es varies approximately as the square of the radius for cloud drop–sized particles. In the example shown in the figure on the previous page, the droplets in the unperturbed cloud sweep up about 64 times the volume of air containing other droplets as the ones in the polluted cloud. Consequently, the polluted cloud would be much less likely to rain.

A complicating factor is the possible presence of giant soluble particles in the pollution, which could act as seeds for large droplets and initiate the precipitation process. Giant soluble particles are used in some modern cloud seeding efforts, and there is evidence of pollution-induced rainfall due to such particles.

Precipitation can also form in clouds below the freezing point, if ice forms. Ice has a lower vapor pressure than liquid water, and ice particles therefore grow rapidly to very large sizes by stealing vapor from surrounding liquid droplets. Large ice crystals then coalesce with liquid droplets to form rain. Ice crystals in the lower atmosphere form on ice nuclei, which constitute less than one out of every thousand particles in the ambient atmosphere. Ice nuclei are often composed of clay minerals. Many pollutant aerosols, such as sulfates, are not ice nuclei. A pollution source could add a few ice nuclei and induce precipitation. Alternatively, it could destroy ambient ice nuclei by coating them in sulfate or add so many ice nuclei that precipitation is suppressed (see the figure on the previous page).

Aerosol modification of marine clouds. A false color image of ship tracks (white streaks) in a boundary layer cloud deck (mottled white) offshore from the northwestern United States (green). Cloud-free ocean is dark blue, high-altitude clouds are light blue. The image was produced with the same type of Advanced Very High Resolution Radiometer satellite data that Rosenfeld (*2*) used to investigate pollution tracks.

Rosenfeld's (*2*) satellite observations indicate substantially reduced precipitation downwind of the pollution source. Multiple types of satellite sensors provide information about the mechanisms for precipitation suppression. Further satellite observations should determine how widespread the influence of aerosols on precipitation may be and whether it varies with the type of pollution or the properties of the clouds. Rosenfeld's work also points to locales where in situ observations should be made to pinpoint the mechanisms by which pollution affects clouds. Such knowledge may allow us to estimate how widespread the aerosol interaction with cloud precipitation may be in our globally polluted world.

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PERSPECTIVES: CELL CYCLE

Piecing Together the p53 Puzzle

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The delays that cells experience in G_1, S, or G_2 phases of the cell cycle after damage to DNA are collectively called DNA integrity checkpoints. There are two manifestations of these delays. The first is the transient arrest seen at G_1, S, or G_2 (independent of the key tumor suppressor protein p53) that gives the DNA repair machinery time to shore up the damage before division continues. The second (dependent on p53) is apoptosis or prolonged, probably permanent, G_1 delay that results in removal of damaged cells from the population. A failure to halt at these checkpoints leads to genomic instability and an increased likelihood that the cell will become cancerous. Studies in yeast have identified a network of DNA integrity checkpoint proteins (including four conserved kinases) that regulate the cell's entry into and exit from these cell cycle checkpoints. The *ATM* gene—mutated in the disease ataxia telangiectasia, which is characterized by a marked predisposition to cancer—is related to the yeast checkpoint kinase Rad3/Mec1. This is consistent with the inability of cells that lack ATM to halt at cell cycle checkpoints after DNA damage.

Mammalian homologs of the four yeast checkpoint kinases have been identified, suggesting that organisms from yeast to human have similar protein pathways for regulating these checkpoints. On page 1824 of this issue, Hirao *et al.* (*1*) report that mouse cells deficient in the checkpoint kinase CHK2—a homolog of yeast Cds1/Rad53—have several defective checkpoints after exposure to ionizing radiation (*1*). They further show that CHK2 stabilizes p53, a key player in regulating the prolonged G_1 arrest checkpoint. In another recent study, Bell *et al.* identified *CHK2* as the gene implicated in a small number of families with the cancer predisposition syndrome Li-Fraumeni, who do not have germ line mutations in *p53* (*2*). Together, these findings emphasize the importance of checkpoint kinases in preventing genomic instability and progression to cancer.

The p53-dependent transcription of target genes responds to a diverse range of cellular signals that affect cell proliferation and DNA integrity checkpoints (*3*). In undamaged cells that are dividing normally, p53 is highly unstable, with a half-life measured in minutes. After DNA damage induced by ionizing radiation (which is the only type of damage discussed here) the half-life increases significantly, leading to accumulation of p53 and transcription of target genes such as p21 and BAX. The outcome of this increased transcription depends on the type of cell but usually is manifest as a very prolonged (possibly irreversible) G_1 arrest or apoptosis (*4*, *5*).

The instability of p53 depends on Mdm2, which binds to its amino terminus and targets it for ubiquitination and degradation. Preventing the interaction of p53 with Mdm2 is sufficient to promote its stabilization. At least 11 posttranslational modifications of p53 have been reported in response to DNA damage, and the relationship between these and p53 stability has attracted much attention. Although there are many conflicting reports in the literature, some data have suggested that phosphorylation of amino acids Ser^{15} and Ser^{20} is involved. In

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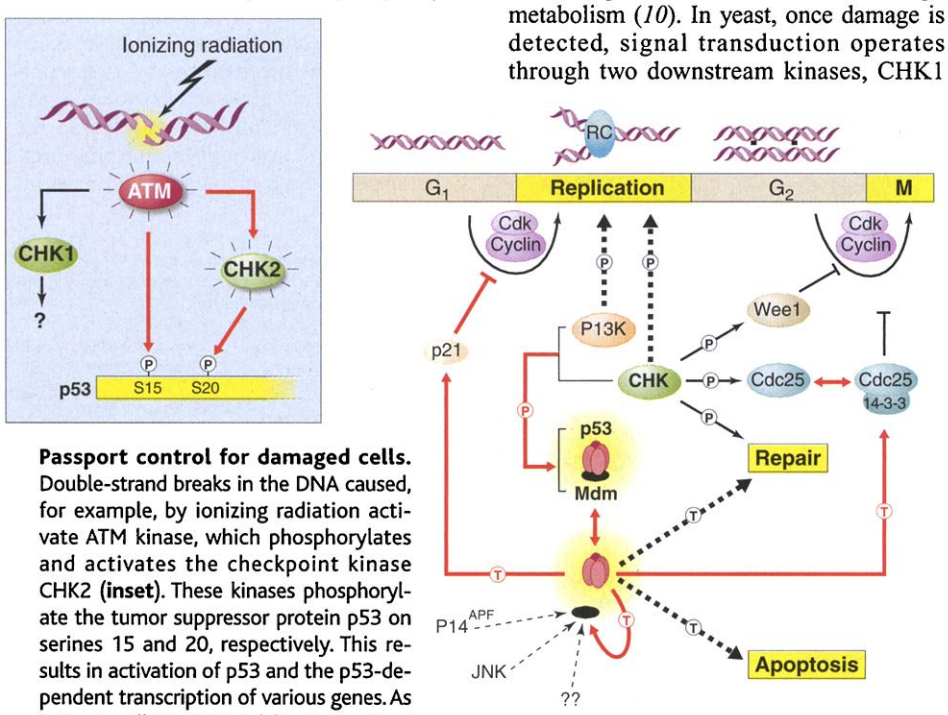
vivo, ATM is required for p53 stabilization and in vitro ATM phosphorylates Ser^{15} but not Ser^{20}. Hirao *et al.* and two other groups (*6, 7*) now provide evidence that CHK2 is the kinase that phosphorylates Ser^{20} of p53 and demonstrate that p53 stabilization is dependent on CHK2. It is reasonable to conclude that CHK2-dependent phosphorylation of Ser^{20} is the p53 modification that is necessary for its stabilization (see the figure). However, modifications of other proteins such as Mdm2 by ATM and CHK2 (*8*) are also likely to be involved.



Passport control for damaged cells. Double-strand breaks in the DNA caused, for example, by ionizing radiation activate ATM kinase, which phosphorylates and activates the checkpoint kinase CHK2 **(inset)**. These kinases phosphorylate the tumor suppressor protein p53 on serines 15 and 20, respectively. This results in activation of p53 and the p53-dependent transcription of various genes. As in yeast cells, transient delays in G_1, S, or G_2 phases of the cell cycle are dependent on ATM and CHK2 but are largely independent of p53. The targeting of p53 by ATM and CHK2 sets in motion other pathways that are not found in yeast. These result in a prolonged (possibly irreversible) G_1 phase arrest or apoptosis (main figure). Additional p53-dependent responses such as transcription of repair genes or genes encoding 14-3-3 proteins may facilitate DNA repair or prolong G_2 arrest. Biochemically well-defined responses are shown in red.

Why do both ATM and CHK2 target p53 when CHK2 is dependent on ATM for its own activation? Does phosphorylation of Ser^{15} and Ser^{20} occur on the same p53 molecule or on distinct molecules in vivo? What is the biological consequence of Ser^{15} phosphorylation? Currently we can only guess at the answers. Perhaps multiple phosphorylation sites and kinases for each site provide a fail-safe mechanism to ensure that p53 only activates prolonged G_1 arrest or cell death when absolutely necessary. Another appealing possibility, for which there is limited evidence (*9*), is that the balance of different phosphorylation events, in addition to activating transcription, may confer promoter selectivity on p53. This would allow the p53 response to DNA damage to be tailored to specific circumstances, such as DNA damage of a particular type or the cell cycle stage during which the damage occurs.

To effect a response, DNA damage must be recognized and a signal transduced. Efficient detection of double-strand breaks in the DNA requires ATM, which may interact with damaged DNA directly or with proteins involved in damage metabolism (*10*). In yeast, once damage is detected, signal transduction operates through two downstream kinases, CHK1 and CHK2. The demonstration that in mice p53-dependent responses to DNA double-strand breaks require CHK2 allows us to speculate that, as multicellular organisms evolved, p53 subsumed existing signaling pathways to control specific and additional responses to DNA damage. It is worth remembering that the response of p53 to DNA damage is not required for cell survival. However, the survival of the whole organism is affected because activation of p53 after DNA damage results in removal of potentially mutant cells from the population by inducing them to enter prolonged arrest or apoptosis. Loss of p53 does not result in cellular radiation sensitivity; its loss actually increases survival rates in many cell types because individual cells escape arrest or apoptosis. Instead, loss of p53 results in decreased genome stability, not because of loss of transient checkpoint controls (these remain substantially intact in cells that lack p53), but because loss of p53 creates an environment that is permissive for genome instability—that is, more damaged cells with chromosome aberrations and mutations survive and propagate. Although p53-dependent transcription may increase the efficiency of DNA repair (*11*), the evidence is compelling that its main role in genome stability is the removal of problematic cells from the population when other systems fail.

The formation of tumors is a multistep process requiring progressive accumulation of genetic alterations. Hence, it is becoming clear why loss of p53 plays such a pivotal role in human cancer (*12*). Accumulating the half-dozen or so mutations necessary for a cell to become carcinogenic requires genetic instability—a consequence of p53 loss. Another important step is relief of the apoptotic barrier that prevents uncontrolled growth—a second consequence of p53 loss. The identification of *CHK2* as a tumor suppressor gene (that when mutated has similar consequences to mutant p53), coupled with the dependency of p53-regulated DNA damage responses on CHK2, is consistent with the much-discussed possibility that DNA integrity checkpoints play a major role in preventing carcinogenesis (*13*).

Hirao *et al.* (*1*) demonstrate that mouse cells deficient in CHK2 have lost both p53-dependent responses (such as apoptosis) and p53-independent responses (such as the G_2 damage checkpoint). Why, then, are checkpoint kinases not more frequently identified as tumor suppressors? On the face of it, the loss of these pathways would be predicted to have more profound effects on genome stability than the loss of p53 alone. It is possible that the combination of defects resulting from the loss of checkpoint kinases is not compatible with viability. The availability of mouse cells that lack CHK2 opens up the possibility of exploring in more detail the relationships among tumor suppressors, conserved DNA damage responses, and the p53-dependent responses that are specific to multicellular organisms.

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