD of YY1 when bound (Figs. 3B, 4D).

YY1 inactivates p53 through other mechanisms as well. YY1 inhibits p300-mediated p53 acetylation and reduces p53-activated transcription (69). Thus, blocking p53 acetylation decreases its transcriptional activity and facilitates Mdm2-mediated p53 ubiquitination and degradation. YY1 directly binds to p14<sup>ARF</sup> by relocating it into the nucleolus or by forming tripartite complex with Hdm2 and p53 (30). In summary, these activities of YY1 result in the same consequence: inactivation of p53 (Fig. 4D). The activity of YY1 is regulated by various post-translational modifications such as phosphorylation (28, 29), sumoylation (70), and glycosylation (71). Smurf2 physically interacts with YY1, induces the

polyubiquitination of YY1 and shortens the half-life of the protein (72, 73), thus relieving the suppression of p53 activity.

As a member of the polycomb group (PcG) protein family, YY1 has been shown to regulate gene silencing without binding to DNA (74). The functional role of YY1 has been characterized in the developmental studies of *Drosophila* using two orthologs of YY1, *pleiohomeotic (pho)* and *pho like (phol)* (75). *Pho* and *phol* recruit the polycomb group silencing complexes to chromatin and establish gene repression (76). The region responsible for recruiting the PcG complex was mapped to the residues 201–226 of human YY1 protein called REPO (Fig. 3B; 29). Enhancer of zeste 2 (Ezh2) was identified as a *bona fide* oncogene, which has been used as a marker of cancers with aggressive potential (77). Ezh2 has been shown to be essential for cancer progression and invasion (78, 79), and thus increases the possibility of therapeutic failure (80). YY1 recruits Ezh2 to target promoters through the REPO domain; its overexpression in cancers promotes the methyltransferase activity of Ezh2 and causes aberrant epigenetics, which augments cancer progression (29, 81, 82).

YY1 influences the E2F activity through RYBP (Ring1- and YY1 binding protein; a member of the PcG group). RYBP has been identified as a molecule that interacts specifically with the E2F2/E2F3, dependent on the marked-box domain (83, Fig. 2B). The authentic YY1 transcriptional target, the *Cdc6* promoter contains adjacent E2F- and YY1-binding sites, and both are required for promoter activity. E2F2, E2F3, YY1, and RYBP bind to the *Cdc6* promoter and stimulate the transcription at G<sub>1</sub>/S phase of the cell cycle (83). Thus the ability of RYBP to mediate an interaction between E2F2/E2F3 and YY1 is an important component of *Cdc6* activation.

## Role of post-translational modifications in p53/Mdm2-TF interactions

The binding between purified Dmp1 $\alpha$  and p53 requires post-translational modification(s) since they bind to each other when they are prepared in Sf9 cells (31). Conversely, E2F1, YB-1, or YY1 binding to p53/Mdm2 does not require any post-translational modification(s) since purified bacterial recombinant proteins bind to p53/Mdm2 (27, 30, 46). Still the Dmp1-p53 binding is physiological since we saw significant binding of endogenous Dmp1 and p53 in thymus from mice injected with doxorubicin (31).

## Conclusive remarks, unanswered questions, and future directions

Although these four TFs directly bind to p53, the modes for interaction and the biological consequences are significantly different as summarized in Fig. 4. Dmp1 and YB-1 bind only to p53 while E2F1 and YY1 bind to both p53 and Mdm2. Dmp1-p53 binding blocks all the known functions for Mdm2 on p53 inhibition, providing a secondary mechanism for tumor suppression by Dmp1 $\alpha$ . While the E2F1-p53 binding provides a checkpoint mechanism to silence hyperactive E2F1, the interaction of YB-1 or YY1 with p53 subverts the activity of p53, contributing to cell cycle progression and tumorigenesis. *In vivo* tumor development assays using drug-inducible transgenic or conditional knockout mice for each TF with/without p53-knockout animals will provide physiological insights for such molecular interactions.

Whether or not these TFs bind to gain-of-function mutant p53 at higher or lower affinity than wild-type should be investigated to translate the research findings to clinical levels. Since more than half of human cancers highly express the YB-1 and/or YY1 proteins, inhibit p53, and confer drug resistance, gene silencing or acceleration of protein degradation of these molecules with small chemicals may be a novel strategy in cancer therapeutics.

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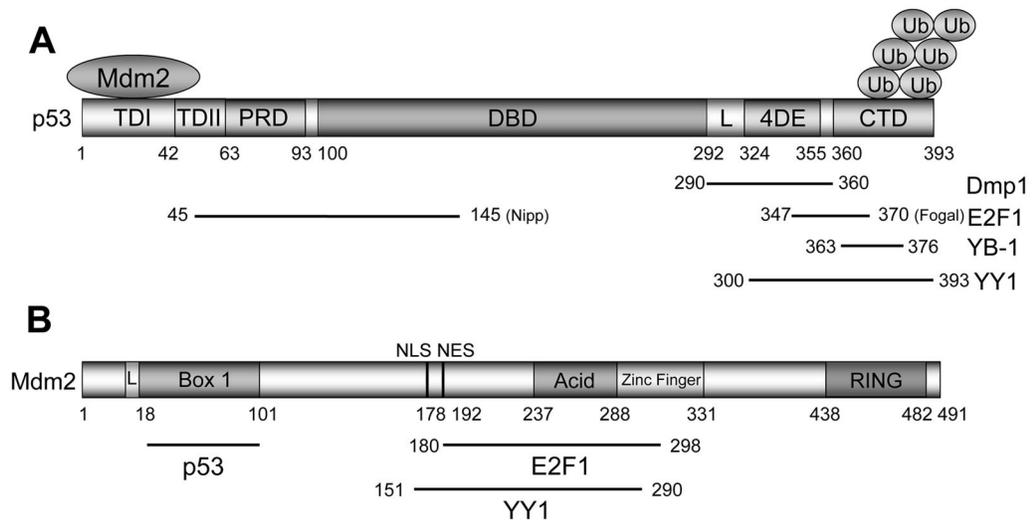
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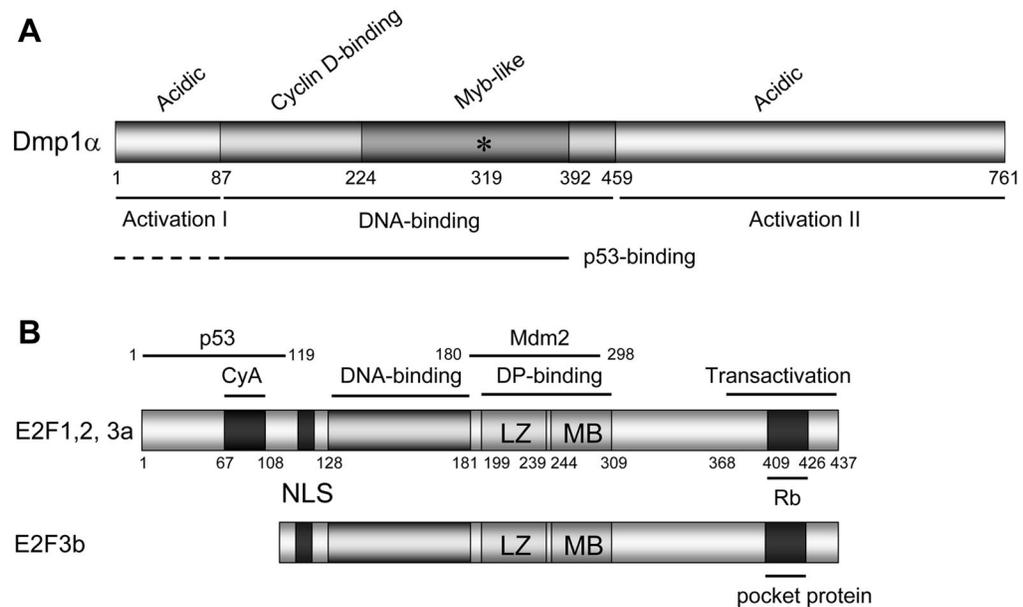
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**Figure 1. The domain structures of p53 and Mdm2 proteins**

(A) Schematic presentation of wild-type p53. Human p53 consists of 393 amino acids with 5 proposed domains. TD1: transactivation domain 1; TD2: transactivation domain 2; PRD: proline-rich domain; DBD: DNA-binding domain; L: nuclear import signal; 4DE: tetramerization domain; CTD: C-terminal regulatory domain. The 2<sup>nd</sup> transactivation domain for p53 was mapped between TD1 and PRD (84). Mdm2 quenches p53 transcriptional activity by occluding the p53 TD1 (a.a. 1–42). It also ubiquitinates lysines in the p53 CTD and accelerates nuclear export of p53.

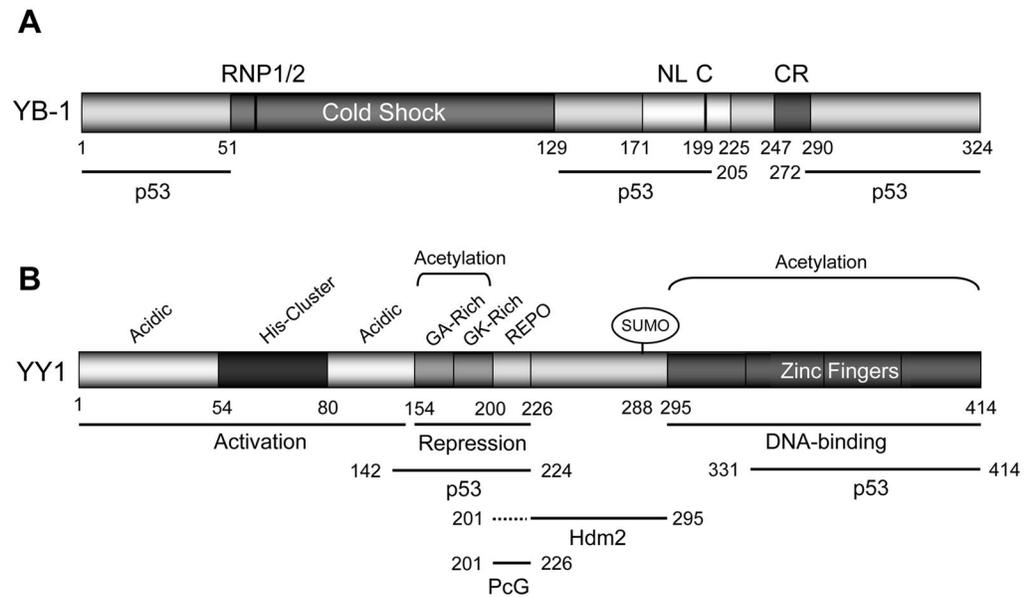
(B) The domain structure for Mdm2. The full-length transcript of the gene encodes a protein of 491 amino acids with a predicted molecular weight of 56kDa. The full-length protein migrates at ~90kDa in SDS-PAGE due to post-translational modification(s) and amino acid composition of the protein (85). This protein contains several structural domains including an N-terminal p53 interaction domain (Box 1). Phosphorylation of S17 in the N-terminal “lid” of Mdm2 is proposed to regulate the binding of p53 (L). The nuclear localization (NLS) and export (NES) signals that are essential for proper nuclear-cytoplasmic shuttling of Mdm2 have been mapped between Box 1 and the acidic domain (a.a. 237–288). The phosphorylation of residues within the acidic domain may stimulate its ability to target p53 for degradation. Another conserved domain within the Mdm2 protein is a zinc finger domain (a.a. 289–331), the function of which is poorly understood. Mdm2 also contains a C-terminal RING domain (a.a. 438–482) that has E3 ubiquitin ligase activity sufficient for auto-ubiquitination. Mdm2 physically interacts with YY1 through a.a. 151–290 that includes the entire acidic residues and N-terminal zinc finger domain.



**Figure 2. The domain structures of Dmp1 $\alpha$  and E2F1-3 proteins**

(A) Schematic presentation of wild-type Dmp1 $\alpha$ . Murine Dmp1 $\alpha$  consists of 761 amino acids (760 amino acids in humans) with the central DNA-binding domain flanked by two transactivation domains. The DNA-binding domain has three tandem Myb-like repeats. Dmp1 loses its DNA-binding activity by substituting Lys-319 into Glu (shown as an asterisk). D-type cyclins interact with Dmp1 through the amino-terminal DNA-binding domain (a.a. 87–224). Thus, Dmp1-cyclin D complexes do not bind to DNA (16). The p53-binding domains have been mapped to the DNA-binding domain for Dmp1 (a.a. 87–392), thus Dmp1-p53 and Dmp1-DNA interactions are mutually exclusive (31).

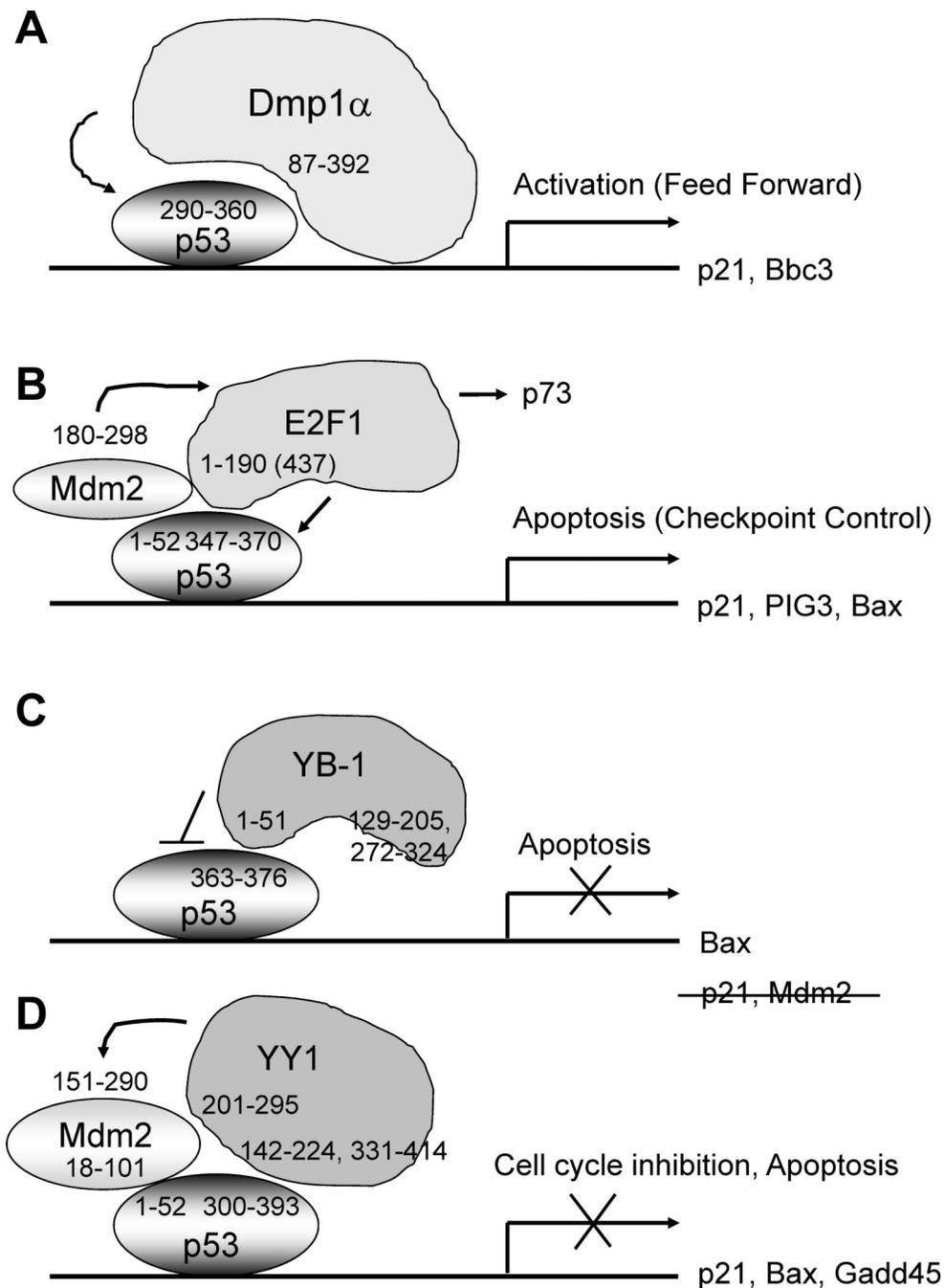
(B) The domain structures for E2F1-3. The E2F proteins have a core domain that mediate DNA-binding (a.a. 128–181) or dimerization with DP proteins (leucine-zipper [LZ: a.a. 199–239] and marked box [MB: a.a. 244–309] motifs). The transactivation and pocket protein-binding domains are present only in E2F1-E2F5 (20). Moreover, E2F1, E2F2, and E2F3a+b share a canonical basic nuclear localization signal that is absent in E2F4-E2F5, which have nuclear export signals instead (20). The major p53-binding domain has been mapped around the cyclin A-binding domain of E2F1.



**Figure 3. The domain structures of YB-1 and YY1 proteins**

(A) The structure of YB-1. YB-1 has a cold-shock DNA-binding domain with two transactivation domains at both amino- and carboxyl-termini through which it binds to p53. The cold-shock domain has RNP1/2-like motifs consisting of a five-stranded  $\beta$ -barrel structure that creates a surface that may act as a large nucleic acid-binding site. The inactive protein is in the cytoplasm (the cytoplasmic retention [CR] signal is at a.a. 247–290). The nuclear localization signals [NL] have been mapped to a.a. 171–225 by Jürchott et al. (56). This protein needs to be cleaved at a.a. 219–220 [C] before nuclear localization. The p53-binding domains have been mapped to N-terminal, middle, and C-terminal domains (a.a. 1–51, 129–205, and 272–324) (27).

(B) The structure for human YY1. Human YY1 consists of 414 amino acids with amino-terminal transactivation domain containing 11x His cluster with a carboxyl terminal zinc finger domain. The GA, GK-rich, and REPO domains have been shown to be responsible for transcriptional repression. p300 and CBP associated factor (PCAF) mediate the acetylation of residues 171–200 while PCAF also acetylates the C-terminal zinc finger domain of YY1. The REPO domain of YY1 binds and recruits PcG transcriptional repressors. p53 binds to YY1 amino acid residues 142–224, and 331–414; Hdm2 binds to YY1 through amino acid residues 226–295; thus YY1 make a ternary complex with p53 and Mdm2, and inactivating p53-mediated transcription (30).



**Figure 4. Schematic presentation of Dmp1 $\alpha$ -p53, E2F1-p53/Mdm2, YB-1-p53, and YY1-p53/Hdm2 interactions and their biological consequences**

(A) Dmp1-p53 interaction. Dmp1 $\alpha$  directly binds to the p53 C-terminus and neutralizes all the known functions for Mdm2 on p53 (31; arrow). This effect is mutually exclusive of DNA-binding of Dmp1 $\alpha$ , and thus independent of Arf.

(B) E2F1-p53/Mdm2 interaction. E2F1 bind to p53 C-terminus through the N-terminal domain (major: a.a. 1-190, minor: a.a. 190-437; 47) or a.a. 1-108 (46). This interaction will provide a checkpoint mechanism to silence aberrant E2F1 signaling to apoptosis to prevent carcinogenesis (46). Mdm2 prolongs the half-life of E2F1 by inhibiting ubiquitination (50,

arrow). Physical interaction of E2F1 and Mdm2 contributes to TAp73 transcriptional activity (horizontal arrow).

(C) YB-1-p53 interaction. YB-1 outside the cold-shock domain binds to p53 C-terminus to prevent the latter from inducing apoptosis genes (27). Thus YB-1 overexpression enhances tumorigenesis by accumulating cells that are not dying.

(D) YY1-p53/Hdm2 interaction. YY1 binds to both Hdm2 and p53, accelerates the polyubiquitination of p53 (arrow) to subvert the activities of p53. Since YY1 interacts with Hdm2 and p53 through different domains, they can form a ternary complex (30). This YY1-p53 interaction does not induce a checkpoint function to cancel the oncogenic activity of YY1.