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REVIEW

Regulation of the G2/M transition by p53

William R Taylor*,[1] and George R Stark[1]

[1]Department of Molecular Biology, Lerner Research Insititute, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44195, USA

p53 protects mammals from neoplasia by inducing apoptosis, DNA repair and cell cycle arrest in response to a variety of stresses. p53-dependent arrest of cells in the G1 phase of the cell cycle is an important component of the cellular response to stress. Here we review recent evidence that implicates p53 in controlling entry into mitosis when cells enter G2 with damaged DNA or when they are arrested in S phase due to depletion of the substrates required for DNA synthesis. Part of the mechanism by which p53 blocks cells at the G2 checkpoint involves inhibition of Cdc2, the cyclin-dependent kinase required to enter mitosis. Cdc2 is inhibited simultaneously by three transcriptional targets of p53, Gadd45, p21, and 14-3-3σ. Binding of Cdc2 to Cyclin B1 is required for its activity, and repression of the *cyclin B1* gene by p53 also contributes to blocking entry into mitosis. p53 also represses the *cdc2* gene, to help ensure that cells do not escape the initial block. Genotoxic stress also activates p53-independent pathways that inhibit Cdc2 activity, activation of the protein kinases Chk1 and Chk2 by the protein kinases Atm and Atr. Chk1 and Chk2 inhibit Cdc2 by inactivating Cdc25, the phosphatase that normally activates Cdc2. Chk1, Chk2, Atm and Atr also contribute to the activation of p53 in response to genotoxic stress and therefore play multiple roles. p53 induces transcription of the *reprimo*, *B99*, and *mcg10* genes, all of which contribute to the arrest of cells in G2, but the mechanisms of cell cycle arrest by these genes is not known. Repression of the *topoisomerase II* gene by p53 helps to block entry into mitosis and strengthens the G2 arrest. In summary, multiple overlapping p53-dependent and p53-independent pathways regulate the G2/M transition in response to genotoxic stress. *Oncogene* (2001) **20,** 1803–1815.

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*Correspondence: WR Taylor
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Effects of p53 on the G2/M transition in response to DNA damage or replication blocks

Effect of eliminating p53 on the G2 checkpoint

Checkpoints are control mechanisms that ensure the proper timing of cell cycle events by enforcing the dependency of late events on the completion of early events (Hartwell and Weinert, 1989). Entry into mitosis is blocked by the G2 checkpoint mechanism when DNA is damaged. To test whether the G2 checkpoint is intact, the number of cells in mitosis after DNA damage can be counted or the DNA content can be measured by using FACScanning. Analysing the importance of p53 in G2 arrest is complicated by p53-independent mechanisms that block entry into mitosis. Thus, many p53-null cell types still arrest in G2 in response to DNA damage (for examples see Kastan *et al*., 1991). However, the importance of p53 in G2 arrest has been observed under a number of conditions.

The human papilloma virus E6 protein (HPV-E6) binds to and inactivates p53. The number of IMR-90 normal lung fibroblasts entering mitosis after exposure to ionizing radiation was much higher after expression of HPV-E6, suggesting that p53 is required for G2 arrest (Thompson *et al*., 1997). In contrast the expression of HPV-E6 in human foreskin fibroblasts did not affect G2 arrest in response to ionizing radiation initially (Filatov *et al*., 1998; Kaufmann *et al*., 1997; Passalaris *et al*., 1999; Paules *et al*., 1995). However, as early as 12 population doublings after the expression of HPV-E6 many cells entered mitosis after exposure to ionizing radiation (Kaufmann *et al*., 1997), and the G2 arrest in response to DNA damage was completely lost upon more extensive *in vitro* propagation (Filatov *et al*., 1998; Kaufmann *et al*., 1997). HPV-E6 appears to allow genetic or epigenetic events to occur that severely impair the G2 checkpoint. Expression of a truncated form of p53 that forms inactive tetramers with endogenous wild-type p53 shortened the G2 delay in IMR-90 fibroblasts, but not as effectively as did HPV-E6 (Thompson *et al*., 1997). HPV-E6 can bind to the ERC-55 calcium binding protein, the human homologue of the *Drosophila* tumor suppressor discs-large, and a putative GTPase-activating protein E6TP1 (Chen *et al*., 1995; Gao *et al*., 1999; Imai *et al*., 1997; Kiyono *et al*., 1997). The different observations made with HPV-E6 and the truncated form of p53

suggests that other targets of HPV-E6 may contribute to the G2 checkpoint.

Overexpression of SV40 large T antigen in IMR-90 fibroblasts shortened the G2 delay stimulated by exposure to ionizing radiation, as determined by counting mitotic cells within several hours of treatment (Chang *et al*., 1997). Large T antigen binds to a number of cellular proteins in addition to p53 including Rb family proteins and the p300 histone acetyl transferase (Avantaggiati *et al*., 1996; DeCaprio *et al*., 1988; Eckner *et al*., 1996; Lane and Crawford, 1979). Inactivation of p53 by large T antigen may be responsible for abrogating the G2 checkpoint. However, the binding of large T antigen to other cellular proteins may also contribute to the loss of this checkpoint.

A role for p53 in the G2 checkpoint was more clearly revealed by using a derivative of the human colorectal tumor cell line HCT116 in which p53 was inactivated by homologous recombination (Bunz *et al*., 1998). Very few of these cells entered mitosis initially after exposure to ionizing radiation, suggesting that the G2 checkpoint is intact. However, arrest was not stable and the number of cells in mitosis eventually rose to a level higher than that for untreated cells. Isogenic cells with wild-type p53 exhibited a prolonged G2 arrest. Therefore, p53 is not required for the initial arrest of HCT116 cells in G2 but is essential for the long-term maintenance of the arrest. While it is possible that p53 also contributes to the initial arrest, it is clear that additional pathways can cause arrest when p53 is missing.

The effect of p53 on the G2/M transition in response to DNA damage depends on the cell type. Reconstitution of p53-null pre-B lymphoblasts with wild-type p53 shortened G2 arrest in response to ionizing radiation and stimulated apoptosis in cells containing micronuclei, a marker for DNA damage (Schwartz *et al*., 1997). Also, bone-marrow cells enriched in myeloblasts from normal mice entered mitosis more frequently than cells from p53-null mice after exposure to ionizing radiation, and this effect occurred before apoptosis was induced (Guillouf *et al*., 1995). The results suggest that p53 acts to shorten the G2 delay in response to DNA damage in these cells. Although hematopoietic cells are highly susceptible to apoptosis in response to DNA damage, reducing the duration of G2 arrest in non-hematopoietic cells can also hasten cell death (Chan *et al*., 2000). By stimulating the escape of hematopoietic cells from the G2 block, p53 may contribute to the susceptibility of hematopoietic cell types to undergo apoptosis.

Effects of caffeine and UCN-01 on the G2 checkpoint

Caffeine is capable of abrogating cell cycle checkpoints in several different mammalian cell types (Schlegel and Pardee *et al*., 1986; Steinmann *et al*., 1991). The protein kinase inhibitor UCN-01 can also overcome cell cycle checkpoints (Wang *et al*., 1996). Caffeine and UCN-01 have been used to help delineate the role of p53 in the G2 arrest that occurs in response to DNA damage.

MCF-7 breast carcinoma cells expressing HPV-E6 still arrest in G2 in response to ionizing radiation, however the arrest is abrogated if they are subsequently exposed to UCN-01 (Wang *et al*., 1996) or the caffeine derivative pentoxifylline (Fan *et al*., 1995). Also, caffeine abrogated G2 arrest in response to ionizing radiation in mouse embryo fibroblasts (MEFs) derived from p53-knockout mice and in rat embryo fibroblasts expressing the dominant negative V143A mutant of human p53, but not in the isogenic mouse or rat cells with wild-type p53 function (Powell *et al*., 1995). These results suggest that two pathways induce G2 arrest in response to DNA damage: one depends on p53 and is relatively insensitive to UCN-01 or caffeine, whereas inactivating the other with UCN-01 or caffeine allows cells lacking p53 to bypass the G2 block.

In yeast, the G2 checkpoint allows more time for repair of DNA damage and enhances survival. Loss of p53 can sensitize mammalian cells to DNA damage when the G2 checkpoint is abrogated by caffeine or UCN-01. For example, pentoxifylline sensitizes MCF-7 cells expressing HPV-E6 to the chemotherapeutic agent cisplatin but not parental cells, which have wild-type p53 (Fan *et al*., 1995). Similarly, UCN-01 and ionizing radiation synergistically kill MCF-7 cells containing HPV-E6 but not parental cells (Wang *et al*., 1996). Therefore, inactivation of both the p53-dependent and the caffeine/UCN-01-sensitive pathways increase tumor cell death. Abrogation of the caffeine/UCN-01-sensitive pathway might kill p53-null tumors selectively, an important finding since many human tumors are defective in p53 function. Although the doses of caffeine effective *in vitro* are too high to be clinically useful, clinical trials are under way to test if UCN-01 can enhance the therapeutic effect of cisplatin (A Eastman, personal communication).

Role of p53 in the S phase completion checkpoint

Inhibitors of DNA synthesis trigger the S phase completion checkpoint, which blocks entry into mitosis. The signal that activates this checkpoint is generated when cells are blocked in S phase. In contrast, the G2 checkpoint is triggered in cells that have completed DNA synthesis but contain damaged DNA. Signals that activate the S phase completion checkpoint may include stalled replication machinery and DNA damage resulting from prolonged inability to synthesize DNA. The S phase completion and G2 checkpoints have in common that both block entry into mitosis. The end results are different in that the S phase completion checkpoint blocks entry into mitosis if DNA synthesis is incomplete, whereas the G2 checkpoint causes cells containing damaged DNA to arrest.

Treatment of some cell types with caffeine, okadaic acid or staurosporine can override the S phase completion checkpoint, causing premature chromatin

condensation, DNA fragmentation and cell death (Ghosh *et al*., 1996; Schlegel and Pardee, 1986; Steinmann *et al*., 1991; Yoshida *et al*., 1997). We observed that p53-null MDAH041 fibroblasts, derived from Li-Fraumeni cells, continued to attempt mitosis when DNA synthesis was inhibited with hydroxyurea, leading to fragmentation of chromatin and cell death. Expression of wild-type p53 in MDAH041 cells prevented them from entering mitosis when DNA synthesis was blocked, showing that p53 contributes to this checkpoint (Taylor *et al*., 1999a). Consistent with these results, caffeine was more efficient in inducing premature chromatin condensation in IMR-90 cells lacking p53 due to expression of HPV-E6 (Thompson *et al*., 1997). A truncated version of p53 that inactivates endogenous p53 was less efficient than HPV-E6 in alleviating the S phase completion checkpoint, similar to the relative effects on the G2 checkpoint (Thompson *et al*., 1997). Even in the absence of p53, many cells do not attempt mitosis when DNA synthesis is blocked, suggesting that both p53-dependent and p53-independent pathways control the S phase completion checkpoint in mammalian cells (Taylor *et al*., 1999a). Most of the p53-null MEFs that entered mitosis when DNA synthesis was blocked aborted mitosis before the cleavage furrow was formed, possibly due to the effect of fragmented DNA (Taylor *et al*., 1999a). Also, p53 blocked entry into mitosis in cells that had completed DNA synthesis when their nucleotide pools were unbalanced by treatment with *N*-(phosphonacetyl)-L-aspartate, an inhibitor of *de novo* pyrimidine biosynthesis (Taylor *et al*., 1999a). DNA synthesis under these conditions creates DNA damage, suggesting that p53 can function to block entry into mitosis if DNA is damaged during S phase.

Effects of overexpressing p53 on the G2/M transition

Studies of the role of p53 in transmitting the effects of DNA damage and unreplicated DNA indicated a role for p53 in regulating the G2/M transition in response to stress. Since DNA damage and inhibition of replication also trigger p53-independent pathways, p53 was overexpressed in the absence of any exogenous stress to define its role in regulating the G2/M transition more clearly. Some groups have used the temperature-sensitive A135V mutant of murine p53, which exhibits wild-type activities at 32°C, but loses the ability to activate transcription and induce growth arrest at 37°C. The A138V mutant of human p53 behaves similarly. Temperature-shift experiments in growing rat fibroblasts expressing the A135V mutant showed that p53 blocked cellular proliferation primarily by inducing arrest in G1, whereas very few cells were arrested in S phase or G2/M (Michalovitz *et al*., 1990).

We have used a different system to study the role of p53 in G2 arrest. p53-null human fibroblasts reconstituted with a tetracyline-regulated p53 minigene were synchronized with mimosine, which blocks them reversibly at the beginning of S phase (Agarwal *et al*., 1995). Mimosine was removed and p53 was induced after most cells had passed the point in G1 where p53 could block entry into S phase. The cells progressed through S phase and up to 60% of them became arrested with a 4N G2/M content of DNA (Agarwal *et al*., 1995). The arrested cells had an interphase morphology with an intact nucleus and no evidence of chromatin condensation (Agarwal *et al*., 1995; Taylor *et al*., 1999a). The level of phosphorylated histone H1b, normally highest during mitosis, was very low in the arrested cells (Taylor *et al*., 1999a). Time-lapse analysis showed that cells expressing low levels of p53 entered mitosis and divided ~20 h after mimosine was removed (Taylor *et al*., 1999b). Cells expressing p53 did not enter mitosis and retained a flat interphase morphology (Taylor *et al*., 1999b). These observations suggest that p53 is involved in G2 arrest and that the arrest is not due to an aborted attempt at mitosis, followed by arrest in G1. Similarly G2 arrest was observed when rat fibroblasts containing the A135V mutant of murine p53 were synchronized by serum starvation and p53 was induced by a temperature shift after the cells had progressed through G1 (Stewart *et al*., 1995). Therefore, overexpression of p53 in the absence of any exogenous stress can arrest cells in G2.

Subsequent studies have confirmed the ability of p53 to arrest a number of different cell types in G2. p53-null H1299 human lung carcinoma cells were transfected with an ecdysone-inducible version of p53. Induction led to arrest in both G1 and G2, and prolonged the G2 arrest that occurred in response to DNA damage created with adriamycin or ionizing radiation (Flatt *et al*., 2000). H1299 cells expressing the temperature-sensitive A138V mutant of human p53 arrested in G2 when p53 was activated by a temperature shift and the cells were simultaneously exposed to ionizing radiation (Winters *et al*., 1998). However, unirradiated cells did not arrest in G2, even at a temperature at which p53 was active (Winters *et al*., 1998). Failure to arrest may be due to lower levels of expression of wild-type p53 using the temperature-sensitive compared to the ecdysone-inducible system. SKOV3 human ovarian carcinoma cells expressing the temperature-sensitive A135V mutant of murine p53 were arrested in both G1 and G2 when incubated at 32°C to induce wild-type p53 function (Innocente *et al*., 1999). Also, infection of EJ bladder carcinoma cells, synchronously moving through S phase, with a recombinant adenovirus that expresses p53 resulted in G2 arrest (Park *et al*., 2000). Thus, p53 can cause G2 arrest on its own and can also prolong the G2 arrest that occurs in response to DNA damage.

Mechanisms of G2 arrest

The mechanism by which p53 regulates the G2/M transition involves regulation of the cyclin dependent kinase Cdc2 which is essential for entry into mitosis (Nurse, 1990, Figure 1). Binding to Cyclin B and phosphorylation at threonine 161 by CDK-activating

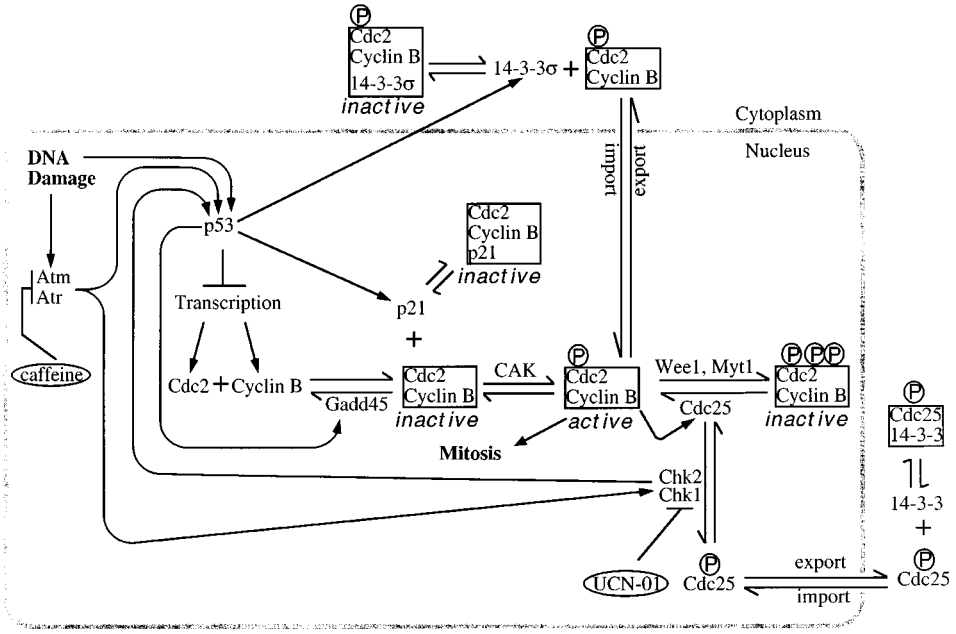

Figure 1 Participation of p53 in regulating Cdc2 in response to DNA damage

kinase (CAK) are required to activate Cdc2 (Pines, 1995). During G2, the Cdc2/Cyclin B complex is kept inactive by phosphorylation on tyrosine 15 and threonine 14 of Cdc2 by the kinases Wee1 and Myt1, respectively (Booher *et al*., 1997; Liu *et al*., 1997; Parker and Piwnica-Worms, 1992). At the onset of mitosis, both of these residues are dephosphorylated by the phosphatase Cdc25 (Draetta and Eckstein, 1997). Cdc2/Cyclin B can then phosphorylate Cdc25, further activating it and initiating a positive feedback loop (Izumi and Maller, 1993). Well defined p53-independent pathways inhibit Cdc2 activity in response to DNA damage. For example, DNA damage stimulates the kinases Atm and Atr, which activate the Chk1 and Chk2 kinases, which then phosphorylate Cdc25, causing it to bind to 14-3-3 proteins, which anchor Cdc25 in the cytoplasm where it cannot activate Cdc2. Also, several of the transcriptional targets of p53 can inhibit Cdc2, including p21, which inhibits Cdc2 directly, 14-3-3σ , which anchors Cdc2 in the cytoplasm where it cannot induce mitosis, and Gadd45, which dissociates Cdc2 from Cyclin B1. Repression of *cyclin B1* and *cdc2* by p53 enforces the arrest. p53 also has other targets that do not affect Cdc2 but also contribute to G2 arrest.

Modulation of Cdc2 phosphorylation by p53-independent pathways

Inhibitory phosphorylation of Cdc2 is essential for the p53-independent G2 arrest that occurs in response to DNA damage, and is dependent on the protein kinases Atm and Atr. Atm is mutated in the ataxia telangiectasia syndrome, which is characterized by acute radiation sensitivity and susceptibility to spontaneous tumors (Spacey *et al*., 2000). These symptoms may be due to control of the normal cellular responses to damaged DNA by Atm. Atm and Atr are members of a family of phosphatidylinositol-3 kinases that are activated by damaged DNA and required for G2 arrest (Cliby *et al*., 1998; Rudolph and Latt, 1989). Atm and Atr can phosphorylate and activate the serine kinases Chk1 and Chk2 (Chaturvedi *et al*., 1999; Liu *et al*., 2000; Matsuoka *et al*., 2000, Figure 1). Chk1 and Chk2 can phosphorylate Cdc25, creating a binding site for proteins of the 14-3-3 family (Chaturvedi *et al*., 1999; Furnari *et al*., 1997; Peng *et al*., 1997; Sanchez *et al*., 1997) that sequester Cdc25 in the cytoplasm where it cannot de-phosphorylate Cdc2/Cyclin B (Kumagai and Dunphy, 1999; Lopex-Girona *et al*., 1999). Thus, Atm and Atr inactivate Cdc2 by increasing phosphorylation of the residues tyrosine 15 and threonine 14, a major p53-independent mechanism that causes G2 arrest in response to DNA damage.

Interestingly, caffeine can inhibit Atm and Atr (Blasina *et al*., 1999; Sarkaria *et al*., 1999; Zhou *et al*., 2000, Figure 1). As discussed above, caffeine can also abrogate the G2 arrest that occurs in response to DNA damage in cells lacking p53. UCN-01, which also abrogates the G2 checkpoint in p53-null cells, can inhibit Chk1 but not Chk2 (Graves *et al*., 2000; Jackson *et al*., 2000, Figure 1). Abrogation of the G2 checkpoint in p53-null cells by caffeine and UCN-01 is expected if Chk1 acts downstream of Atm and Atr in a p53-independent G2 arrest pathway. However, these pathways are not completely independent because Chk1 and Chk2 can phosphorylate serine 20 in the amino-terminal region of p53 (Hirao *et al*., 2000; Shieh *et al*., 2000, Figure 1). Atm and Atr can also

phosphorylate serines 15 and 37 of p53 in response to DNA damage (Canman *et al*., 1998; Chehab *et al*., 1999; Tibbetts *et al*., 1999). Phosphorylation of the amino-terminal region of p53 causes it to accumulate and stimulates its ability to bind to specific DNA sequences, essential steps in activating p53 in response to stress (Lakin and Jackson, 1999). Thus, Atm and Atr play multiple roles in regulating the responses to DNA damage.

Effects of p53 on the phosphorylation of Cdc2

p53 can reduce the tyrosine phosphorylation of Cdc2. However, the impact of this event on Cdc2 activity depends on the cell type. Human fibroblasts arrested in G2 due to overexpression of p53 showed a reduction in the phosphorylation of Cdc2 on tyrosine (Taylor *et al*., 1999b). These G2-arrested cells contain low levels of Cdc2 kinase activity, consistent with their inability to enter mitosis (Taylor *et al*., 1999b). Since a loss of tyrosine phosphorylation is expected to activate Cdc2, other effects of p53 probably caused the inactivation of Cdc2. For example, the G2-arrested cells contain low levels of Cyclin B1 (Taylor *et al*., 1999b). Loss of Cyclin B1 would result in loss of Cdc2 activity. Downregulation of Cyclin B1 levels could also cause a drop in the extent of tyrosine phosphorylation of Cdc2, since the Cdc2/Cyclin B1 complex is a better substrate for the tyrosine 15 kinase Wee1 than is Cdc2 alone (Watanabe *et al*., 1995).

A temperature shift to activate the A135V mutant of murine p53 in rat fibroblasts also caused a reduction in the level of phosphorylation of Cdc2 on tyrosine (Leach *et al*., 1998). The cells arrested primarily in G1, with a minor population in G2, upon induction of p53. Cdc2 activity was gradually reduced after induction of p53, attributed to a loss of Cyclin B1 protein. A different outcome was observed when the A135V mutant of murine p53 was expressed in murine T-cell lymphoma cells (Leach *et al*., 1998), where the induction of p53 caused a drop in tyrosine phosphorylation of Cdc2 and activation of its kinase activity. Thus, the effects of p53 on Cdc2 activity are cell-type dependent. In both rat fibroblast and T-cell lymphoma cells the induction of p53 led to a dramatic loss of expression of the Wee1 protein, which could explain the dephosphorylation of Cdc2 on tyrosine. However, the level of Wee1 in human fibroblasts was not reduced when p53 was overexpressed using the tetracycline-regulated system (Taylor *et al*., 1999b). Downregulation of Wee1 may occur more readily in rodent cells, or may require more p53 than was expressed in the human fibroblasts tested.

Role of CAK

CAK is composed of Cdk7, Cyclin H and Mat1 and activates Cdc2 by phosphorylating it on threonine 161. Purified wild-type human p53 binds to and inhibits CAK *in vitro* but the tumor-derived R175H mutant of p53 had no effect (Schneider *et al*., 1998). Exposure of p21-null MEFs to ultraviolet radiation inhibited endogenous CAK activity, an effect not observed with p53-null MEFs (Schneider *et al*., 1998). These results show that the inhibition of CAK by p53 may contribute to G2 arrest. However, overexpression of p53 using a tetracycline-regulated system did not inhibit CAK, although the cells were arrested in G2 (Taylor *et al*., 1999b). Perhaps the tetracycline-regulated system did not produce enough p53 to inhibit CAK in human fibroblasts. Thus, alternative effects of p53 were sufficient to drive G2 arrest under conditions where CAK was active.

Role of p21

Rodent fibroblasts arrested in G2 using a temperature-sensitive p53 protein, combined with exposure to ionizing radiation, contained low levels of Cdc2 activity without changes in the level of tyrosine phosphorylation of Cdc2 or the level of Cyclin B1 (Winters *et al*., 1998). This observation suggests that p53 might induce the synthesis of an inhibitor of Cdc2. p21 is a candidate for such an inhibitor because it is a transcriptional target of p53 and can inhibit cyclin-dependent kinases (CDKs). Induction of p21 using a tetracycline-regulated system caused a number of different cell lines to arrest in both G1 and G2. G2 arrest was observed in human Hela cervical carcinoma cells, Saos-2 and U2OS osteosarcoma cells, RKO colorectal carcinoma cells, H1299 lung carcinoma cells and in Rat 1 fibroblast cells (Bates *et al*., 1998; Medema *et al*., 1998; Niculescu *et al*., 1998). The arrested cells did not contain condensed chromosomes, suggesting that they were in G2.

p21 also participates in the G2 checkpoint. HCT116 colorectal tumor cells lacking p21 did not arrest stably in G2 after exposure to ionizing radiation, similarly to HCT116 cells lacking p53 (Bunz *et al*., 1998). Failure to arrest was associated with levels of Cdc2 kinase activity higher than those observed in cells with p21. Re-entry of irradiated p53-null and p21-null HCT116 cells into mitosis did not cause a reduction in the number of cells containing a 4N G2/M complement of DNA, as expected if the cells were dividing (Bunz *et al*., 1998). Close examination of irradiated p21-null tumor cells showed that they failed to complete mitosis, leading to arrest in a post-mitotic interphase state. Many cells contained multiple spindle poles, which may have hindered the segregation of chromosomes and interfered with cytokinesis.

Additional evidence linking p21 to the G2/M transition comes from its abundance and localization during the cell cycle. p21 is present during G1 but its levels go down during S phase and back up during G2 (Dulic *et al*., 1998). Near the end of G2, but before prophase (defined by the first appearance of condensed chromatin), Cyclin B is synthesized and accumulates in the cytoplasm. Abrupt relocalization of Cyclin B to the nucleus occurs shortly before chromatin condensation and entry into mitosis. Thus, $\sim$80% of the cells containing nuclear Cyclin B1 did not have condensed

chromatin. Following the entry of Cyclin B into the nucleus, p21 re-accumulates in the nucleus until mitosis, when the levels are reduced (Dulic *et al*., 1998). In contrast, all p21-null cells with Cyclin B in the nucleus have also begun chromatin condensation. Cells containing nuclear Cyclin B but without evidence of chromatin condensation were not observed (Dulic *et al*., 1998). Loss of p21 may shorten G2 and alter the relative timing of the nuclear accumulation of Cyclin B and chromatin condensation.

Four mechanisms have been postulated for how p21 participates in inhibiting Cdc2 activity to cause G2 arrest. First, p21 inhibits CDK activity by binding directly to CDK/Cyclin complexes (Boulaire *et al*., 2000, Figure 1). p21 was found in Cyclin B1 immunoprecipitates in cells overexpressing either p53 or p21 (Medema *et al*., 1998; Taylor *et al*., 1999b). However, *in vitro* studies have shown that p21 is much less efficient in binding to and inhibiting Cdc2 compared to other CDKs (Harper *et al*., 1995). In some studies, it was not found in Cyclin B1 immunoprecipitates, even though Cdc2 was inactive (Bates *et al*., 1998; Dulic *et al*., 1998). Perhaps binding to Cdc2/Cyclin B1 is only observed when there are very high levels of p21 or in particular cell types. A second mechanism for inhibiting Cdc2 is suggested by experiments in *Xenopus* showing that active Cdk2 is involved in generating active Cdc2. p21, by inhibiting Cdk2, causes loss of Cdc2 activity (Guadagno and Newport, 1996). It is not known how Cdk2 activates Cdc2 in *Xenopus* or whether a comparable pathway is functional in mammals.

A third mechanism of Cdc2 inhibition by p21 was uncovered by using a tetracycline-regulated p21 expressed in human U20S cells (Smits *et al*., 2000). Overexpression of p21 caused G2 arrest accompanied by low levels of Cdc2 activity. However, Cdc2 immunoprecipitated from the arrested cells was re-activated by adding recombinant CAK. Also, phospho-tryptic peptide mapping showed that overexpression of p21 reduced the phosphorylation of Cdc2 on threonine 161, the site of phosphorylation by CAK (Smits *et al*., 2000). Thus, p21 can interfere with the activating phosphorylation of Cdc2 by CAK. It is not known whether p21 reduces CAK activity in U2OS cells; however, p21 was not required for down-regulation of CAK activity in MEFs exposed to ultraviolet radiation (Schneider *et al*., 1998). Binding of p21 to Cdk2 can block access to CAK, and a similar effect may underlie the inhibition of Cdc2 by p21 (Hitomi *et al*., 1998).

A fourth mechanism depends on the fact that p21 binds to PCNA, a processivity factor for DNA polymerases δ and ε, required for DNA synthesis and repair (Waga *et al*., 1994). Expression of a mutant of p21 that binds to PCNA but not CDKs arrests DLD 1 colorectal carcinoma cells in G2 (Cayrol *et al*., 1998). However, a mutant of p21 that binds to CDKs but not PCNA does not cause G2 arrest, although the activities of Cdk2 and Cdc2 are reduced (Cayrol *et al*., 1998). Presumably there was enough CDK activity for cells to progress through the cell cycle in the latter experiment. How does the binding of p21 to PCNA cause G2 arrest? Since PCNA is required for DNA synthesis, p21 might inactivate it, causing DNA damage during S phase and thus leading to inhibition of Cdc2 and G2 arrest by p53-independent mechanisms. p21 may contribute to G2 arrest in several different ways with the predominating mechanisms determined by the cellular background.

Regulation of Cdc2/Cyclin subunit assembly by Gadd45

Another transcriptional target of p53 implicated in regulating the G2/M transition is Gadd45. Microinjection of this protein into primary human fibroblasts arrests them with partially condensed chromatin and an intact nuclear membrane. The cells are also positive for the mitosis-specific antigens recognized by the monoclonal antibody MPM-2 (Wang *et al*., 1999). The arrest, which appears to be in early prophase, was not observed in MDAH041 Li-Fraumeni fibroblasts lacking p53, suggesting that Gadd45 requires the presence of p53, directly or indirectly, to cause this arrest (Wang *et al*., 1999). The fraction of wild-type mouse lymphocytes in mitosis after exposure to ultraviolet radiation was reduced to 25% of the fraction for untreated cells. However, ultraviolet radiation did not affect mitotic entry in lymphocytes from Gadd45-null mice. Loss of Gadd45 did not affect the G2 arrest in response to ionizing radiation (Wang *et al*., 1999). Microinjection of Cyclin B1 and Cdc25C abrogated the arrest caused by Gadd45, suggesting that Cdc2 was an important target (Wang *et al*., 1999).

The effect of Gadd45 on the G2/M transition may be due to its ability to dissociate complexes of Cyclin B1 and Cdc2. Amino acids 65–84 of Gadd45 are required for it to inhibit the activity of Cdc2/Cyclin B1 *in vitro* (Jin *et al*., 2000; Zhan *et al*., 1999). Gadd45 did not efficiently inhibit Cdk2/Cyclin E, which may explain why it did not cause G1 arrest when microinjected (Zhan *et al*., 1999). Interestingly, immunoprecipitation of Gadd45 revealed co-associated Cdc2 but not Cyclin B1. Addition of recombinant Gadd45 released Cdc2 from the Cdc2/Cyclin B1 complex, suggesting that Gadd45 inhibits Cdc2 by blocking its binding to Cyclin B (Zhan *et al*., 1999, Figure 1). However, if Gadd45 acts directly on Cdc2/Cyclin B1, it is not clear why p53 is required for the arrest. One possibility is that Gadd45 may cooperate with other downstream targets of p53, such as p21, whose constitutive expression is lost upon deletion of p53 (Tang *et al*., 1998).

The role of Gadd45 in regulating Cdc2 may be important in maintaining genomic stability. Cells from Gadd45-null mice rapidly become aneuploid (Hollander *et al*., 1999), possibly due to the presence of supernumerary centrosomes, an effect also observed in p53-null cells (Fukasawa *et al*., 1996). Cells entering mitosis with more than two centrosomes cannot form a bipolar spindle, leading to losses and gains of chromosomes if cytokinesis is completed. Gadd45-null

mice are also more prone to radiation-induced tumors than wild-type mice, which may be the result of genomic instability caused by defects in G2 checkpoints (Hollander *et al*., 1999).

Role of 14-3-3σ in the nuclear targeting of Cdc2/Cyclin B

An additional mechanism by which p53 causes G2 arrest involves regulation of the subcellular localization of Cdc2. For Cdc2 to induce mitosis it must enter the nucleus, a process mediated by binding of Cyclin B1 to importin β which, along with importin α, translocates the Cdc2/Cyclin B1 complex into the nucleus (Moore *et al*., 1999; Takizawa *et al*., 1999). However, Cdc2/Cyclin B1 is rapidly removed from the nucleus by the nuclear export factor Crm1, which binds to Cyclin B1 (Hagting *et al*., 1998; Toyoshima *et al*., 1998; Yang *et al*., 1998). Cells enter mitosis when residues in the Crm1 binding site of Cyclin B become phosphorylated, blocking the binding of Crm1 and the export of Cdc2/Cyclin B1 from the nucleus (Yang *et al*., 1998).

The protein 14-3-3σ, which can bind to Cdc2/Cyclin B1 and sequester it in the cytoplasm, is a direct transcriptional target of p53 (Figure 1). 14-3-3σ is induced by ionizing radiation in the colorectal tumor cells lines Lim2405, LoVo, RKO and HCT116, which contain wild-type p53, but not the DLD-1, HT29 or SW480 colorectal tumor cell lines, which lack p53-dependent functions (Hermeking *et al*., 1997). Overexpression of 14-3-3σ in HCT116 cells, using a recombinant adenovirus, caused most cells to arrest in G2 (Hermeking *et al*., 1997). The arrested cells eventually synthesized DNA without dividing, leading to the production of cells with DNA contents larger than 4N. Time-lapse analysis revealed that many of the arrested cells underwent an abortive mitosis. It is not known why cytokinesis is blocked in cells overexpressing 14-3-3σ. Cells arrested by 14-3-3σ may eventually re-enter S phase because they do not express other inhibitors of S phase entry. For example, ionizing radiation causes a G2 arrest in the same cells that is not followed by re-synthesis of DNA, possibly because an increase in p21 blocks progression into S (Hermeking *et al*., 1997).

14-3-3σ controls the duration of G2 arrest in response to DNA damage in the epithelial colorectal tumor cell line HCT116. HCT116 cells in which 14-3-3σ is inactivated by homologous recombination initially arrest in G2 after treatment with adriamycin, which causes DNA damage and induces p53 (Chan *et al*., 1999). Eventually the treated 14-3-3σ-null cells enter a state with a disassembled nuclear membrane and fragmented chromatin, similar to the condition of cells undergoing mitotic catastrophe as a result of entering mitosis before completing DNA synthesis (Chan *et al*., 1999). Mitotic catastrophe in treated 14-3-3σ-null cells was preceded by entry of Cdc2/Cyclin B1 into the nucleus, whereas the Cdc2/Cyclin B1 complex remains in the cytoplasm in adriamycin-treated cells containing 14-3-3σ. 14-3-3σ co-precipitated with Cdc2 but not with Cdc25C, suggesting that the binding of 14-3-3σ might block entry into mitosis by anchoring the Cdc2/Cyclin B1 complex in the cytoplasm.

The involvement of 14-3-3σ in p53-mediated G2 arrest appears to be cell-lineage specific. 14-3-3σ was originally described as an epithelial cell specific protein (Leffers *et al*., 1993). Interestingly, 14-3-3σ is down-regulated in keratinocytes expressing SV40 large T antigen, suggesting that p53 contributes to the basal expression of 14-3-3σ, although other effects of large T antigen cannot be ruled out (Leffers *et al*., 1993). 14-3-3σ was not expressed in fibroblasts upon overexpression of p53 and experiments with an antibody broadly reactive to 14-3-3 proteins failed to uncover upregulation of any 14-3-3 protein in response to p53 (Taylor *et al*., 1999b). Thus, 14-3-3σ appears not to contribute to G2 arrest in the fibroblasts tested, and 14-3-3 proteins are not induced by overexpression of p53 in these cells.

There is evidence that the subcellular localization of Cdc2/Cyclin B1 may be important in p53-dependent G2 arrest in human fibroblasts that do not express 14-3-3σ. G2 arrest of fibroblasts induced by overexpression of p53 was not abrogated after overexpression of wild-type Cyclin B1 alone or in combination with the Cdc2 T14A, Y15F double mutant, which cannot be inactivated by phosphorylation (Taylor *et al*., 1999b). However, expression of Cdc2 T14A, Y15F in combination with a variant Cyclin B1 carrying the nuclear localization signal of SV40 large T antigen caused the arrested cells to enter mitosis with highly fragmented chromatin (Taylor *et al*., 1999b). The large T antigen nuclear localization signal causes Cyclin B1 to be in the nucleus constitutively (Jin *et al*., 1998). p53 may cause Cdc2/Cyclin B1 to be anchored in the cytoplasm by a mechanism that does not involve 14-3-3σ. Alternatively, fibroblasts arrested in G2 by overexpression of p53 may contain high levels of Cdc2/Cyclin B1 inhibitors such as p21. Overexpression of wild-type Cyclin B1 together with active Cdc2 may overcome these inhibitors only partially. Forcing this partially active complex into the nucleus may allow it to induce mitosis. Overexpression of wild-type Cyclin B1 (without the large T antigen nuclear localization signal) in combination with Cdc2 T14A, Y15F was sufficient to abrogate G2 arrest imposed by p53 in EJ bladder carcinoma cells (Park *et al*., 2000). Thus, again the requirements for abrogating p53-dependent G2 arrest seem to be cell-type dependent.

Overexpression of p53 along with ionizing radiation leads to G2 arrest in H1299 human lung carcinoma cells. In these arrested cells, Cdc2/Cyclin B1 is inactive, but it is in the nucleus in ~50% of the cells (Winters *et al*., 1998). Furthermore, overexpression of p21 on its own causes Cdc2/Cyclin B1 to accumulate in the nucleus (Winters *et al*., 1998). A potential explanation is suggested by the identification of the protein Carb, which can bind to Cyclin B1 (McShea *et al*., 2000). Carb and Cyclin B1 are co-localized to the centrosome during G2. Overexpression of Carb arrests p21-null cells in both G1 and G2, but arrest was not evident in

cells containing p21. Furthermore, p21 can bind to Carb and block its ability to bind to Cyclin B1 (McShea *et al*., 2000). Perhaps Carb anchors Cyclin B1 to the centrosome, causing G2 arrest, but expression of p21 inhibits the binding of Carb and Cyclin B1, allowing Cyclin B1 to enter the nucleus. Therefore, regulation of the nuclear-cytoplasmic shuttling of Cdc2/Cyclin B1 is complex, involving multiple transcriptional targets of p53.

Role of Reprimo in p53-dependent G2 arrest

Reprimo, a glycosylated, cytoplasmic protein, was identified by using differential display to find genes induced by ionizing radiation in a p53-dependent manner (Ohki *et al*., 2000). Saos-2 cells infected with an adenovirus overexpressing Reprimo were arrested with a 4N G2/M content of DNA without condensed chromatin and with Cyclin B in the cytoplasm, suggesting that the cells were in G2 (Ohki *et al*., 2000). Cells arrested by the overexpression of Reprimo contained inactive Cdc2, highly phosphorylated on tyrosine (Ohki *et al*., 2000). Reprimo was not observed to bind to either Cyclin B1 or Cdc2 and its mechanism of action is not known.

Trans-repression of cdc2 and cyclin B1

p53 activates the transcription of many genes that mediate its downstream functions and can also repress the transcription of different genes through several distinct mechanisms. Repression can be due to squelching, whereby high levels of p53 bind to TATA-binding protein-associated factors (TAFs), sequestering them away from the promoters of genes that do not contain p53-binding sites (Farmer *et al*., 1996; Mack *et al*., 1993; Ragimov *et al*., 1993). Repression can also be due to binding of p53 to the p300 histone acetyl transferase, blocking the binding of this protein to genes that depend on histone acetylation for activity (Avantaggiati *et al*., 1997). p53 can also interfere with sequence-specific transcriptional activators to cause repression. For example, the binding of p53 to the *bcl2* promoter inhibits transcription driven by the simultaneously bound transcription factor Brn-3a (Budhram-Mahadeo *et al*., 1999). Repression of certain genes by p53 has important biological consequences. For example, repression of *bcl2* by p53 is important for the induction of apoptosis.

Inhibition of Cdc2 activity in human fibroblasts overexpressing p53 was due in part to a reduced level of Cyclin B1 protein (Taylor *et al*., 1999b). The loss of Cyclin B1 occurred before most cells had arrested in G2, was due to transcriptional repression by p53 and mapped to the −123 to −287 region of the *cyclin B1* promoter (Taylor *et al*., 1999b, Figure 1). Although a reporter construct containing sequences up to −123 was not repressed, the basal activity of this construct was similar to larger constructs that were repressed. Thus, repression does not appear to be due to squelching because repression was lost when basal activity was maintained (Taylor *et al*., 1999b). Repression of the *cyclin B1* promoter was also observed in the SKOV3 human ovarian cancer cells after the temperature-sensitive A135V mutant of murine p53 was activated by a temperature shift (Innocente *et al*., 1999). Induction of wild-type p53 in these cells also caused G2 arrest with low levels of Cdc2 activity. Overexpression of Cyclin B1 on its own could abrogate the G2 arrest induced by p53, indicating that reduction of Cyclin B1 is an integral part of the arrest mechanism (Innocente *et al*., 1999).

Transient transfection of p21-null MEFs with p53 and a *cyclin B1* reporter construct indicated that the repression of *cyclin B1* by p53 was independent of p21 (Passalaris *et al*., 1999). However, ionizing radiation caused a reduction of *cyclin B1* mRNA in wild-type but not p21-null MEFs (de Toledo *et al*., 1998). A major difference between these two approaches is that transient transfection causes the production of much higher levels of p53 than does ionizing radiation. Squelching of the *cyclin B1* promoter by high levels of p53 might obscure a p21-dependent effect. Alternatively, p21 may contribute to the post-transcriptional regulation of *cyclin B1*. Together, these results show that *cyclin B1* is repressed by p53. The mechanism of repression is not known, but there are binding sites for the E2F, MyoD1, USF1, and SP1 transcription factors within the region of the *cyclin B1* promoter required for repression, and p53 may target the proteins binding to these sites (Taylor *et al*., 1999b).

Reduction in the level of Cdc2 protein is another part of the mechanism of G2 arrest by p53, although the loss of Cdc2 occurs after the loss of Cyclin B1. Loss of the Cdc2 protein in fibroblasts arrested in G2 by p53 is due to loss of the mRNA (Taylor *et al*., 1999b). Ionizing radiation reduced the level of *cdc2* mRNA in wild-type but not p53-null MEFs (Azzam *et al*., 1997; de Toledo *et al*., 1998) and in normal diploid human fibroblasts but not those expressing HPV-E6 (de Toledo *et al*., 1998). Overexpression of p53 in human fibroblasts, using the tetracycline-regulated system, and transient transfections in p53-null MEFs revealed that p53 represses the *cdc2* promoter (Taylor *et al*., 1999b; Passalaris *et al*., 1999, Figure 1). A fragment of the *cdc2* promoter extending from −104 to the start of transcription could drive basal transcription and was repressed by p53. Deletion to −74 caused a 40-fold drop in basal promoter activity and a complete loss of repression (Taylor *et al*., 1999b). The deleted region contains an inverted CCAAT box, which binds NF-Y, a transcriptional activator consisting of NF-A, NF-B, and NF-C (Yun *et al*., 1999). Overexpression of a dominant negative version of NF-A reduced the basal activity of the *cdc2* promoter and abolished repression by p53 (Yun *et al*., 1999). Thus, p53 inhibits the ability of NF-Y to activate transcription of the *cdc2* gene. However, it was not demonstrated that this was due to a direct effect of p53 on NF-Y.

Repression of the *cdc2* promoter by p53 requires a second region of ~20 bp very close to the start of transcription, the R box, which acts as a silencer that can confer p53-dependent repression on heterologous promoters containing upstream AP-1 or SP1 binding sites (Taylor *et al*., 2001). Replacement of the R box region in the *cdc2* promoter with a region from the same area of the prolactin promoter eliminated repression by p53, suggesting that p53 does not squelch the *cdc2* promoter but might stimulate the binding of a repressor to the R box that can interfere with NF-Y-driven transcription. Further evidence against squelching is that *cdc2* mRNA is not downregulated in MEFs lacking p21, unlike the situation in wild-type cells (de Toledo *et al*., 1998). Squelching by p53, due to its direct binding to basal factors, is independent of p21. Repression of the intact *cdc2* promoter did not occur in cells lacking p21, and overexpression of p21 alone reduced *cdc2* promoter activity and mRNA levels, indicating an important role for this protein in repressing the transcription of *cdc2* (Chang *et al*., 2000; Taylor *et al*., 2001).

How does p53 utilize p21 to repress the *cdc2* promoter? Repression maps to two sequence elements in the R box, one resembling an E2F site and another called the cell cycle genes homology region (CHR) (Sugarman *et al*., 1995; Tommasi and Pfeifer, 1995; Zwicker *et al*., 1995, Figure 2). Mutations in either the E2F site of CHR reduce repression by p53 (Taylor *et al*., 2001). Furthermore, the E2F4 DNA-binding protein and the Rb family member p130 bind to the R box in response to overexpression of p53 only if the E2F site and the CHR are intact (Taylor *et al*., 2001). E2F4/p130 is an efficient repressor of transcription, especially in cells arrested in Go (Smith *et al*., 1996; Vairo *et al*., 1995). p130 has overlapping functions in transcriptional regulation with p107, another Rb family member, whereas Rb regulates a partially distinct set of genes (Hurford *et al*., 1997). All three family members are required for repression of *cdc2* by p53 in mouse cells. Treatment of MEFs with adriamycin activates p53, leading to down-regulation of Cdc2 protein levels. Downregulation of Cdc2 protein was less efficient in MEFs lacking Rb, and completely abrogated in MEFs lacking both p130 and p107 (Taylor *et al*., 2001). A requirement for both p107 and p130 in repression of the *cdc2* promoter is consistent with cross-linking experiments showing that both p107 and p130 are bound to the R box of the *cdc2* promoter in NIH3T3 mouse fibroblasts (Wells *et al*., 2000). In human cells, p53 appears to repress the *cdc2* promoter by causing the p130/E2F4 repressor to accumulate and thus bind to the R box (Figure 2). Repression is dependent on induction of p21 by p53, leading to the inactivation of CDKs which would otherwise phosphorylate p130, causing the dissociation of E2F4 and loss of the repressor complex. In mouse cells, p107 and Rb may replace p130 to facilitate repression of *cdc2*. p107 and Rb may contribute to the repression of the human *cdc2* promoter even though they do not appear to bind to the R box *in vitro*. Based on its crystal structure, E2F4 is not expected to interact with the CHR element of the *cdc2* promoter (Zheng *et al*., 1999), although this region is required for repression by p53. Therefore, additional proteins may bind to the CHR, stabilizing the binding of E2F and thus contributing to repression (Taylor *et al*., 2001, Figure 2).

Consistent with observations on the *cdc2* promoter, it has been found that Rb family proteins are required for prolonged G2 arrest in response to DNA damage. Prolonged arrest of H1299 cells in G2 exposed to high levels of p53, in combination with treatment with adriamycin or ionizing radiation, is associated with a reduction in the levels of Cdc2 and Cyclin B1 proteins and mRNAs (Flatt *et al*., 2000). Treatment of HCT116 colorectal tumor cells with adriamycin or ionizing radiation also caused a loss of Cdc2 and Cyclin B1 protein. DNA damage did not cause the downregulation of either Cdc2 or Cyclin B1 if either p53 or p21 were inactivated by homologous recombination, showing that p53 and p21 are responsible for the loss of these proteins (Flatt *et al*., 2000). Interestingly, expression of the HPV-E7 protein (which binds to and inactivates Rb, p130 and p107) in RKO colorectal

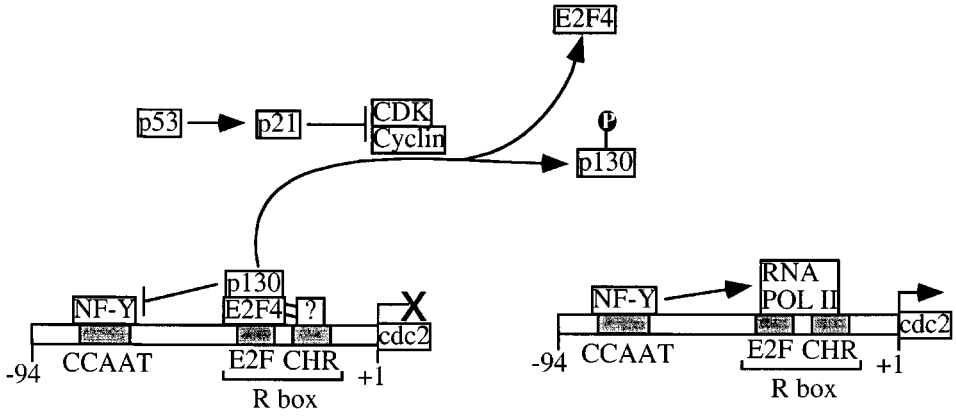

Figure 2 The mechanism of repression of the *cdc2* promoter by p53

tumor cells resulted in fewer cells arrested in G2 in response to adriamycin or ionizing radiation compared to parental cells. HPV-E7-expressing cells contained more Cdc2 and Cyclin B1 protein and higher Cdc2 activity after treatment with adriamycin than did parental cells (Flatt *et al*., 2000). Therefore, Rb family members are required to downregulate Cdc2 and Cyclin B1, which is necessary to maintain prolonged G2 arrest.

Repression of topoisomerase II, a mechanism of G2 arrest that does not involve Cdc2

In addition to modulating Cdc2 activity, p53 affects several genes that are important for the G2/M transition but do not directly affect Cdc2. Topoisomerase II, an essential enzyme that regulates chromatin topology, binds to DNA, creates double-strand breaks, and allows torsional strain to be removed from chromatin by allowing rotation of the broken ends. Once proper topology is attained, the double-strand break is resealed. Topoisomerase II is required during the G2/M transition, where it helps to bring about the higher order compaction of chromatin to form highly condensed mitotic chromosomes (reviewed in Withoff *et al*., 1996). Inhibition of topoisomerase II is sufficient to arrest cells in G2 (Anderson and Roberge, 1996). p53 was found to cause downregulation of topoisomerase II, by repressing its promoter (Sandri *et al*., 1996; Wang *et al*., 1997). Repression was mapped to several inverted CCAAT boxes in the topoisomerase II promoter (Wang *et al*., 1997). Downregulation of topoisomerase II may be important for G2 arrest by p53. However, the effect of overexpressing topoisomerase II on p53-dependent G2 arrest has not been tested.

G2 arrest by the p53 target genes B99 and MCG10

The B99 protein may cause G2 arrest independently of Cdc2 (Utrera *et al*., 1998). The B99 gene can be induced by overexpression of p53 and is upregulated in a p53-dependent manner by DNA damage. B99 contains a region with homology to the microtubule network-associated protein Map4. B99 is also localized to the microtubule network, and overexpression of B99 in the p53-null murine Balb/c (10)1 cell line can cause G2 arrest (Utrera *et al*., 1998). One possibility is that B99 triggers G2 arrest by interfering with microtubule rearrangements that are required to enter mitosis, such as the formation of a spindle.

MCG10 may also contribute to G2 arrest in a Cdc2-independent manner (Zhu and Chen, 2000). MCG10 can be induced in H1299 cells by overexpressing wild-type p53 but not the R249S tumor-derived mutant of p53 or a mutant of p53 with a deletion in the proline-rich domain (Zhu and Chen, 2000). Both of these mutants are defective in inducing other transcriptional targets of p53. MCG10 is induced by DNA damage in the colorectal tumor cell line RKO but not if p53 is inactivated by HPV-E6. Also DNA damage induces MCG10 in MCF-7 breast cancer cells and the colorectal tumor cell lines LS174T and HCT116, but not in HCT116 cells with a deletion of the p53 gene (Zhu and Chen, 2000). Overexpression of MCG10 inhibits the proliferation of H1299 cells by causing cell cycle arrest in G1 and G2, and induces apoptosis (Zhu and Chen, 2000). MCG10 contains two regions of homology to the RNA binding domains of heterogenous nuclear ribonucleoprotein K, an RNA-binding protein with a number of functions relevant to the regulation of gene expression (Bomsztyk *et al*., 1997). Both RNA binding domains are required to inhibit proliferation, and to bind to RNA *in vitro* (Zhu and Chen, 2000). MCG10 may regulate the expression of genes important in cell cycle progression and apoptosis since other proteins with similar RNA binding properties can regulate transcription, translation or mRNA stability (Kiledjian *et al*., 1995; Michelotti *et al*., 1996; Ostareck *et al*., 1997).

Physiological significance and future directions

What conditions have provided the selective pressures to drive the evolution of such a highly elaborate mechanism of G2 arrest? Blocking damaged cells in G2 provides time to repair DNA damage or an opportunity to permanently arrest cells with severe damage in G2. Both of these responses are important to protect organisms from tumor formation driven by the accumulation of mutations. Why do so many pathways converge to inhibit entry into mitosis when DNA is damaged? Perhaps the individual pathways are inherently unable to provide a prolonged arrest. For example, cell cycle checkpoints in yeast that block entry into mitosis in response to DNA damage eventually become attenuated, and the cells eventually do enter mitosis, presumably by reactivating Cdc2, without having repaired the damaged DNA (Toczyski *et al*., 1997). Although checkpoint attenuation may be tolerated in populations of unicellular organisms where proliferation is more important than eliminating every damaged cell, the selective pressure to eliminate damaged cells is much greater in mammals, where a single aberrant cell may develop into a neoplasm. One way to ensure that damaged mammalian cells do not continue to proliferate even if checkpoint pathways become attenuated is for p53 to cause the elimination of Cdc2, Cyclin B1 and topoisomerase II proteins required for entry into mitosis.

Although much has been learned about the regulation of the G2/M transition by p53, many questions remain. What is the molecular basis for the profound differences in the cell-cycle responses of different types of human cells, and are these differences important in the specialized functions of these cells? As observed in *Xenopus* embryos does Cdk2 help to activate Cdc2 in mammalian cells, and, if so, how? Does p53 play a role in the nuclear-cytoplasmic shuttling of Cdc2/Cyclin B1 in fibroblasts and does this role ensure G2 arrest? Mammalian cells contain kinases similar to NimA which may function parallel to Cdc2 in pathways

necessary for mitosis (Lu and Hunter, 1995). In fact, p53-null fibroblasts treated with adriamycin arrest in G2 with active Cdc2 in the nucleus (Passalaris *et al*., 1999). Do p53-dependent or independent pathways triggered by DNA damage ensure G2 arrest by inhibiting NimA-related kinases as well as Cdc2? Finally, how does regulation of the G2/M transition by p53 contribute to neoplasia? The fact that Gadd45-null mice, with defects in the control of this transition, get tumors in response to DNA damage underscores the importance of regulating the G2/M transition for tumor suppression. Of course, p53-null mice also get tumors spontaneously and both p53 and Gadd45 have multiple functions in addition to participating in G2 arrest. Another challenge is to dissect the specific roles of these functions in neoplasia.

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