

Analyzing the G2/M Checkpoint

George R. Stark and William R. Taylor

Summary

The G2 checkpoint prevents cells from entering mitosis when DNA is damaged, providing an opportunity for repair and stopping the proliferation of damaged cells. Because the G2 checkpoint helps to maintain genomic stability, it is an important focus in understanding the molecular causes of cancer. Many different methods have been used to investigate the G2 checkpoint and uncover some of the underlying mechanisms. Because cell-cycle controls are highly conserved, a remarkable synergy between the genetic power of model organisms and biochemical analyses is possible and has uncovered control mechanisms that operate in many diverse species, including humans. Cdc2, the cyclin-dependent kinase that normally drives cells into mitosis, is an important target of pathways that mediate rapid arrest in G2 in response to DNA damage. Additional pathways ensure that the arrest is stably maintained. When mammalian cells contain damaged DNA, the p53 tumor suppressor and the Rb family of transcriptional repressors work together to downregulate a large number of genes that encode proteins required for G2 and M. Elimination of these essential cell cycle proteins helps to keep the cells arrested in G2.

Key Words: Cdc2; cyclin; CDK; mitosis; p53; DNA damage.

1. Introduction

An understanding of the G2/M transition in molecular terms began 15 yr ago. Different experimental approaches revealed that one master regulatory kinase, p34cdc2 (also called Cdk1), controlled entry into mitosis. Genetic screens in fission and budding yeast uncovered temperature-sensitive cell division cycle (Cdc) mutants (*1,2*). Cdc mutants are arrested at a specific cell cycle stage at the restrictive temperature. Importantly, the *cdc28* gene in *S. cerevisiae* was found to be essential for cells to pass START, a major control point in G1 (*1*). *cdc28* was found to be interchangeable with *cdc2* from *S. pombe*, which was needed for cells to pass from G1 into S phase and from G2 into mitosis (*3,4*). Interestingly, mutants of *cdc2* could give rise to the “cdc” phenotype

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characterized by lengthening of a cell cycle phase, but also to a “wee” phenotype characterized by the shortening of the cell cycle and division at a smaller size (5). Additional studies with *S. pombe* uncovered *cdc25* and *wee1*, and indicated that *cdc25* encoded an activator of the *cdc2* gene product, while *wee1* encoded an inhibitor (6–8). The isolation of *wee1* mutants was significant because *wee1* is a gene whose inactivation caused acceleration of the cell cycle, and encoded an inhibitor of a rate-limiting process. Cdc mutants, on the other hand, could map to any gene that was required for cell cycle progression, not necessarily part of the regulatory network.

Studies in yeast involving ingenious and laborious genetic screens laid important groundwork for the analysis of the G2 phase. Equally amazing was the convergence of experimental approaches that allowed the realization that the same protein could control entry into mitosis in yeast, mammals, frogs, starfish, and many other organisms. Studies in the large oocytes of frogs and starfish identified a factor that could induce entry into mitosis when microinjected, called maturation promoting factor (MPF) (9–11). The components of MPF were not to be identified for many years. One problem was that the high concentration of MPF required to induce mitosis using microinjection precluded its purification. This problem was overcome with the development of cell-free extracts that recapitulated many of the mitotic processes when MPF was added (12–14). Highly purified MPF was found to contain two proteins of molecular weight 34 and 45 kDa (15). Immunoblotting and immunoprecipitation using antibodies raised against the *S. pombe cdc2* gene product were used to show that the 34 kDa subunit was identical to Cdc2 (16,17). *S. pombe* Cdc2 was known to bind to the product of the *suc1* gene, which was exploited to show that MPF could be depleted using Suc1 purified from bacteria and immobilized on agarose beads (18,19). This result added further evidence that MPF contained Cdc2.

A separate line of investigation resolved the identity of the 45 kDa component of MPF. Sea urchin oocytes were found to contain proteins that oscillated during the cell cycle with highest levels in mitosis; these are called *cyclins* (20). *cyclin* mRNA could induce entry in mitosis when injected into frog oocytes, and was the only mRNA needed to drive a frog oocyte extract into mitosis (21–23). Subsequent western blotting and immunoprecipitation studies with antibodies raised against frog cyclin proved that the 45kDa subunit of MPF was a cyclin (24). This was consistent with studies documenting the interaction between p34Cdc2 and cyclins in the clam *Spisula solidissima* (25).

Additional proof for a universal controller of mitosis came from studies of histone phosphorylation. The growth-associated H1 histone kinase was observed to vary during the cell cycle in many types of cells, including sea urchin, starfish and frog oocytes, and mammalian fibroblasts, and the highest levels of activity were detected in mitosis (26–30). Interestingly, a partially

purified H1 kinase could accelerate mitosis in *Physarium*, leading the authors to suggest that their H1 kinase was a conserved regulator of mitotic entry (31). The identification of the H1 kinase as the product of the *cdc2* gene was made many years later, using a combination of chromatographic purification and an antibody against the cloned *cdc2* gene product (18). The realization that Cdc2 is a conserved inducer of mitosis illustrates an important idea in cell cycle research. By combining a diversity of methods and using information from diverse organisms, considerable progress was made.

2. Our Current Understanding of Cdc2 Regulation

Many of the regulatory steps that control Cdc2 activity have been elucidated and have been conserved during evolution, although some details are different (reviewed in 32,33). Cdc2 is active only at the G2/M border and is turned off as cells enter the anaphase stage of mitosis. The first step in generating active Cdc2 is its association with a cyclin (Fig. 1). In animal cells, Cdc2 associates with an A-type or a B-type cyclin, in fission yeast with Cdc13, and in budding yeast with the CLB proteins. The cyclins that bind to Cdc2 accumulate as cells progress through G2 and are degraded when cells progress from metaphase to anaphase, thus extinguishing Cdc2 kinase activity.

During G2, Cdc2/cyclin B is actively excluded from the nucleus, where it must go to phosphorylate the substrates that will bring about the various steps of mitosis (34–36). During G2, the Cdc2–cyclin B1 complex is kept in the cytoplasm by nuclear export, mediated by binding of the cyclin subunit to the exportin protein CRM1 (34,36,37) (Fig. 1). Export by Crm1 counterbalances the constitutive import of the complex mediated by binding of the cyclin subunit to importin β (38,39). As cells approach the G2/M boundary, cyclin B1 becomes phosphorylated in its Crm1 binding site, which blocks binding and stops export, allowing the accumulation of the Cdc2–cyclin B complex in the nucleus, where it can induce entry into mitosis (37) (Fig. 1). The Crm1 binding site of cyclin B can be phosphorylated by Cdc2 and also by Plk1, an enzyme found in many organisms including *Drosophila*, frogs, and yeast, and known to be required for multiple events during mitosis (40–42).

Cdc2 must be phosphorylated at threonine 161 to be active, and in animal cells this process is catalyzed by cyclin-dependent kinase (CDK)-activating kinase (CAK) (43,44) (Fig. 1). As animal cells approach the G2/M boundary, the accumulating Cdc2–cyclin B complex is kept inactive by two inhibitory phosphorylations on the Cdc2 subunit at tyrosine 15, catalyzed by Wee1, and at threonine 14, catalyzed by Myt1 (45–47) (Fig. 1). In fission yeast, only the conserved tyrosine residue is phosphorylated to turn off the kinase (48). In budding yeast, although the conserved tyrosine of Cdc28 is phosphorylated, this is not an important factor in the regulation of the kinase (49,50).

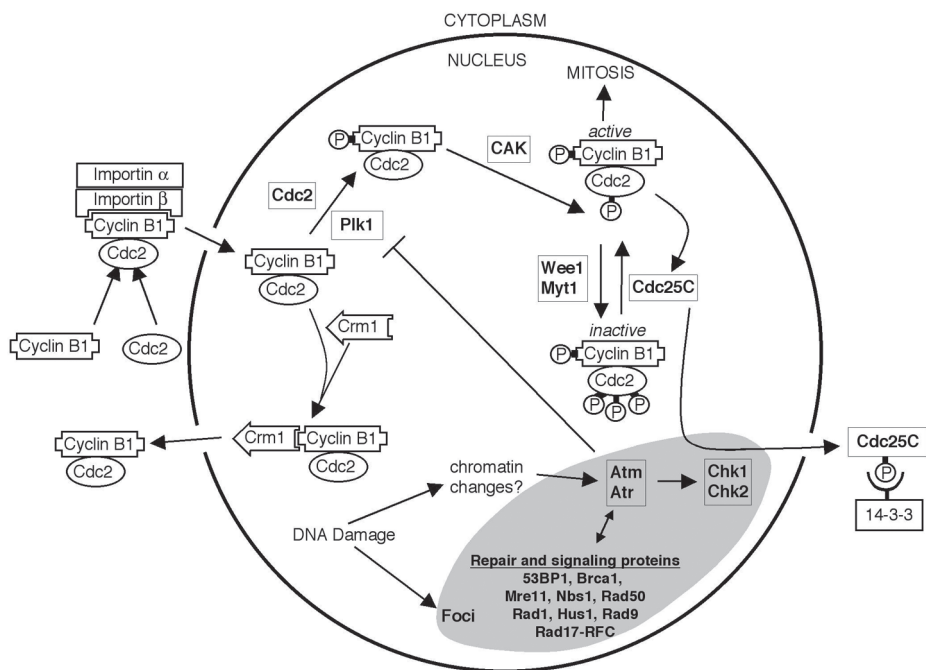


Fig. 1. Regulation of Cdc2 activity. Several steps lead to the assembly of active Cdc2 (as described in the text). These steps include binding to newly synthesized cyclin B, import into the nucleus, phosphorylation of Cdc2 by CAK, and cyclin B by Plk1 and Cdc2. Cdc25, Wee1, and Myt1 maintain a balance of phosphorylation on threonine 14 and tyrosine 15, which shifts to dephosphorylation and drives cells into mitosis. p53-independent pathways can rapidly initiate G2 arrest and involve Atm, Atr-dependent activation of Chk1 and Chk2, which phosphorylate Cdc25 and stimulate its binding to 14-3-3 proteins, which anchor it in the cytoplasm. The mechanisms leading to the activation of Atm and Atr may involve changes in chromatin topology and interactions, within nuclear foci (shown in gray), with DNA damage repair and recognition proteins. Nuclear foci occupy a very tiny area of the nucleus, but here are shown much larger for ease of presentation.

The Cdc2–cyclin B complex must not only enter the nucleus and be phosphorylated at threonine 161, but must also be activated by dephosphorylation of the Cdc2 subunit. At the G2/M boundary, tyrosine 15 and threonine 14 of Cdc2 are rapidly dephosphorylated by the conserved Cdc25 phosphatase (51) (Fig. 1). The rapid activation of Cdc2 is aided by a positive feedback loop involving Cdc25. Cdc2 phosphorylates and further activates Cdc25, allowing it rapidly to activate Cdc2 (51). Whereas yeast have a single Cdc25, mammals contain Cdc25A, which functions during the G1 to S transition, and Cdc25B

and Cdc25C, which dephosphorylate Cdc2 (**51**). The initial trigger for the activation of the Cdc25–Cdc2 loop is not known, but there is evidence that Cdc25B is important in this step in mammals. Unlike Cdc25C, which is concentrated in the nucleus, Cdc25B is found in the cytoplasm and may dephosphorylate cytoplasmic Cdc2 before the nuclear export has been turned off (**52**).

3. Cell Cycle Responses to DNA Damage

Early studies with mammalian cells uncovered a delay in G2 that occurred within 2 h of exposure to ionizing radiation (reviewed in **53**). This delay was found to be transient, with cells entering mitosis after a time that depends on the dose of ionizing radiation. A division delay in response to DNA damage was observed in diverse cell types, including amoeba, yeast, sea urchin eggs, and mammalian cervical cancer cells (**53**). Part of the basis for the division delay is that cells are blocked in G2 in response to DNA damage. The early method of determining the G2 delay was to count mitotic cells shortly after irradiation or adding DNA-damaging chemicals. G2 delay is characterized by a rapid reduction in the number of mitotic cells. Mitosis must be analyzed quickly to eliminate possible contributions of alternate points of arrest in the cell cycle. For example, if the reduction in mitotic cells occurs after 8 or 10 h (significantly longer than the G2/M period of approx 4–5 h) the blockage may be in an earlier cell cycle phase, with all the cells in G2 passing through mitosis before the block is manifest as a drop in mitosis. Mitotic cells were originally identified based on the morphology of chromatin, and the standard method involves dropping cells that are hypotonically swollen and fixed, onto glass slides. This method causes chromosomes to be spread out when they hit the slide and allows the unambiguous identification of mitotic cells (for example, **54**). More recent methods rely on markers present in mitotic cells such as phosphorylated histones, or MPM-2 antigens (**55,56**). By analyzing these markers with specific antibodies it is now feasible to quantify the number of cells in mitosis using the fluorescence-activated cell sorter (FACS) (**57**).

4. The Molecular Basis of G2 Arrest

To understand how the cell cycle is regulated in response to DNA damage, mutants of budding and fission yeast were identified in which the cell cycle was no longer delayed in response to DNA damage (**58**). This approach led to the concept of the cell cycle checkpoint, and uncovered many genes that form an important part of what we know about the workings of the DNA-damage response. Cell cycle checkpoints are regulatory mechanisms that ensure that cell cycle processes occur at the right time and in the right order. Early studies in budding yeast with the *rad9* mutant indicated that G2 delay was owing to intracellular signaling that blocked entry into mitosis in the presence of DNA

damage (58). A number of other “Rad” mutants that fail to arrest in the cell cycle in response to ionizing radiation have been uncovered, and orthologs in various organisms including mammals have been identified (reviewed in 59–61). Studies on the components of the DNA-damage response have uncovered three main groups of proteins involved in initiating G2 arrest. The *sensors* recognize damaged DNA, and the *transducers* transmit the signal downstream to the *effectors*, whose activity is modulated to bring about arrest (61,62). Although these groups of proteins are sufficient to induce the arrest, in time, many checkpoints become spontaneously inactivated, and additional mechanisms are used to maintain the arrest.

4.1. Effectors

Studies with mammalian cells in tissue culture showed that Cdc2 is an important effector for the G2 checkpoint. Ionizing radiation and other forms of DNA damage blocked the dephosphorylation of Cdc2 at tyrosine 15 and threonine 14, causing it to remain inactive (63,64). Inhibition of Cdc2 activity occurs rapidly after inducing DNA damage. For example, the loss of activity can be detected within an hour of adding the DNA-damaging agent etoposide to Chinese hamster ovary cells (63). One of the main effects of DNA damage is to interfere with the dephosphorylation of Cdc2 by the Cdc25C phosphatase.

4.2. Transducers

The mechanism by which Cdc25C is inactivated in response to DNA damage involves its phosphorylation by Chk1 and Chk2 (65–67) (Fig. 1). Chk1 and Chk2 (also known as Cds1) were originally identified by genetic screens in *S. pombe*, where Chk1 is required for cell cycle arrest in response to damaged DNA and Chk2–Cds1 is required for arrest in response to unreplicated DNA (68,69). Chk1 and Chk2 are protein kinases whose activity increases in response to damaged or unreplicated DNA in yeast and mammals (reviewed in 70). Cdc25C is phosphorylated by either Chk1 or Chk2, which creates a binding site on Cdc25C for proteins of the 14-3-3 family (65–67,71). Binding to 14-3-3 sequesters Cdc25C in the cytoplasm and blocks its ability to dephosphorylate Cdc2 (Fig. 1).

Two kinases, Atm and Atr, are responsible for the activation of Chk1 and Chk2 in response to stress (71–73) (Fig. 1). In mammals, it appears that both Atm and Atr can phosphorylate either Chk1 or Chk2 as well as other substrates (reviewed in 74). Parallels between mammals and yeast extend upstream of Chk1 and Chk2–Cds1. Budding yeast Mec1 and fission yeast Rad3 are the orthologs of Atr and are required for the activation of Chk1 and Chk2–Cds1 in response to DNA damage or unreplicated DNA (61). Tel1 in budding and fission yeast is an Atm ortholog.

Studies in mammalian cells on the phosphorylation of the p53 tumor suppressor by Atm and Atr have indicated that these kinases respond to different types of damage. For example, the phosphorylation of p53 on serine 15 in response to ionizing radiation is significantly, but not completely, reduced in cells lacking Atm, whereas there is no defect in the phosphorylation of p53 on serine 15 in response to ultraviolet radiation (75–77). Inactivation of Atr using a dominant negative version of the protein severely reduced p53 phosphorylation in response to ultraviolet radiation, and the initial phosphorylation of p53 was normal when cells were exposed to ionizing radiation (78,79). This suggests that Atr primarily mediates the response to ultraviolet radiation and Atm mediates the response to ionizing radiation. Although p53 was phosphorylated in response to ionizing radiation in cells expressing the dominant-negative Atr, this phosphorylation was lost much faster than in parental cells (79). This suggests that Atr may also provide a backup function in response to ionizing radiation. Atr has also been implicated in the response to unreplicated DNA caused by blocking DNA synthesis with hydroxyurea (78). Atm was originally identified as the gene mutated in the recessive autosomal disease ataxia telangiectasia (80). Among a number of symptoms, patients with ataxia telangiectasia are prone to cancer, which probably reflects the roles played by Atm in the cellular response to DNA damage.

There is evidence that Chk1 and Chk2 are not the only kinases that phosphorylate Cdc25 in response to DNA damage. For example, the p38 stress-activated kinase can phosphorylate both Cdc25B and Cdc25C, leading to their increased binding to 14-3-3 proteins in vitro (81). Also, the immediate G2 arrest that normally occurs after ultraviolet radiation was attenuated in cells treated with SB202190, a chemical inhibitor of p38 (81). In vivo studies showed that the binding of Cdc25B to 14-3-3 in cells exposed to ultraviolet radiation was reduced by treatment with the p38 inhibitor. However, the inhibitor had no effect on the binding of Cdc25C to 14-3-3 proteins in vivo, suggesting that Cdc25B is the main target of p38 in the G2 arrest response (81).

There is also evidence that inhibiting Cdc25 is not the only way by which Atm and Atr block the activation of Cdc2. Division of fission yeast normally requires Cdc25, but cells with the hypermorphic *cdc2-3w* allele can survive if Cdc25 is deleted (82). Induction of DNA damage in a *cdc2-3w*, Δ *cdc25c* mutant still caused a mitotic delay, showing that Cdc25 is not the only determinant of this response (82). Further studies pinpointed the Mik1 kinase as an important target of this Cdc25-independent arrest. Deletion of either Chk1 or Mik1 abrogated the residual arrest that occurred in the *cdc2-3w*, Δ *cdc25c* mutant. Overexpressing Chk1 caused an arrest that was dependent on Mik1, suggesting that Mik1 acts downstream of Chk1. Also, upregulation of Mik1 proteins in response to DNA damage was found to depend on Chk1 and Rad3 (82).

Similarly to Wee1, Mik1 phosphorylates tyrosine 15 of Cdc2, suggesting that DNA damage not only turns off the Cdc25 phosphatase that targets tyrosine 15 of Cdc2, but also turns on a kinase that phosphorylates this residue. Upregulation of the rate of inhibitory phosphorylation of Cdc2 has not been implicated as a mechanism of G2 arrest in mammals.

An additional substrate of the Atm and Atr kinases has been uncovered that may contribute to G2 arrest. Plk1 is inactivated in response to DNA damage by Atm/Atr-dependent phosphorylation (83). Because Plk1 can phosphorylate cyclin B1 to block export of cyclin B1 from the nucleus, one interesting possibility is that inactivation of Plk1 leaves cyclin B1 stranded in the cytoplasm (40,41). Because Cdc2 can also phosphorylate cyclin B1 in its nuclear export signal, the regulation of cyclin B1 localization is likely to be more complicated (42) (Fig. 1).

4.3. Sensors

Evidence is accumulating regarding how Atm, Atr, Chk1, and Chk2 are activated in response to DNA damage. There is clearly an involvement of damage sensors in the activation of at least some of these kinases (Fig. 1). There are several major damage-sensing machines, one of which shows striking similarity to the PCNA and RFC complexes needed for processive DNA synthesis (reviewed in 59–61). PCNA forms a homotrimeric clamp around the double helix, which is loaded onto DNA by the heteropentameric RFC complex composed of the RFC1–5 subunits. The DNA damage response involves the Rad17 protein, which forms a complex with RFC2, 3, 4, and 5, and by homology has been suggested to form a clamp loader (84,85). Rad1, Hus1, and Rad9 proteins form a trimer with structural similarity to the PCNA sliding clamp complex (86). Interactions between Rad17 and components of the Rad1–Hus1–Rad9 trimer suggest that the Rad17 complex may load the Rad1–Hus1–Rad9 complex onto DNA at sites of DNA damage (87,88). Rad1, Rad9, Rad17, and Hus1 are all required for the cell cycle delay in response to DNA damage and for the activation of checkpoint proteins such as Chk1 (59,89). Thus, the Rad17–RFC complex may recognize damaged DNA and load the Rad1–Hus1–Rad9 complex. Because Rad1 is a 3'-5' exonuclease, the Rad1–Hus1–Rad9 complex may function to increase the amount of single-stranded DNA at sites of damage to facilitate signaling to the checkpoint transducer proteins (90).

Additional complexes act proximal to DNA damage and are important in allowing the signal transducers to become activated. Brcal, originally identified as a locus of susceptibility in human breast cancer, is localized to sites of DNA damage and may be needed to recruit other proteins, such as Atm, to these sites (91–93). Atm and Atr can be found in a large complex containing Brcal, called Brcal-associated genome surveillance complex (BASC) (94).

BASC also contains the proteins Mre11, Nbs1, and Rad50, which are essential for the recognition and repair of double-strand breaks in DNA (94). Mre11, Nbs1, and Rad50 form a complex that is recruited to sites of DNA damage and helps to repair the break through nonhomologous end-joining (95,96). Studies in yeast have shown that these three proteins are needed for proper checkpoint function, but the detailed mechanism of how these proteins signal to downstream signal transducers has not been uncovered (97).

Rad3, the Atr ortholog in budding yeast, is activated in response to DNA damage, and this process is independent of the Rad17–RFC and Rad1–Hus1–Rad9 complexes (98). This observation is important, because it argues against a simple model in which these proximal DNA damage-sensing complexes signal to the Atm/Atr proteins. Also important is the fact that the activation of Chk1 does require the Rad17–RFC and Rad1–Hus1–Rad9 complexes (89). The phosphorylation of Chk1 appears to be directly catalyzed by Rad3 (99). If Rad3 is active in cells lacking Rad17, why can't it phosphorylate Chk1? One possibility is that Rad17 provides a docking site for Chk1 and activated Rad3, either directly or by allowing damaged DNA to be processed into foci where DNA damage-signaling proteins accumulate.

A similar situation may occur in mammalian cells. Atm exists as an inactive dimer or oligomer in unstressed cells (100). Atm is phosphorylated at an autophosphorylation site and dissociates into a monomeric active kinase very rapidly (within approx 30 s) after cells are exposed to ionizing radiation. This rapid phosphorylation occurs on approx 50% of the Atm in the cell and happens at doses of radiation that would create fewer than 20 double-strand breaks (100). These data suggest that it is unlikely that every Atm dimer must diffuse to the site of damage to be activated. This suggestion is consistent with literature on budding yeast showing that Rad3 does not need the damage-sensing Rad proteins to be activated. An alternative model has been proposed in which DNA strand breaks cause changes in the higher order topology of chromatin, which can act at a distance to signal the activation of Atm. This model is supported by the observation that chloroquine and trichostatin A, drugs that can alter higher order chromatin topology, can also induce the phosphorylation of Atm without causing detectable DNA damage (100). Once activated, Atm may then diffuse to foci in the nucleus that contain damage recognition and repair complexes like BASC (Fig. 1). At those sites, Atm could phosphorylate some of its downstream targets that mediate G2 arrest, such as Chk1 and Brcal.

Additional proteins may act as adapters to bring substrate and enzyme together at damage-induced nuclear foci. For example, the 53BP1 protein shows regions of homology to Brcal, and is relocalized to damage-induced foci (101). 53BP1 binds to Chk1 and Brcal in unstressed cells, and these associations are disrupted by ionizing radiation at the same time as Chk1 and Brcal

become phosphorylated (**102**). 53BP1 was identified because it binds to p53 (**103**). Reduction of 53BP1 levels with small interfering RNA molecules reduces the accumulation of p53 and the phosphorylation of Chk1 that occurs in response to ionizing radiation (**102**). One interpretation is that 53BP1 is an adapter that brings Atm substrates to damage-induced foci, where they are phosphorylated by monomeric active Atm that has diffused to those same sites.

Biochemical and genetic approaches have uncovered a large amount of information about how the cell responds to DNA damage. An important branch of this response ultimately leads to the inactivation of Cdc2 and G2 arrest. As described above, rapid events lead to the inactivation of Cdc2 through inactivation of Cdc25C. Recent experiments in mammalian cells suggest that the p53 tumor suppressor participates in pathways that help to maintain G2 arrest in response to DNA damage.

5. The Function of p53

The p53 tumor suppressor was originally identified as a protein bound to the large T antigen of the SV40 tumor virus (**104**). Early studies on p53 function suggested that it was an oncogene, because the first clones of p53 could immortalize primary cells and cooperate with other oncogenes such as Ha-*ras* to induce neoplastic transformation (**105–108**). The true function of p53 as a tumor suppressor was established in a number of landmark studies showing that (1) transforming p53 alleles contained mutations, and wild-type p53 was inactive in transformation assays (**109**); (2) p53 was mutated during the progression of colorectal cancer (**110**); and (3) elimination of p53 function in the mouse greatly increased the frequency of spontaneous tumors (**111**). Thus, the elucidation of the function of p53 in neoplasia relied on biochemical approaches, mapping of disease loci using the methods of human genetics, as well as mouse studies involving homologous recombination to inactivate the p53 gene.

The important tumor suppressor function of p53 is illustrated by its widespread inactivation in many types of human cancer (**112**). Also, germline inactivation of p53 is responsible for the cancer susceptibility syndrome described by Li and Fraumeni (**113,114**). The tumor suppressor function of p53 lies in its ability to induce either cell cycle arrest or cell death by apoptosis in response to genotoxic stress (reviewed in **115,116**). Many types of stress, including DNA damage, arrest of transcription, arrest of DNA synthesis, hypoxia, and oncogene activation, can activate p53. Activation of p53 depends on its post-translational modification in response to stress (reviewed in **117**). In particular, serine and threonine residues are phosphorylated and lysine residues are acetylated or sumoylated in response to stress, provoking the activation of p53.

In unstressed cells, p53 is present at a low level due to its degradation by Mdm2, which binds to the N-terminus of p53 and catalyzes the transfer of ubiquitin to lysine residues of p53, generating a signal for degradation by the proteasome (reviewed in *118*). As mentioned above, serine 15 of p53 is phosphorylated by Atm and Atr in response to DNA damage (*76,79,119*). In addition, Chk1 and Chk2 can phosphorylate p53 on serine 20 (*120,121*). Phosphorylation of these and other residues in the N-terminus of p53 blocks the binding of Mdm2, allowing p53 to accumulate to high levels (*122*). Once activated, p53 carries out its downstream arrest or apoptosis functions by activating the expression of a large number of genes. p53 binds in a sequence-specific manner to the promoters of its target genes and uses its acidic N-terminus to activate transcription (*115*).

Interestingly, the release of Mdm2 correlates with the binding of the CBP/p300 histone acetyl transferases to the phosphorylated N-terminus of p53 (*123*). CBP/p300 acetylates lysine residues in the C-terminal region of p53, stimulating its ability to bind to DNA (*124*). These studies have relied on gel shift assays, where acetylated or non-acetylated p53 is added to radio-labeled oligonucleotides containing the p53 consensus sequence (*124*). DNA binding is indicated by a shift in the mobility of the probe and the formation of a novel band. There is recent evidence that when the probe is complexed with histones, the stimulation of DNA binding observed after acetylating p53 is abolished (*125*). This has led to the suggestion that p53 does not need to be acetylated to bind to DNA that is packaged into chromatin, a more physiological substrate than naked DNA. However, acetylation-specific antibodies show that p53 is acetylated in vivo (*123*). Because the lysine residues that are acetylated are also ubiquitinated, simply mutating these residues will not be helpful in clearing up this issue. If CBP/p300 are not needed to acetylate p53, perhaps their role is to acetylate histones in the vicinity of p53 target genes to aid in their transcriptional induction.

6. Contribution of p53 to G2 Arrest

The first clue that p53 was important for DNA-damage responses was that the levels of p53 protein were elevated when cells were exposed to ultraviolet radiation (*126*). Proof for this idea was provided by the observation that p53 was required for the G1 arrest that occurred in response to ionizing radiation (*127*). Human cells lacking p53 bypass the G1 checkpoint, progress through S phase, and accumulate in G2, showing that G2 arrest still occurs in cells lacking p53 (*127*). However, we found that when p53 is overexpressed in the absence of any other stress, this could also arrest cells in G2 (*128*). These and other studies showed that p53-independent pathways are sufficient to induce G2 arrest, and that p53 is also involved, probably to ensure the long-term sta-

bility of the arrest (reviewed in *129*). In our work using p53 overexpression, we relied on a number of methods to determine cell cycle position. In our first studies, cellular DNA was stained with propidium iodide (PI) and cell cycle distribution assessed using the FACS to measure PI fluorescence (*128*). Because p53 is capable of inducing G1 arrest, we used synchronized cells to investigate its functions in G2. We used mimosine to reversibly arrest cells in S phase and a cell line containing a tetracycline inducible p53. Cells were released from the mimosine block and tetracycline was removed to induce p53. On the basis of DNA content, p53 overexpression did not affect progression through S phase after mimosine removal but caused approx 60% of the cells to become arrested with a 4N content of DNA (*128*). Cells with a 4N DNA content might be either in G2 or mitosis; however, direct examination indicated that mitotic cells were rare, suggesting that the cells were in G2. Similar results were obtained by another group using a temperature-sensitive allele of p53 and serum starvation/stimulation to synchronize the cells (*130*).

One possible alternative explanation of the accumulation of cells with a 4N DNA content was that p53 did not block cells in G2, but somehow altered progression through mitosis in such a way that cells exited mitosis by decondensing their chromatin, resulting in p53-dependent arrest in a tetraploid G1 state. To test this possibility, we released cells from a mimosine block and removed tetracycline to induce p53. Entry into mitosis was directly observed using time-lapse microscopy (*131*). This technique involves maintaining cells in an environment that allows them to grow (i.e., at 37°C with humidity and elevated CO₂) while on a microscope stage (**Figs. 2 and 3**). Current approaches involve capturing images of the cells using digital cameras, with image capture controlled by computer software. We use Metamorph software to capture images and to control shutters, which shield cells from light during the interval between image capture. To analyze cell cycle events, we capture images every 15–20 min. Time-lapse experiments are run for several days to allow an analysis of delayed effects of specific treatments. When we applied this method to the study of G2 arrest by p53, we found that few cells released from the mimosine block entered mitosis (*131*). Many control cells released from the mimosine block in the presence of tetracycline to repress the p53 transgene entered mitosis and divided. These studies show that p53 prevents cells from entering mitosis, causing them to arrest in G2. Additional support for a role for p53 in G2 arrest was provided by studies of a derivative of the HCT116 colorectal tumor cell line in which p53 was inactivated by homologous recombination (*132*). These p53-null cells initially arrested in G2 when DNA was damaged with adriamycin, but the arrest could not be maintained and the cells eventually did enter mitosis. These studies indicate that p53 is very important in maintaining G2 arrest in response to DNA damage.

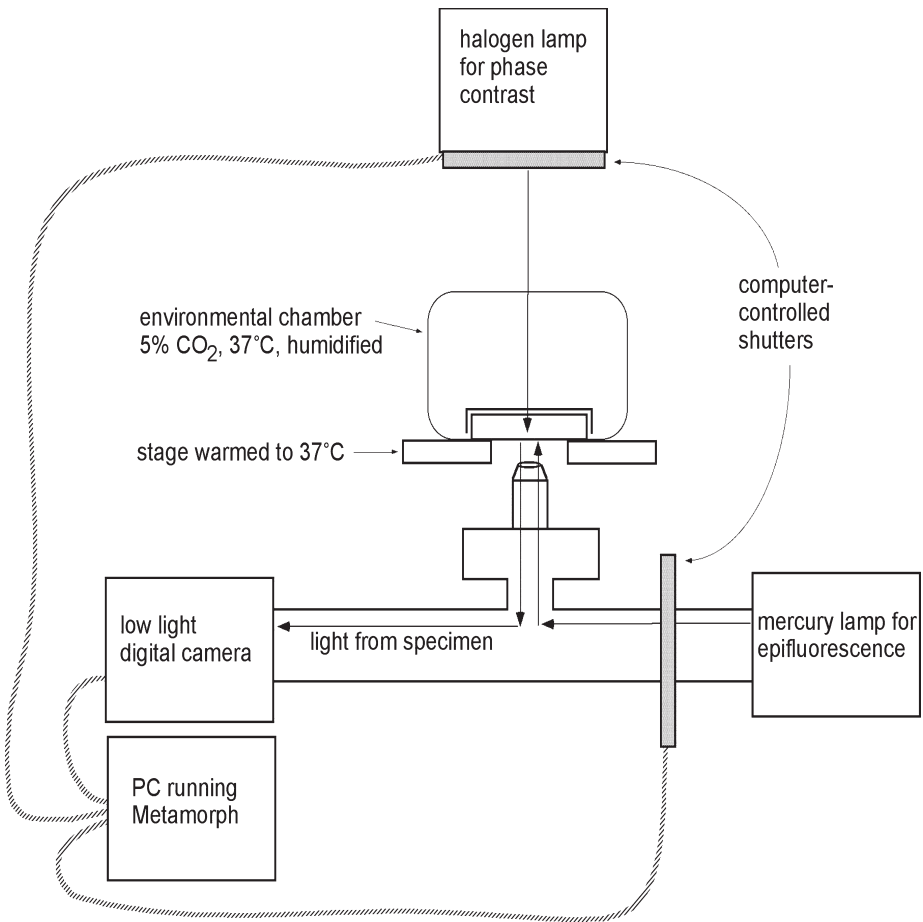


Fig. 2. Equipment used for time-lapse microscopy. The basic arrangement is shown. Cells are viewed on an inverted microscope using phase contrast or epifluorescence. Shutters allow light from the halogen lamp (for phase contrast) or the mercury lamp (for epifluorescence) to hit the sample. Metamorph software is used to open the shutters for image capture with a low-light digital camera. For a typical experiment in which phase contrast and epifluorescence are combined, Metamorph is programmed to open the phase shutter and capture an image, close the shutter, and then open the epifluorescence shutter, capture an image, and close the shutter. This series usually takes approx 1–2 s and is repeated every 10 min. The result is a time series of both phase and epifluorescence images, which can be viewed as separate movies.

7. Mechanisms of G2 Arrest by p53

The contribution of p53 to G2 arrest involves some of its transcriptional targets. p53 activates many genes that encode proteins which carry out its bio-

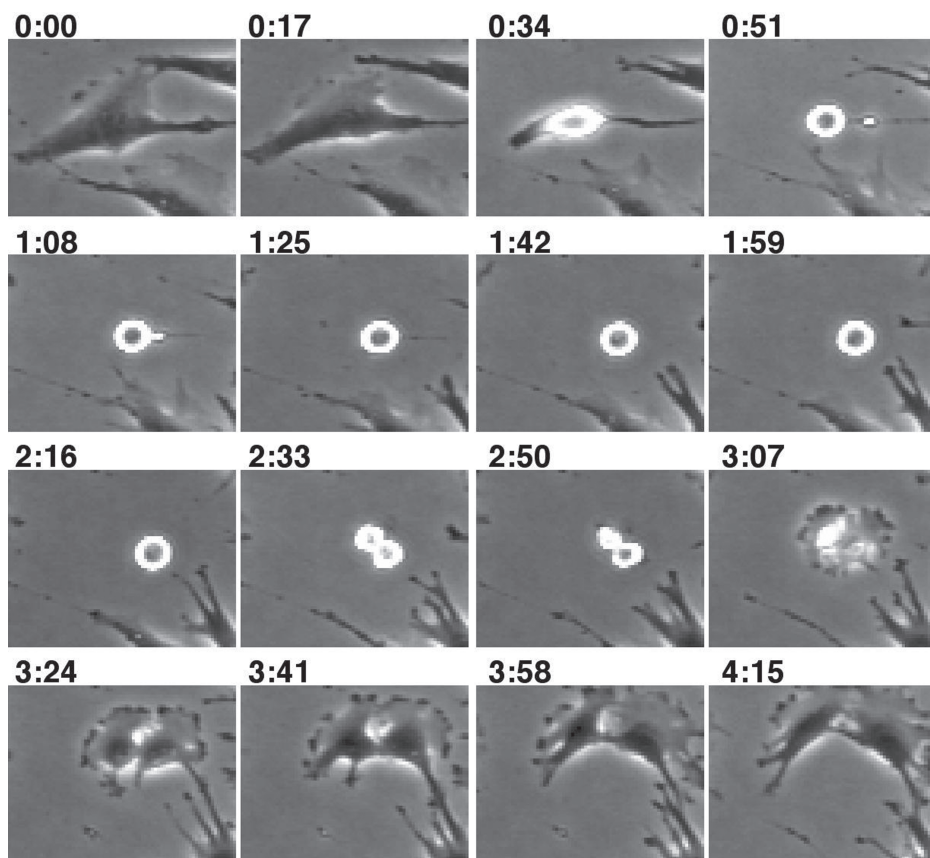


Fig. 3. Time-lapse images of the mitotic process. Phase contrast images of a human fibroblast entering and progressing through mitosis are shown. An image was taken every 17 min. By viewing approx 100 cells at a time, the mitotic activity of a culture can be assessed over time.

logical functions, including Bax, Puma, Pig3, and Noxa, which are important in inducing apoptosis, and p21/waf1, Gadd45, and 14-3-3 σ , which are inducers of growth arrest (*116*). p21/waf1 is a major target of p53 that is essential for G1 arrest. Unlike yeast, in which Cdc2 can control both the G1/S and G2/M transitions, mammalian cells have a family of kinases with homology to Cdc2. These kinases require a cyclin subunit for activity, are called cyclin-dependent kinases (CDKs), and control the major mammalian cell cycle transitions. p21/waf1 is an efficient inhibitor of CDK2, 4, and 6, which explains its ability to block cells at the G1/S boundary (*133*). p21/waf1 can bind only poorly to Cdc2, yet can arrest cells in G2 when overexpressed (*133–136*). In addition, HCT116

cells lacking p21/waf1 arrest only transiently in G2 in response to adriamycin, adding further support for the involvement of this protein in stabilizing G2 arrest (*132*). One mechanism that has been proposed for G2 arrest by p21/waf1 involves its ability to block the activating phosphorylation of Cdc2 at threonine 161, but the mechanism by which p21/waf1 reduces this phosphorylation is not known (*137*). Our work has uncovered an additional mechanism by which p21/waf1 contributes to the stability of G2 arrest.

Gadd45 has also been implicated in G2 arrest induced by p53. Gadd45 can bind to Cdc2 and dissociate the cyclin B subunit, thus inactivating the complex (*138,139*). The G2 delay that occurs in response to ultraviolet radiation is reduced in lymphocytes when Gadd45 is deleted (*140*). The 14-3-3 σ target of p53 can cause G2 arrest when overexpressed (*141*). It appears to do so by binding to the Cdc2–cyclin B complex and anchoring it in the cytoplasm, where it is unable to induce mitosis (*142*). Deletion of 14-3-3 σ from HCT116 cells caused the cells to escape from the G2 arrest induced by adriamycin and enter mitosis in the presence of large amounts of damaged DNA (*142*). The 14-3-3 σ -null cells were very sensitive to killing by adriamycin, probably owing to the catastrophic mitosis. Inactivation of both p21/waf1 and 14-3-3 σ in HCT116 led to a severe defect in G2 arrest: such cells were killed much more efficiently by adriamycin than cells lacking either gene alone (*143*).

7.1. The Role of Transcriptional Repression in G2 Arrest

To understand how the overexpression of p53 could induce G2 arrest, we focused on the regulation of Cdc2, a likely candidate to mediate this effect. In cells arrested in G2 by p53, Cdc2 activity was low, but CAK activity was unaffected (*131*). Thus, our results suggest that the reduction in threonine 161 phosphorylation of Cdc2 in response to p21/waf1 is not owing to direct inactivation of CAK, and other mechanisms must be at play. Immunoblotting also showed that when cells were just reaching the G2/M boundary (approx 20 h after removal of mimosine), the phosphorylation of Cdc2 at either tyrosine 15 or threonine 14 was not increased by high levels of p53, compared to low levels of p53 (*131*). However, the level of cyclin B1 was reduced at this time point, and when cells were arrested in G2 for 48 or 72 h, the amount of Cdc2 protein was also downregulated. Loss of both proteins was caused by repression of both promoters by p53 (*131*). We did not detect 14-3-3 σ in these experiments because it appears not to be expressed in fibroblasts. Combining the available data, p53 initially reduces Cdc2 activity by inducing Gadd45, by blocking threonine 161 phosphorylation by way of p21/waf1, and by repressing cyclin B1. Eventually, Cdc2 protein is also downregulated. Interestingly, overexpression of cyclin B1 was sufficient to overcome G2 arrest induced by p53 in an ovarian cancer cell line (*144*). In our studies, G2 arrest was abrogated

only if we expressed a Cdc2 mutant protein that could not be phosphorylated at its inhibitory sites in combination with a cyclin B1 mutant that is constitutively present in the nucleus (*131*). Although our studies agree with the conclusion that Cdc2–cyclin B1 is an important target in G2 arrest induced by p53, the effects of p53 may be more complicated. The fact that a constitutively nuclear cyclin B1 was needed to abrogate the arrest suggests that p53 may alter the nuclear/cytoplasmic shuttling of cyclin B1, although now, there is no direct evidence to support this idea.

The downregulation by p53 of Cdc2 and cyclin B1, two proteins essential for cells to enter mitosis, helps to explain the effects of caffeine on the cell cycle. Caffeine can abrogate cell cycle checkpoints, including the G2 arrest that occurs in response to DNA damage (*145*). However, p53 can block the effect of caffeine on G2 arrest. For example, a breast cancer cell line with wild-type p53 arrests in G2 in response to DNA damage, and this effect could not be overcome by caffeine (*146*). However, when p53 was inactivated by expressing the E6 protein of the human papilloma virus, caffeine could abrogate the G2 arrest (*146*). These results suggested that p53 has to be inactivated to unleash the effects of caffeine because it downregulates both Cdc2 and cyclin B1. We recently reinvestigated this phenomenon and found that the inactivation of p53 allows caffeine to abrogate G2 arrest induced by etoposide in HT1080 fibrosarcoma cells, which have wild-type p53 (*147*). The stable arrest of the parental cells in the presence of caffeine was highly correlated with the p53-dependent transcriptional repression of both *cdc2* and *cyclin B1*. To determine the effects of caffeine on mitosis in HT1080 cells, we used time-lapse microscopy, as well as Western analysis for histone H3 phosphorylated on serine 10, a marker for mitosis (*147*). A major breakthrough in understanding the effects of caffeine on the cell cycle was the observation that it inhibits both Atm and Atr by directly binding to these proteins (*148–150*). In cells lacking p53 function, G2 arrest still occurs, even though Cdc2 and cyclin B1 are present, presumably because of the Atm/Atr-dependent inactivation of Cdc25C. Caffeine can inactivate this pathway, allowing cells lacking p53 to overcome the arrest.

Although we detected many mitotic cells when caffeine was added to the cells lacking p53 function, we did not detect major changes in the DNA content of the cells (*147*). If cells were entering and completing mitosis, we expected the number of cells with a 4N content of DNA to decrease and the number of cells in G1, with a 2N DNA content, to increase. To examine the fate of cells in more detail, we used a fusion protein comprised of histone H2B and green fluorescent protein (GFP) (*151*). This fusion protein associates stably with chromatin, allowing DNA to be visualized in live cells. We used time-lapse epifluorescent imaging to analyze chromatin dynamics as cells were

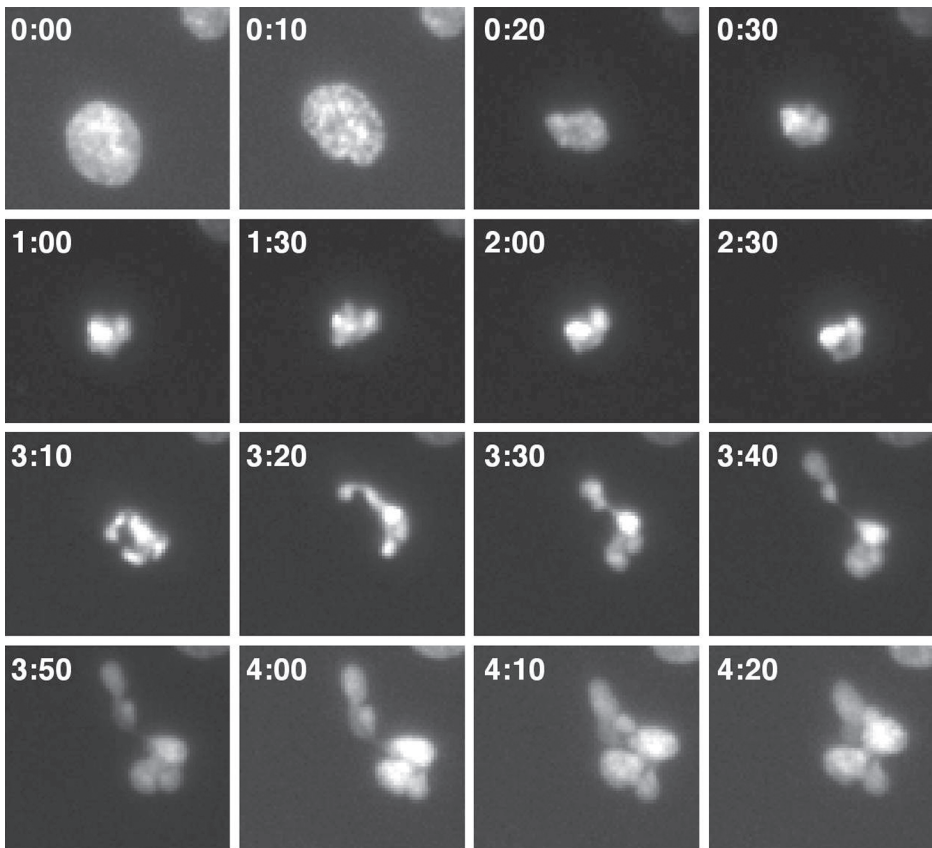


Fig. 4. Time-lapse analysis of chromatin dynamics. In the cell shown, time-lapse was used to visualize a defective mitosis, which occurred in the presence of DNA damage and caffeine. The cell type shown lacks p53 function. It was treated with etoposide to induce DNA damage and with caffeine to override the G2 checkpoint. The cells were stably transfected with a histone H2B-GFP fusion protein, which associates with chromatin. Images were captured every 10 min (not all frames are shown). At the 0:10 time point, the cell has just entered prophase and chromatin condensation has started. The cell is in metaphase for approx 3 h, decondenses its chromatin, and exits mitosis without dividing and with an aberrant nuclear morphology.

driven into mitosis with caffeine (**Figs. 2 and 4**) (*147*). Interestingly, the cells spent much more time in metaphase than untreated cells and were able to divide into two viable daughter cells only infrequently. In some cells, cytokinesis could not be completed, owing to the presence of chromatin bridges between partially divided nuclei. In other cells, a prolonged metaphase was immediately followed by death. These results suggest that, although caffeine can

induce mitosis in cells lacking p53, the cells cannot divide, which explains why we do not observe newly divided cells in G1.

7.2. The Mechanism of *cdc2* Repression by p53

We relied on classical mutation and deletion analyses to find the region of the *cdc2* promoter that mediated repression by p53 (**152**). This region was narrowed down to the R box, previously identified as a binding site for a transcriptional repressor (**152,153**). The R box is composed of a cell cycle-dependent element (CDE) and a cell cycle-homology region (CHR) (**153**). Both CDE and CHR are found in a number of cell cycle-regulated promoters and cause repression of these genes during G1 (**154**). Derepression occurs as cells enter S and G2. A repressor that can bind to the CDE/CHR element of the *cdc2* promoter contains an E2F subunit and a member of the Rb family of proteins (**155**).

E2Fs were originally identified as cellular proteins required for the transcription of the E2 gene of adenovirus during infection (**156**). There are eight E2Fs (E2F1 to 6 and DP1 and 2) (reviewed in **157**). An E2F forms a heterodimer with a DP to form a functional transcription factor, with DP1 and 2 relatively interchangeable. E2F1, 2, and 3 bind to promoters in a sequence-specific manner and activate the transcription of the corresponding genes. E2F4 and 5 bind to the same sequences but are more important in repression. E2F1, 2, and 3 contain transcriptional activation domains, which allow them to induce transcription. Repression by E2F4 and 5 is mediated by binding to a protein of the Rb family, which consists of three proteins: Rb, p130, and p107. E2F6 binds to the E2F element but lacks the transcriptional activation and Rb-binding domains, and thus can block activation as well as repression.

Rb was the first protein identified as a tumor suppressor, and its inactivation causes retinoblastoma with high penetrance (**158**). p130 and p107 have significant structural and functional homology to Rb. Rb-family proteins bind to E2Fs and recruit histone-modifying enzymes, which cause repression of E2F target genes (reviewed in **159,160**). Whether an E2F target gene is repressed or activated depends in large part on the phosphorylation state of Rb family members. When highly phosphorylated, all three Rb proteins do not interact with E2F, and active transcription predominates. When hypophosphorylated, Rb proteins form a tight complex with E2F4 and 5 to cause repression. The major kinases that phosphorylate the Rb proteins are CDK2, 4, and 6. In early G1, when CDK activity is low, Rb proteins are poorly phosphorylated, are bound to E2F4 and 5 and are engaged in the repression of E2F targets. As cells progress through the cell cycle and CDK activity rises, the phosphorylation of Rb proteins reverses repression, clearing the way for E2F1, 2, and 3 to activate some of the same genes.

The CDE element of the *cdc2* promoter is similar in sequence to an E2F element (152,155). We reasoned that p53 represses *cdc2* by an indirect mechanism involving p21/waf1, which, by inhibiting CDK activity, elevates the level of hypophosphorylated Rb, which in turn binds to E2F, interacts with the CDE, and represses transcription. Based on a variety of biochemical assays, we conclude that this model is likely to explain the repression of *cdc2* by p53. In particular, we could identify a complex containing E2F4 and p130 that interacted with an oligonucleotide corresponding to the CDE/CHR only in cells expressing high levels of p53 (152). In addition, Cdc2 was not downregulated in response to the DNA damage caused by adriamycin in cells lacking p130 and p107, unlike wild-type cells in which Cdc2 was eliminated after 48 h of treatment (152). Cdc2 was still downregulated in cells lacking either p130 or p107 alone, suggesting that their functions overlap. We did not detect p107 binding to the *cdc2* oligonucleotide in vitro, even though it must be deleted to abrogate repression (152). It is possible that p107-containing complexes are important for repression but are present at too low a level to be detected by gel shift analysis.

Several groups have reported that the long-term maintenance of G2 arrest after DNA damage requires transcriptional repression mediated by Rb-family members. The E7 protein from types 16 and 18 human papilloma virus inactivates all three Rb proteins by binding to them and causing their degradation. Cells expressing E7 can still arrest in G2 in response to adriamycin. If adriamycin is removed, the parental cells do not enter mitosis, whereas cells with E7 do enter mitosis, suggesting that Rb is required to stabilize the G2 arrest (161). Also, expression of a mutant E2F that neither binds to Rb proteins nor activates transcription, but still binds DNA, can reduce G2 arrest after DNA damage (162). This result suggests that active repression is required for proper G2 arrest. Our early studies suggested that one of the targets of Rb during p53-dependent G2 arrest is *cdc2*, although our recent experiments suggest that the Rb family has wider effects on the expression of genes required for progression through G2 and M (Taylor, W. R., and Stark, G. R., unpublished observations).

7.3. Large-Scale Reprogramming of Transcription in Response to DNA Damage

With the knowledge that p130 and p107 are required to downregulate Cdc2 in response to DNA damage, we used Affymetrix microarrays to compare gene expression profiles in p130/p107-null and wild-type mouse embryo fibroblasts (MEFs) (Taylor and Stark, unpublished observations). Untreated cells were compared with cells treated with adriamycin for either 12 or 24 h. Although the expression of a large number of genes changed, we focused on genes that were repressed in the wild-type but not in the p130/p107-null cells, just like *cdc2*.

Many genes required for DNA synthesis were repressed similarly to *cdc2*, including MCM genes that encode proteins that initiate DNA synthesis at origins of replication; ribonucleotide reductase and thymidine kinase, which provide substrates for DNA synthesis; and others (Taylor and Stark, unpublished observations). This observation was not surprising, because genes involved in S phase were previously known to be transcriptional targets of E2F, thus explaining how E2F encourages progression from G1 into S phase (for example, *see 163*). We also observed that many genes required for G2 and M were repressed in a p130/p107-dependent manner, including genes whose products are required to drive cells into mitosis, such as *cdc2*, *cyclin b1*, *cdc25c*, and *plk1*, as well as a large number of genes that do not serve a regulatory role but nonetheless are required for progression through mitosis (Taylor and Stark, unpublished observations) (**Fig. 5**). This latter group includes a number of kinesin motor proteins (which help to move chromosomes during alignment at the metaphase plate), proteins required for the assembly of the mitotic spindle, and proteins required for cytokinesis (**Fig. 5**). Thus, p130 and p107 are responsible for repressing different classes of genes that are required for cells to enter and progress through mitosis. This function has presumably evolved to ensure that mammalian cells are arrested stably in G2 in response to DNA damage. Interestingly, this control does not exist in unicellular yeast cells, possibly because the worst consequence of a cell escaping G2 arrest before DNA damage is repaired is death of a single cell. In mammals, a single cell propagating with damaged DNA could potentially develop into a neoplasm and threaten the survival of the entire organism.

Our work showing that p130 and p107 are required to repress genes involved in G2 and M is consistent with recent reports using microarrays that show that E2F on its own can activate the transcription of some of these same genes (*164–166*). In addition, the overexpression of p21/waf1 has been shown to lead to the repression of a number of genes required for G2 and M, some of which are the same as we have uncovered (*167*). Together, these results suggest a model in which p53, induced by DNA damage, upregulates p21/waf1, which inhibits CDK activity to cause the formation of repressive Rb–E2F complexes (**Fig. 5**). These complexes repress a large number of genes required for G2 and M to ensure that arrest is stably maintained. Our work has highlighted the specific importance of p130 and p107 in this response. In addition, we identified some genes that, although repressed in wild-type cells, were not downregulated at all in p130/p107-null cells, indicating an important role for p130 and p107 in the downregulation of these genes (Taylor and Stark, unpublished observations). Other mitotic genes were still downregulated in p130/p107-null cells, but to a lesser extent than in wild-type cells. The genes must be subject to

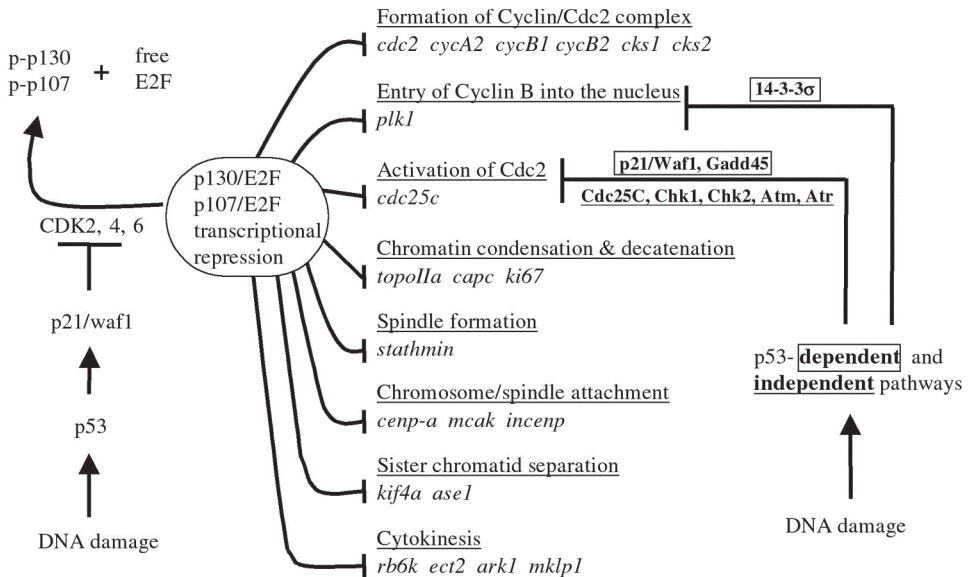


Fig. 5. Downregulation of mitotic genes by p53 and Rb-dependent pathways. p53 induces p21/waf1, which stops CDKs from phosphorylating Rb proteins, leading to the formation of repressive Rb/E2F complexes. p130 and p107 play an essential role in downregulating many genes required for multiple steps in mitosis when DNA is damaged. DNA damage also blocks the activation of Cdc2 and its entry into the nucleus by p53-dependent and independent pathways.

p130/p107-independent mechanisms of repression. One likely possibility is that p105Rb is responsible for the residual repression of these genes.

8. Conclusions

Multiple mechanisms ensure that cells arrest rapidly and stably in G2 when DNA is damaged. Initiation of arrest results primarily from the inactivation of Cdc2 by pathways driven by posttranslational modification. Posttranslational modification is well suited for the initiation of the arrest, because changes in the activity of the components of the pathway occur rapidly. However, posttranslational modifications can be reversed, and stable arrest requires the transcriptional repression of genes that encode parts of the cell cycle engine. Downregulation of these components takes more time than the initial pathway, because it involves posttranslational modification of p53, accumulation of p21/waf1, dephosphorylation of the Rb family, transcriptional repression of target genes, and the decay of the encoded mRNAs and proteins. By discouraging

inappropriate entry into mitosis, stable G2 arrest helps to protect the genome and suppress tumorigenesis.

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References

1. Hartwell, L. H., Culotti, J., Pringle, J. R., and Reid, B. J. (1974) Genetic control of the cell division cycle in yeast. *Science* **183**, 46–51.
2. Nurse, P., Thuriaux, P., and Nasmyth, K. (1976) Genetic control of the cell division cycle in the fission yeast *Schizosaccharomyces pombe*. *Molecular and General Genetics* **146**, 167–178.
3. Beach, D., Durkacz, B., and Nurse, P. (1982) Functionally homologous cell cycle control genes in budding and fission yeast. *Nature* **300**, 706–709.
4. Nurse, P., and Bissett, Y. (1981) Gene required in G1 for commitment to cell cycle and in G2 for control of mitosis in fission yeast. *Nature* **292**, 558–560.
5. Nurse, P., and Thuriaux, P. (1980) Regulatory genes controlling mitosis in the fission yeast *Schizosaccharomyces pombe*. *Genetics* **96**, 627–637.
6. Fantes, P. A. (1979) Epistatic gene interactions in the control of division in fission yeast. *Nature* **279**, 428–430.
7. Nurse, P. (1975) Genetic control of cell size at cell division in yeast. *Nature* **256**, 547–551.
8. Russell, P., and Nurse, P. (1986) *cdc25+* functions as an inducer in the mitotic control of fission yeast. *Cell* **45**, 145–153.
9. Masui, Y., and Markert, C. L. (1971) Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *J. Exp. Zool.* **177**, 129–145.
10. Smith, L. D., and Ecker, R. E. (1971) The interaction of steroids with *Rana pipiens* Oocytes in the induction of maturation. *Dev. Biol.* **25**, 232–247.
11. Kishimoto, T., and Kanatani, H. (1976) Cytoplasmic factor responsible for germinal vesicle breakdown and meiotic maturation in starfish oocyte. *Nature* **260**, 321–322.
12. Lohka, M. J., and Masui, Y. (1983) Formation in vitro of sperm pronuclei and mitotic chromosomes induced by amphibian ooplasmic components. *Science* **220**, 719–721.
13. Lohka, M. J., and Maller, J. L. (1985) Induction of nuclear envelope breakdown, chromosome condensation, and spindle formation in cell-free extracts. *J. Cell Biol.* **101**, 518–523.
14. Miake-Lye, R., and Kirschner, M. W. (1985) Induction of early mitotic events in a cell-free system. *Cell* **41**, 165–175.
15. Lohka, M. J., Hayes, M. K., and Maller, J. L. (1988) Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. *Proc. Natl. Acad. Sci. U S A* **85**, 3009–3013.

16. Labbe, J. C., Lee, M. G., Nurse, P., Picard, A., and Doree, M. (1988) Activation at M-phase of a protein kinase encoded by a starfish homolog of the cell cycle control gene *cdc2+*. *Nature* **335**, 251–254.
17. Gautier, J., Norbury, C., Lohka, M., Nurse, P., and Maller, J. (1988) Purified maturation-promoting factor contains the product of a *Xenopus* homolog of the fission yeast cell cycle control gene *cdc2+*. *Cell* **54**, 433–439.
18. Arion, D., Meijer, L., Brizuela, L., and Beach, D. (1988) *cdc2* is a component of the M phase-specific histone H1 kinase: evidence for identity with MPF. *Cell* **55**, 371–378.
19. Dunphy, W. G., Brizuela, L., Beach, D., and Newport, J. (1988) The *Xenopus* *cdc2* protein is a component of MPF, a cytoplasmic regulator of mitosis. *Cell* **54**, 423–431.
20. Evans, T., Rosenthal, E. T., Youngblom, J., Distel, D., and Hunt, T. (1983) Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell* **33**, 389–396.
21. Swenson, K. I., Farrell, K. M., and Ruderman, J. V. (1986) The clam embryo protein cyclin A induces entry into M phase and the resumption of meiosis in *Xenopus* oocytes. *Cell* **47**, 861–870.
22. Murray, A. W., Solomon, M. J., and Kirschner, M. W. (1989) The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. *Nature* **339**, 280–286.
23. Murray, A. W., and Kirschner, M. W. (1989) Cyclin synthesis drives the early embryonic cell cycle. *Nature* **339**, 275–280.
24. Gautier, J., Minshull, J., Lohka, M., Glotzer, M., Hunt, T., and Maller, J. L. (1990) Cyclin is a component of maturation-promoting factor from *Xenopus*. *Cell* **60**, 487–494.
25. Draetta, G., Luca, F., Westendorf, J., Brizuela, L., Ruderman, J., and Beach, D. (1989) Cdc2 protein kinase is complexed with both cyclin A and B: evidence for proteolytic inactivation of MPF. *Cell* **56**, 829–838.
26. Hardie, D. G., Matthews, H. R., and Bradbury, E. M. (1976) Cell-cycle dependence of two nuclear histone kinase enzyme activities. *Eur. J. Biochem.* **66**, 37–42.
27. Meijer, L., and Pondaven, P. (1988) Cyclic activation of histone H1 kinase during sea urchin egg mitotic divisions. *Exp. Cell Res.* **174**, 116–129.
28. Picard, A., Peaucellier, G., le Bouffant, F., Le Peuch, C., and Doree, M. (1985) Role of protein synthesis and proteases in production and inactivation of maturation-promoting activity during meiotic maturation of starfish oocytes. *Dev. Biol.* **109**, 311–320.
29. Cicirelli, M. F., Pelech, S. L., and Krebs, E. G. (1988) Activation of multiple protein kinases during the burst in protein phosphorylation that precedes the first meiotic cell division in *Xenopus* oocytes. *J. Biol. Chem.* **263**, 2009–2019.
30. Lake, R. S., Goidl, J. A., and Salzman, N. P. (1972) F1-histone modification at metaphase in Chinese hamster cells. *Exp. Cell Res.* **73**, 113–121.
31. Bradbury, E. M., Inglis, R. J., and Matthews, H. R. (1974) Control of cell division by very lysine rich histone (F1) phosphorylation. *Nature* **247**, 257–261.

32. Pines, J. (1995) Cyclins and cyclin-dependent kinases: a biochemical view. *Biochem. J.* **308**, 697–711.
33. Smits, V. A., and Medema, R. H. (2001) Checking out the G(2)/M transition. *Biochim. Biophys. Acta.* **1519**, 1–12.
34. Hagting, A., Karlsson, C., Clute, P., Jackman, M., and Pines, J. (1998) MPF localization is controlled by nuclear export. *EMBO J.* **17**, 4127–4138.
35. Pines, J., and Hunter, T. (1991) Human cyclins A and B1 are differentially located in the cell and undergo cell cycle-dependent nuclear transport. *J. Cell Biol.* **115**, 1–17.
36. Toyoshima, F., Moriguchi, T., Wada, A., Fukuda, M., and Nishida, E. (1998) Nuclear export of cyclin B1 and its possible role in the DNA damage-induced G2 checkpoint. *EMBO J.* **17**, 2728–2735.
37. Yang, J., Bardes, E. S., Moore, J. D., Brennan, J., Powers, M. A., and Kornbluth, S. (1998) Control of cyclin B1 localization through regulated binding of the nuclear export factor CRM1. *Genes Dev.* **12**, 2131–2143.
38. Moore, J. D., Yang, J., Truant, R., and Kornbluth, S. (1999) Nuclear import of Cdk/cyclin complexes: identification of distinct mechanisms for import of Cdk2/cyclin E and Cdc2/cyclin B1. *J. Cell Biol.* **144**, 213–224.
39. Takizawa, C. G., Weis, K., and Morgan, D. O. (1999) Ran-independent nuclear import of cyclin B1–Cdc2 by importin beta. *Proc. Natl. Acad. Sci. U S A* **96**, 7938–7943.
40. Collyer, T., Hardy, C. F., and Yuan, J. (1999) Cooperative phosphorylation including the activity of polo-like kinase 1 regulates the subcellular localization of cyclin B1. *Mol. Cell. Biol.* **19**, 4270–4278.
41. Kaiser, B. K., Swanson, C., Jackson, P. K., and Toyoshima-Morimoto, F. (2001) Polo-like kinase 1 phosphorylates cyclin B1 and targets it to the nucleus during prophase.[erratum appears in Nature 2001; 410(6830):847]. *J. Cell Biol.* **152**, 1267–1278.
42. Borgne, A., Ostvold, A. C., Flament, S., and Meijer, L. (1999) Intra-M phase-promoting factor phosphorylation of cyclin B at the prophase/metaphase transition. *J. Biol. Chem.* **274**, 11977–11986.
43. Poon, R. Y., Yamashita, K., Adamczewski, J. P., Hunt, T., and Shuttleworth, J. (1993) The cdc2-related protein p40MO15 is the catalytic subunit of a protein kinase that can activate p33cdk2 and p34cdc2. *EMBO J.* **12**, 3123–3132.
44. Fesquet, D., Labbe, J. C., Derancourt, J., Capony, J. P., Galas, S., Girard, F., Lorca, T., Shuttleworth, J., Doree, M., and Cavadore, J. C. (1993) The MO15 gene encodes the catalytic subunit of a protein kinase that activates cdc2 and other cyclin-dependent kinases (CDKs) through phosphorylation of Thr161 and its homologs. *EMBO J.* **12**, 3111–3121.
45. Booher, R. N., Holman, P. S., and Fattaey, A. (1997) Human Myt1 is a cell cycle-regulated kinase that inhibits Cdc2 but not Cdk2 activity. *J. Biol. Chem.* **272**, 22300–22306.
46. Liu, F., Stanton, J. J., Wu, Z., and Piwnica-Worms, H. (1997) The human Myt1 kinase preferentially phosphorylates Cdc2 on threonine 14 and localizes to the endoplasmic reticulum and Golgi complex. *Mol. Cell. Biol.* **17**, 571–583.

47. Parker, L. L., and Piwnica-Worms, H. (1992) Inactivation of the p34cdc2-cyclin B complex by the human WEE1 tyrosine kinase. *Science* **257**, 1955–1957.
48. Gould, K. L., and Nurse, P. (1989) Tyrosine phosphorylation of the fission yeast cdc2+ protein kinase regulates entry into mitosis. *Nature* **342**, 39–45.
49. Amon, A., Surana, U., Muroff, I., and Nasmyth, K. (1992) Regulation of p34CDC28 tyrosine phosphorylation is not required for entry into mitosis in *S. cerevisiae*. *Nature* **355**, 368–371.
50. Sorger, P. K., and Murray, A. W. (1992) S-phase feedback control in budding yeast independent of tyrosine phosphorylation of p34cdc28. *Nature* **355**, 365–368.
51. Draetta, G., and Eckstein, J. (1997) Cdc25 protein phosphatases in cell proliferation. *Biochim. Biophys. Acta*. **1332**, M53–63.
52. De Souza, C. P., Ellem, K. A., and Gabrielli, B. G. (2000) Centrosomal and cytoplasmic Cdc2/cyclin B1 activation precedes nuclear mitotic events. *Exp. Cell Res.* **257**, 11–21.
53. Maity, A., McKenna, W. G., and Muschel, R. J. (1994) The molecular basis for cell cycle delays following ionizing radiation: a review. *Radiother. Oncol.* **31**, 1–13.
54. Smith, K. A., Gorman, P. A., Stark, M. B., Groves, R. P., and Stark, G. R. (1990) Distinctive chromosomal structures are formed very early in the amplification of CAD genes in Syrian hamster cells. *Cell* **63**, 1219–1227.
55. Xu, B., Kim, S. T., Lim, D. S., and Kastan, M. B. (2002) Two molecularly distinct G(2)/M checkpoints are induced by ionizing irradiation. *Mol. Cell. Biol.* **22**, 1049–1059.
56. van Vugt, M. A., Smits, V. A., Klompmaier, R., and Medema, R. H. (2001) Inhibition of Polo-like kinase-1 by DNA damage occurs in an ATM- or ATR-dependent fashion. *J. Biol. Chem.* **276**, 41656–41660.
57. Taylor, W. R. (2004) FACS-based detection of phosphorylated histone H3 for the quantitation of mitotic cells. In *Checkpoint Controls and Cancer, Volume 2: Activation and Regulation Protocols* (A. H. Schönthal, ed.). Humana, Totowa, NJ: pp. 293–300.
58. Weinert, T. A. (1989) The RAD9 gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. *Science* **246**, 629–634.
59. O’Connell, M. J., Walworth, N. C., and Carr, A. M. (2000) The G2-phase DNA-damage checkpoint. *Trends Cell Biol.* **10**, 296–303.
60. Lowndes, N. F., and Murguia, J. R. (2000) Sensing and responding to DNA damage. *Curr. Opin. Gen. Dev.* **10**, 17–25.
61. Nyberg, K. A., Michelson, R. J., Putnam, C. W., and Weinert, T. A. (2002) Toward maintaining the genome: DNA damage and replication checkpoints. *Ann. Rev. Genet.* **36**, 617–656.
62. Elledge, S. J. (1996) Cell cycle checkpoints: preventing an identity crisis. *Science* **274**, 1664–1672.
63. Lock, R. B., and Ross, W. E. (1990) Inhibition of p34cdc2 kinase activity by etoposide or irradiation as a mechanism of G2 arrest in Chinese hamster ovary cells. *Cancer Res.* **50**, 3761–3766.

64. Kharbanda, S., Saleem, A., Datta, R., Yuan, Z. M., Weichselbaum, R., and Kufe, D. (1994) Ionizing radiation induces rapid tyrosine phosphorylation of p34cdc2. *Cancer Res.* **54**, 1412–1414.
65. Furnari, B., Rhind, N., and Russell, P. (1997) Cdc25 mitotic inducer targeted by chk1 DNA damage checkpoint kinase [see comments]. *Science* **277**, 1495–1497.
66. Peng, C. Y., Graves, P. R., Thoma, R. S., Wu, Z., Shaw, A. S., and Piwnica-Worms, H. (1997) Mitotic and G2 checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on serine-216 [see comments]. *Science* **277**, 1501–1505.
67. Sanchez, Y., Wong, C., Thoma, R. S., Richman, R., Wu, Z., Piwnica-Worms, H., and Elledge, S. J. (1997) Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25 [see comments]. *Science* **277**, 1497–1501.
68. Walworth, N., Davey, S., and Beach, D. (1993) Fission yeast chk1 protein kinase links the rad checkpoint pathway to cdc2. *Nature* **363**, 368–371.
69. Murakami, H., and Okayama, H. (1995) A kinase from fission yeast responsible for blocking mitosis in S phase. *Nature* **374**, 817–819.
70. McGowan, C. H. (2002) Checking in on Cds1 (Chk2): A checkpoint kinase and tumor suppressor. *Bioessays* **24**, 502–511.
71. Chaturvedi, P., Eng, W. K., Zhu, Y., et al. (1999) Mammalian Chk2 is a downstream effector of the ATM-dependent DNA damage checkpoint pathway. *Oncogene* **18**, 4047–4054.
72. Liu, Q., Guntuku, S., Cui, X. S., et al. (2000) Chk1 is an essential kinase that is regulated by Atr and required for the G(2)/M DNA damage checkpoint. *Genes Dev.* **14**, 1448–1459.
73. Matsuoka, S., Rotman, G., Ogawa, A., Shiloh, Y., Tamai, K., and Elledge, S. J. (2000) Ataxia telangiectasia-mutated phosphorylates chk2 in vivo and in vitro. *Proc. Natl. Acad. Sci. U S A* **97**, 10389–10394.
74. Abraham, R. T. (2001) Cell cycle checkpoint signaling through the ATM and ATR kinases. *Genes Dev.* **15**, 2177–2196.
75. Banin, S., Moyal, L., Shieh, S., et al. (1998) Enhanced phosphorylation of p53 by ATM in response to DNA damage. *Science* **281**, 1674–1677.
76. Canman, C. E., Lim, D. S., Cimprich, K. A., et al. (1998) Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science* **281**, 1677–1679.
77. Siliciano, J. D., Canman, C. E., Taya, Y., Sakaguchi, K., Appella, E., and Kastan, M. B. (1997) DNA damage induces phosphorylation of the amino terminus of p53. *Genes Dev.* **11**, 3471–3481.
78. Cliby, W. A., Roberts, C. J., Cimprich, K. A., et al. (1998) Overexpression of a kinase-inactive ATR protein causes sensitivity to DNA-damaging agents and defects in cell cycle checkpoints. *EMBO J.* **17**, 159–169.
79. Tibbetts, R. S., Brumbaugh, K. M., Williams, J. M., et al. (1999) A role for ATR in the DNA damage-induced phosphorylation of p53. *Genes Dev.* **13**, 152–157.
80. Savitsky, K., Bar-Shira, A., Gilad, S., et al. (1995) A single ataxia telangiectasia gene with a product similar to PI-3 kinase. *Science* **268**, 1749–1753.

81. Bulavin, D. V., Higashimoto, Y., Popoff, I. J., et al. (2001) Initiation of a G2/M checkpoint after ultraviolet radiation requires p38 kinase. *Nature* **411**, 102–107.
82. Baber-Furnari, B. A., Rhind, N., Boddy, M. N., Shanahan, P., Lopez-Girona, A., and Russell, P. (2000) Regulation of mitotic inhibitor Mik1 helps to enforce the DNA damage checkpoint. *Mol. Biol. Cell* **11**, 1–11.
83. Smits, V. A., Klompmaaker, R., Arnaud, L., Rijksen, G., Nigg, E. A., and Medema, R. H. (2000) Polo-like kinase-1 is a target of the DNA damage checkpoint. *Nat. Cell Biol.* **2**, 672–676.
84. Griffiths, D. J., Barbet, N. C., McCready, S., Lehmann, A. R., and Carr, A. M. (1995) Fission yeast rad17: a homolog of budding yeast RAD24 that shares regions of sequence similarity with DNA polymerase accessory proteins. *EMBO J.* **14**, 5812–5823.
85. Green, C. M., Erdjument-Bromage, H., Tempst, P., and Lowndes, N. F. (2000) A novel Rad24 checkpoint protein complex closely related to replication factor C.[erratum appears in Curr Biol 2000;10(4):R171]. *Curr. Biol.* **10**, 39–42.
86. St Onge, R. P., Udell, C. M., Casselman, R., and Davey, S. (1999) The human G2 checkpoint control protein hRAD9 is a nuclear phosphoprotein that forms complexes with hRAD1 and hHUS1. *Mol. Biol. Cell* **10**, 1985–1995.
87. Lindsey-Boltz, L. A., Bermudez, V. P., Hurwitz, J., and Sancar, A. (2001) Purification and characterization of human DNA damage checkpoint Rad complexes. *Proc. Natl. Acad. Sci. U S A* **98**, 11236–11241.
88. Venclovas, C., and Thelen, M. P. (2000) Structure-based predictions of Rad1, Rad9, Hus1 and Rad17 participation in sliding clamp and clamp-loading complexes. *Nucleic Acids Res.* **28**, 2481–2493.
89. Walworth, N. C., and Bernards, R. (1996) rad-dependent response of the chk1-encoded protein kinase at the DNA damage checkpoint. *Science* **271**, 353–356.
90. Parker, A. E., Van de Weyer, I., Laus, M. C., Oostveen, I., Yon, J., Verhasselt, P., and Luyten, W. H. (1998) A human homolog of the *Schizosaccharomyces pombe* rad1+ checkpoint gene encodes an exonuclease. *J. Biol. Chem.* **273**, 18332–18339.
91. Miki, Y., Swensen, J., Shattuck-Eidens, D., et al. (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* **266**, 66–71.
92. Futreal, P. A., Liu, Q., Shattuck-Eidens, D., et al. (1994) BRCA1 mutations in primary breast and ovarian carcinomas. *Science* **266**, 120–122.
93. Scully, R., Chen, J., Ochs, R. L., et al. (1997) Dynamic changes of BRCA1 subnuclear location and phosphorylation state are initiated by DNA damage. *Cell* **90**, 425–435.
94. Wang, Y., Cortez, D., Yazdi, P., Neff, N., Elledge, S. J., and Qin, J. (2000) BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. *Genes Dev.* **14**, 927–939.
95. Dolganov, G., Maser, R., Novikov, A., et al. (1996) Human Rad50 is physically associated with human Mre11: identification of a conserved multiprotein complex implicated in recombinational DNA repair. *Mol. Cell. Biol.* **16**, p4832–4841.
96. Maser, R., Monsen, K., Nelms, B., and Petrini, J. (1997) hMre11 and hRad50 nuclear foci are induced during the normal cellular response to DNA double-strand breaks. *Mol. Cell. Biol.* **17**, p6087–6096.

97. D'Amours, D., and Jackson, S. (2002) The Mre11 complex: at the crossroads of dna repair and checkpoint signalling. *Nat. Rev. Mol. Cell Biol.* **3**, p317–327.
98. Edwards, R. J., Bentley, N. J., and Carr, A. M. (1999) A Rad3-Rad26 complex responds to DNA damage independently of other checkpoint proteins. *Nat. Cell Biol.* **1**, 393–398.
99. Lopez-Girona, A., Tanaka, K., Chen, X. B., Baber, B. A., McGowan, C. H., and Russell, P. (2001) Serine-345 is required for Rad3-dependent phosphorylation and function of checkpoint kinase Chk1 in fission yeast. *Proc. Natl. Acad. Sci. U S A* **98**, 11289–11294.
100. Bakkenist, C., and Kastan, M. (2003) DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* **421**, 499–506.
101. Schultz, L., Chehab, N., Malikzay, A., and Halazonetis, T. (2000) p53 binding protein 1 (53BP1) is an early participant in the cellular response to DNA double-strand breaks. *J. Cell Biol.* **151**, 1381–1390.
102. Wang, B., Matsuoka, S., Carpenter, P., and Elledge, S. (2002) 53BP1, a mediator of the DNA damage checkpoint. *Science* **298**, 1435–1438.
103. Iwabuchi, K., Bartel, P., Li, B., Marraccino, R., and Fields, S. (1994) Two cellular proteins that bind to wild-type but not mutant p53. *Proc. Natl. Acad. Sci. U S A* **91**, 6098–6102.
104. Lane, D. P., and Crawford, L. V. (1979) T antigen is bound to a host protein in SV40-transformed cells. *Nature* **278**, 261–263.
105. Lane, D. (1984) Cell immortalization and transformation by the p53 gene. *Nature* **312**, p596–597.
106. Jenkins, J., Rudge, K., and Currie, G. (1984) Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53. *Nature* **312**, 651–654.
107. Parada, L., Land, H., Weinberg, R., Wolf, D., and Rotter, V. (1984) Cooperation between gene encoding p53 tumour antigen and ras in cellular transformation. *Nature* **312**, 649–651.
108. Eliyahu, D., Raz, A., Gruss, P., Givol, D., and Oren, M. (1984) Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. *Nature* **312**, 646–649.
109. Finlay, C., Hinds, P., Tan, T., Eliyahu, D., Oren, M., and Levine, A. (1988) Activating mutations for transformation by p53 produce a gene product that forms an hsc70-p53 complex with an altered half-life. *Mol. Cell. Biol.* **8**, 531–539.
110. Fearon, E., and Vogelstein, B. (1990) A genetic model for colorectal tumorigenesis. *Cell* **61**, 759–767.
111. Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A., Jr., Butel, J. S., and Bradley, A. (1992) Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356**, 215–221.
112. Greenblatt, M. S., Bennett, W. P., Hollstein, M., and Harris, C. C. (1994) Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res.* **54**, 4855–4878.

113. Srivastava, S., Zou, Z., Pirollo, K., Blattner, W., and Chang, E. (1990) Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. *Nature* **348**, 747–749.
114. Malkin, D., Li, F., Strong, L., et al. (1990) Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* **250**, 1233–1238.
115. Ko, L. J., and Prives, C. (1996) p53: puzzle and paradigm. *Genes Dev.* **10**, 1054–1072.
116. Vogelstein, B., Lane, D., and Levine, A. J. (2000) Surfing the p53 network. *Nature* **408**, 307–310.
117. Appella, E., and Anderson, C. W. (2001) Post-translational modifications and activation of p53 by genotoxic stresses. *Eur. J. Biochem.* **268**, 2764–2772.
118. Momand, J., Wu, H. H., and Dasgupta, G. (2000) MDM2—master regulator of the p53 tumor suppressor protein. *Gene* **242**, 15–29.
119. Chehab, N. H., Malikzay, A., Stavridi, E. S., and Halazonetis, T. D. (1999) Phosphorylation of Ser-20 mediates stabilization of human p53 in response to DNA damage. *Proc. Natl. Acad. Sci. U S A* **96**, 13777–13782.
120. Hirao, A., Kong, Y. Y., Matsuoka, S., et al. (2000) DNA damage-induced activation of p53 by the checkpoint kinase Chk2 [see comments]. *Science* **287**, 1824–1827.
121. Shieh, S. Y., Ahn, J., Tamai, K., Taya, Y., and Prives, C. (2000) The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites [published erratum appears in *Genes Dev* 2000;14(6):750]. *Genes Dev.* **14**, 289–300.
122. Shieh, S. Y., Ikeda, M., Taya, Y., and Prives, C. (1997) DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. *Cell* **91**, 325–334.
123. Sakaguchi, K., Herrera, J. E., Saito, S., et al. (1998) DNA damage activates p53 through a phosphorylation-acetylation cascade. *Genes Dev.* **12**, 2831–2841.
124. Gu, W., and Roeder, R. G. (1997) Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* **90**, 595–606.
125. Espinosa, J. M., and Emerson, B. M. (2001) Transcriptional regulation by p53 through intrinsic DNA/chromatin binding and site-directed cofactor recruitment. *Mol. Cell* **8**, 57–69.
126. Maltzman, W., and Czyzyk, L. (1984) UV irradiation stimulates levels of p53 cellular tumor antigen in nontransformed mouse cells. *Mol. Cell. Biol.* **4**, 1689–1694.
127. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B., and Craig, R. W. (1991) Participation of p53 protein in the cellular response to DNA damage. *Cancer Res.* **51**, 6304–6311.
128. Agarwal, M. L., Agarwal, A., Taylor, W. R., and Stark, G. R. (1995) p53 controls both the G2/M and the G1 cell cycle checkpoints and mediates reversible growth arrest in human fibroblasts. *Proc. Natl. Acad. Sci. U S A* **92**, 8493–8497.
129. Taylor, W. R., and Stark, G. R. (2001) Regulation of the G2/M transition by p53. *Oncogene* **20**, 1803–1815.

130. Stewart, N., Hicks, G. G., Paraskevas, F., and Mowat, M. (1995) Evidence for a second cell cycle block at G2/M by p53. *Oncogene* **10**, 109–115.
131. Taylor, W. R., DePrimo, S. E., Agarwal, A., et al. (1999) Mechanisms of G2 arrest in response to overexpression of p53. *Mol. Biol. Cell.* **10**, 3607–3622.
132. Bunz, F., Dutriaux, A., Lengauer, C., et al. (1998) Requirement for p53 and p21 to sustain G2 arrest after DNA damage. *Science* **282**, 1497–1501.
133. Harper, J. W., Elledge, S. J., Keyomarsi, K., et al. (1995) Inhibition of cyclin-dependent kinases by p21. *Mol. Biol. Cell.* **6**, 387–400.
134. Bates, S., Ryan, K. M., Phillips, A. C., and Vousden, K. H. (1998) Cell cycle arrest and DNA endoreduplication following p21Waf1/Cip1 expression. *Oncogene* **17**, 1691–1703.
135. Medema, R. H., Klompmaker, R., Smits, V. A., and Rijksen, G. (1998) p21waf1 can block cells at two points in the cell cycle, but does not interfere with processive DNA-replication or stress-activated kinases. *Oncogene* **16**, 431–441.
136. Niculescu, A. B., 3rd, Chen, X., Smeets, M., Hengst, L., Prives, C., and Reed, S. I. (1998) Effects of p21(Cip1/Waf1) at both the G1/S and the G2/M cell cycle transitions: pRb is a critical determinant in blocking DNA replication and in preventing endoreduplication [published erratum appears in Mol Cell Biol 1998;18(3):1763]. *Mol. Cell. Biol.* **18**, 629–643.
137. Smits, V. A., Klompmaker, R., Vallenius, T., Rijksen, G., Makela, T. P., and Medema, R. H. (2000) p21 inhibits thr161 phosphorylation of cdc2 to enforce the G2 DNA damage checkpoint. *J. Biol. Chem.* **275**, 30638–30643.
138. Jin, S., Antinore, M. J., Lung, F. D., et al. (2000) The GADD45 inhibition of Cdc2 kinase correlates with GADD45-mediated growth suppression. *J. Biol. Chem.* **275**, 16602–16608.
139. Zhan, Q., Antinore, M. J., Wang, X. W., et al. (1999) Association with Cdc2 and inhibition of Cdc2/Cyclin B1 kinase activity by the p53-regulated protein Gadd45. *Oncogene* **18**, 2892–2900.
140. Wang, X. W., Zhan, Q., Coursen, J. D., et al. (1999) GADD45 induction of a G2/M cell cycle checkpoint. *Proc. Natl. Acad. Sci. U S A* **96**, 3706–3711.
141. Hermeking, H., Lengauer, C., Polyak, K., et al. (1997) 14-3-3 sigma is a p53-regulated inhibitor of G2/M progression. *Mol. Cell* **1**, 3–11.
142. Chan, T. A., Hermeking, H., Lengauer, C., Kinzler, K. W., and Vogelstein, B. (1999) 14-3-3Sigma is required to prevent mitotic catastrophe after DNA damage [see comments]. *Nature* **401**, 616–620.
143. Chan, T. A., Hwang, P. M., Hermeking, H., Kinzler, K. W., and Vogelstein, B. (2000) Cooperative effects of genes controlling the G(2)/M checkpoint. *Genes Dev.* **14**, 1584–1588.
144. Innocente, S. A., Abrahamson, J. L., Cogswell, J. P., and Lee, J. M. (1999) p53 regulates a G2 checkpoint through cyclin B1. *Proc. Natl. Acad. Sci. U S A* **96**, 2147–2152.
145. Lau, C. C., and Pardee, A. B. (1982) Mechanism by which caffeine potentiates lethality of nitrogen mustard. *Proc. Natl. Acad. Sci. U S A* **79**, 2942–2946.

146. Fan, S., Smith, M. L., Rivet, D. J., 2nd, et al. (1995) Disruption of p53 function sensitizes breast cancer MCF-7 cells to cisplatin and pentoxifylline. *Cancer Res.* **55**, 1649–1654.
147. Clifford, B., Beljin, M., Stark, G. R., and Taylor, W. R. (2003) G2 arrest in response to topoisomerase II inhibitors: the role of p53. *Cancer Res.* ??, in press.
148. Blasina, A., Price, B. D., Turenne, G. A., and McGowan, C. H. (1999) Caffeine inhibits the checkpoint kinase ATM. *Curr. Biol.* **9**, 1135–1138.
149. Sarkaria, J. N., Busby, E. C., Tibbetts, R. S., et al. (1999) Inhibition of ATM and ATR kinase activities by the radiosensitizing agent, caffeine. *Cancer Res.* **59**, 4375–4382.
150. Zhou, B. B., Chaturvedi, P., Spring, K., et al. (2000) Caffeine abolishes the mammalian G(2)/M DNA damage checkpoint by inhibiting ataxia-telangiectasia-mutated kinase activity. *J. Biol. Chem.* **275**, 10342–10348.
151. Kanda, T., Sullivan, K. F., and Wahl, G. M. (1998) Histone-GFP fusion protein enables sensitive analysis of chromosome dynamics in living mammalian cells. *Curr. Biol.* **8**, 377–385.
152. Taylor, W. R., Schöenthal, A. H., Galante, J., and Stark, G. R. (2001) p130/E2F4 binds to and represses the cdc2 promoter in response to p53. *J. Biol. Chem.* **276**, 1998–2006.
153. Sugarman, J. L., Schöenthal, A. H., and Glass, C. K. (1995) Identification of a cell-type-specific and E2F-independent mechanism for repression of cdc2 transcription. *Mol. Cell. Biol.* **15**, 3282–3290.
154. Zwicker, J., Lucibello, F. C., Wolfrum, L. A., et al. (1995) Cell cycle regulation of the cyclin A, cdc25C and cdc2 genes is based on a common mechanism of transcriptional repression. *EMBO J.* **14**, 4514–4522.
155. Tommasi, S., and Pfeifer, G. P. (1995) In vivo structure of the human cdc2 promoter: release of a p130–E2F–4 complex from sequences immediately upstream of the transcription initiation site coincides with induction of cdc2 expression. *Mol. Cell. Biol.* **15**, 6901–6913.
156. Kovesdi, I., Reichel, R., and Nevins, J. R. (1987) Role of an adenovirus E2 promoter binding factor in E1A-mediated coordinate gene control. *Proc. Natl. Acad. Sci. U S A* **84**, 2180–2184.
157. Trimarchi, J. M., and Lees, J. A. (2002) Sibling rivalry in the E2F family. *Nat. Rev. Mol. Cell Biol.* **3**, 11–20.
158. Friend, S. H., Bernards, R., Rogelj, S., et al. (1986) A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature* **323**, 643–646.
159. Harbour, J. W., and Dean, D. C. (2000) Rb function in cell-cycle regulation and apoptosis. *Nat. Cell. Biol.* **2**, E65–67.
160. Lipinski, M. M., and Jacks, T. (1999) The retinoblastoma gene family in differentiation and development. *Oncogene* **18**, 7873–7882.
161. Flatt, P. M., Tang, L. J., Scatena, C. D., Szak, S. T., and Pieterpol, J. A. (2000) p53 regulation of G(2) checkpoint is retinoblastoma protein dependent. *Mol. Cell. Biol.* **20**, 4210–4223.

162. Polager, S., and Ginsberg, D. (2003) E2F mediates sustained G2 arrest and down-regulation of Stathmin and AIM-1 expression in response to genotoxic stress. *J. Biol. Chem.* **278**, 1443–1449.
163. Dou, Q. P., Zhao, S., Levin, A. H., Wang, J., Helin, K., and Pardee, A. B. (1994) G1/S-regulated E2F-containing protein complexes bind to the mouse thymidine kinase gene promoter. *J. Biol. Chem.* **269**, 1306–1313.
164. Ishida, S., Huang, E., Zuzan, H., et al. (2001) Role for E2F in control of both DNA replication and mitotic functions as revealed from DNA microarray analysis. *Mol. Cell. Biol.* **21**, 4684–4699.
165. Polager, S., Kalma, Y., Berkovich, E., and Ginsberg, D. (2002) E2Fs up-regulate expression of genes involved in DNA replication, DNA repair and mitosis. *Oncogene* **21**, 437–446.
166. Ren, B., Cam, H., Takahashi, Y., Volkert, T., Terragni, J., Young, R. A., and Dynlacht, B. D. (2002) E2F integrates cell cycle progression with DNA repair, replication, and G(2)/M checkpoints. *Genes Dev.* **16**, 245–256.
167. Chang, B. D., Broude, E. V., Fang, J., V., K. T., Abdryashitov, R., Poole, J. C., and Roninson, I. B. (2000) p21Waf1/Cip1/Sdi1-induced growth arrest is associated with depletion of mitosis-control proteins and leads to abnormal mitosis and endoreduplication in recovering cells. *Oncogene* **19**, 2165–2170.



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