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Cancer Cell Cycles

Charles J. Sherr

Uncontrolled cell proliferation is the hallmark of cancer, and tumor cells have typically acquired damage to genes that directly regulate their cell cycles. Genetic alterations affecting $p16^{INK4a}$ and cyclin D1, proteins that govern phosphorylation of the retinoblastoma protein (RB) and control exit from the G_1 phase of the cell cycle, are so frequent in human cancers that inactivation of this pathway may well be necessary for tumor development. Like the tumor suppressor protein p53, components of this "RB pathway," although not essential for the cell cycle per se, may participate in checkpoint functions that regulate homeostatic tissue renewal throughout life.

The fundamental task of the cell cycle is to ensure that DNA is faithfully replicated once during S phase and that identical chromosomal copies are distributed equally to two daughter cells during M phase (*1*). The machinery for DNA replication and chromosome segregation is insulated from interruption by extracellular signals, and its essential and autonomous nature implies that damage to the pivotal components would be highly debilitating, if not fatal, to cells. Therefore, genes commanding these processes should not be frequent targets of mutation, deletion, or amplification in cancer.

Oncogenic processes exert their greatest effect by targeting particular regulators of G_1 phase progression (*2*, *3*). During the G_1 phase, cells respond to extracellular signals by either advancing toward another division or withdrawing from the cycle into a resting state (G_0) (*4*, *5*). Unlike transit through the S, G_2, and M phases, G_1 progression normally relies on stimulation by mitogens and can be blocked by antiproliferative cytokines. Cancer cells abandon these controls and tend to remain in cycle, and because cell cycle exit can facilitate maturation and terminal differentiation, these processes are subverted as well. The decision to divide occurs as cells pass a restriction point late in G_1, after which they become refractory to extracellular growth regulatory signals and instead commit to the autonomous program that carries them through to division (*4*, *5*). An appreciation of restriction point control is central to our understanding of how and why cancer cells continuously cycle.

The author is at the Howard Hughes Medical Institute, Department of Tumor Cell Biology, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38105, USA. E-mail: sherr@stjude.org

Restriction Point Control and the G_1-S Transition

Passage through the restriction point and entry into S phase is controlled by cyclin-dependent protein kinases (CDKs) that are sequentially regulated by cyclins D, E, and A (Fig. 1). In general, CDK activity requires cyclin binding, depends on both positive and negative regulatory phosphorylations (*6*), and can be constrained by at least two families of CDK inhibitory proteins (*7*).

D-type cyclins act as growth factor sensors, with their expression depending more on extracellular cues than on the cell's position in the cycle (*8*). As cells enter the cycle from quiescence (G_0), one or more D-type cyclins (D1, D2, and D3) are induced as part of the delayed early response to growth factor stimulation, and both their synthesis and assembly with their catalytic partners, CDK4 and CDK6, depend on mitogenic stimulation (*5*). The catalytic activities of the assembled holoenzymes are first manifest by mid-G_1, increase to a maximum near the G_1-S transition, and persist through the first and subsequent cycles as long as mitogenic stimulation continues. Conversely, mitogen withdrawal leads to cessation of cyclin D synthesis; the D cyclins are labile proteins, and because their holoenzyme activities decay rapidly, cells rapidly exit the cycle. Specific polypeptide inhibitors of CDK4 and CDK6—so-called INK4 proteins—can directly block cyclin D–dependent kinase activity and cause G_1 phase arrest (*9*). The four known 15- to 19-kD INK4 proteins ($p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$, and $p19^{INK4d}$) bind and inhibit CDK4 and CDK6, but not other CDKs. Like the three D-type cyclins, the *INK4* genes are expressed in distinct tissue-specific patterns, suggesting that they are not strictly redundant.

A loss of cyclin D1–dependent kinase activity before the restriction point prevents many cultured cell lines from entering S phase, but its absence later in the cell cycle is without effect (*10*, *11*). Hence, cyclin D–dependent kinases must phosphorylate some substrate or substrates whose modification is required for G_1 exit, and the retinoblastoma tumor suppressor protein (RB) is one such target (*12*). Notably, cyclin D–dependent kinases are dispensable for passage through the restriction point in cultured cells that lack functional RB, and in this setting, ectopic expression of INK4 proteins does not induce G_1 phase arrest (*13*). Thus, INK4 proteins inhibit cyclin D–dependent kinases that, in turn, phosphorylate RB (Fig. 2). Disruption of this "RB pathway" is important in cancer.

RB and other RB-like proteins (p130, p107) control gene expression mediated by a family of heterodimeric transcriptional regulators, collectively termed the E2Fs (*14*, *15*), which can transactivate genes whose products are important for S phase entry (*14*, *16*) (Fig. 2). In its hypophosphorylated form, RB binds to a subset of E2F complexes, converting them to repressors that constrain expression of E2F target genes (*17*). Phosphorylation of RB frees these E2Fs, enabling them to transactivate the same genes, a process initially triggered by the cyclin D–dependent kinases (*5*, *12*, *13*) and then accelerated by the cyclin E–CDK2 complex (*18*–*20*) (Fig. 2).

In proliferating cells, the expression of cyclin E is normally periodic and maximal at the G_1-S transition (Fig. 1), and throughout this interval, cyclin E enters into active

complexes with its catalytic partner CDK2. Because the cyclin E gene is itself E2F-responsive, cyclin E–CDK2 acts through positive feedback to facilitate progressive rounds of RB phosphorylation and E2F release (*16*, *19*) (Fig. 2). In addition, E2F-1 stimulates its own transcription. Positive cross-regulation of E2F and cyclin E produces a rapid rise of both activities as cells approach the G_1-S boundary. In concert with the irreversible commitment to enter S phase, RB inactivation shifts from being mitogen-dependent (cyclin D–driven) to mitogen-independent (cyclin E–driven). Inactivation of RB by phosphorylation or by direct genetic damage to the *RB* gene itself shortens the G_1 phase, reduces cell size, and decreases, but does not eliminate, the cell's requirements for mitogens and adhesive signals (*11*, *21–23*). Because RB-negative cells retain some requirements for growth factors, events in addition to RB phosphorylation must contribute to restriction point control.

Cyclin A– and cyclin B–dependent kinases probably maintain RB in its hyperphosphorylated state as the cycle moves ahead (Fig. 1), and RB is not dephosphorylated until cells complete mitosis and reenter the G_1 phase (or G_0). The onset of cyclin A synthesis late in G_1 is important for the G_1-S transition, because inhibition of cyclin A function in cultured cells can also inhibit S phase entry (*24*). Many cells exhibit a dual requirement for growth factors and adhesive signals to enter S phase, and not only RB phosphorylation but also cyclin A gene expression is adhesion-dependent (*23*, *25*). Substrates for cyclin E–CDK2 and cyclin A–CDK2 could include proteins at replication origins (Fig. 2) whose phosphorylation might promote DNA synthesis or prevent reassembly of preinitiating complexes (*1*, *26*).

Once cells enter S phase, the timely inactivation of cyclin E and E2F activities may be equally crucial for cell cycle progression. Rapid turnover of cyclin E is mediated by ubiquitin-dependent proteolysis, and its phosphorylation by its own catalytic partner, CDK2, signals its destruction (*27*). E2F-1 transactivation activity also decreases once cells enter S phase, as cyclin A–CDK2 complexes accumulate (Fig. 1). Cyclin A–CDK2 binds to the RB-regulated E2Fs and phosphorylates one of their heterodimeric components (DP-1), thereby precluding DNA binding (*28*). Because the cyclin E–CDK2 complex lacks this function, the reversal of E2F-mediated transactivation during S phase depends on the appearance of cyclin A–CDK2.

Cyclin D–, E–, and A–dependent kinases are negatively regulated by a distinct family of CDK inhibitors that include at least three proteins: $p21^{CIP1}$, $p27^{KIP1}$, and $p57^{KIP2}$ (*29–31*). The single most remarkable feature in relation to cancer is the inducibility of the *CIP1* gene by the tumor suppressor p53 [(*29*), and see below], although these genes also respond to many other types of stimuli during terminal differentiation (*7*). *KIP1* may be the most directly involved in restriction point control. In quiescent cells, $p27^{KIP1}$ levels are high, but once cells enter the cycle, they fall (Fig. 1) (*32*). Residual $p27^{KIP1}$ is sequestered into complexes with excess cyclin D–CDK complexes (*31*, *32*), alleviating $p27^{KIP1}$-mediated repression of cyclin E–CDK2 and cyclin A–CDK2 activity in cycling cells. The level of $p27^{KIP1}$ is largely controlled by translational (*33*) and posttranslational (*34*) mechanisms, and because its turnover can be accelerated by cyclin E–CDK2–mediated phosphorylation (*35*), cyclin E–CDK2 and $p27^{KIP1}$ may oppose each other's function (Fig. 2). When proliferating fibroblasts are deprived of serum mitogens, synthesis of $p27^{KIP1}$ not only increases, but the inhibitor is released from cyclin D–CDK complexes as cyclin D is degraded. The loss of cyclin D–dependent kinase activity coupled with $p27^{KIP1}$-mediated inhibition of CDK2 induces arrest in G_1-G_0 within a single cycle (Fig. 1). Antisense inhibition of p27 synthesis in cycling cells can prevent them from becoming quiescent (*36*). Mice nullizygous for the gene encoding p27 grow faster than littermate controls and exhibit frank organomegaly, with all tissues containing increased numbers of smaller cells (*37*). This phenotype underscores the importance of $p27^{KIP1}$ in regulating both cell size and cell number.

The RB Pathway in Cancer Cells

Cyclin D1 is overexpressed in many human cancers as a result of gene amplification or translocations targeting the *D1* locus (formally designated *CCND1*) on human chromosome 11q13 (*2*, *3*). The gene encoding its catalytic partner CDK4, located on chromosome 12q13, is also amplified in sarcomas and gliomas, although several other potential oncogenes, including the p53 antagonist *MDM2*, map to this region. In the first studies to implicate cyclin D1 in cancer, Motokura *et al.* isolated *D1* (originally designated *PRAD1*) linked to the parathyroid hormone gene in parathyroid adenomas containing an inversion of human chromosome 11 [inv(11)(p15;q13)] (*38*). They recognized the position of *D1* in relation to a recurrent chromosomal amplification unit at 11q13 and to the previously described *BCL1* breakpoint in the translocation 11;14 (q13;q32). The latter, characteristically observed in B lineage mantle cell lymphomas, moves the immunoglobulin heavy chain enhancer into the cyclin *D1* locus, leaving the *D1* coding sequences uninterrupted. B lymphocytes normally express only cyclins D2 and D3, but all lymphoma cells containing t(11;14) ectopically synthesize cyclin D1, which is sufficient to provide a growth advantage.

Amplification of chromosome 11q13 is frequent in a broad spectrum of common adult cancers, including squamous cell carcinomas of the head and neck (43% of cases on average), esophageal carcinomas (34%), bladder cancer (15%), primary breast carcinoma (13%), small-cell lung tumors, and hepatocellular carcinomas (~10% each) (*3*). The amplicons are large, but evidence that *D1* is the critical target gene stems

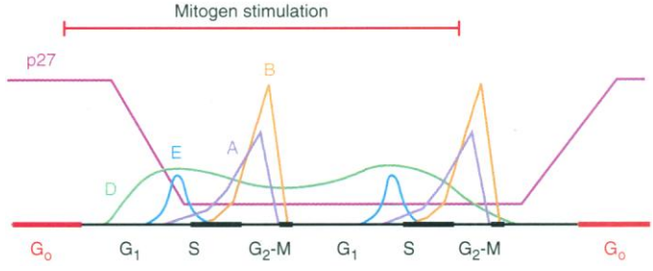

Fig. 1. Fluctuations of cyclins and $p27^{KIP1}$ during the cell cycle. Expression of cyclins E, A, and B (mitotic cyclin) is periodic (*6*). D-type cyclins are expressed throughout the cycle in response to mitogen stimulation (the period indicated by the top bar), and a less idealized scheme would indicate that different ones (D1, D2, and D3) are induced by various signals in a cell lineage–specific manner (*8*). The cyclins assemble with more stably expressed CDKs to temporally regulate their activities. D-type cyclins form complexes with CDK4 and CDK6; cyclin E with CDK2; cyclin A with CDK2 (in S phase) and with CDC2 (CDK1) (in late S and G_2); and cyclin B with CDC2. The holoenzymes can be negatively regulated by phosphorylation, so that even though cyclin B–CDC2 complexes progressively assemble as B cyclins accumulate, their catalytic activity is restricted to mitosis (*6*). p27 levels are high in quiescent cells, fall in response to mitogenic stimulation, remain at lower threshold levels in proliferating cells, and increase again when mitogens are withdrawn. In proliferating cells, most p27 is complexed with cyclin D–CDK complexes (*7*, *31*).

from its frequency of involvement compared with those of flanking markers and from its selective and consistent overexpression in tumor tissues. In esophageal, hepatic, and head and neck cancers, there is a correlation between *D1* amplification and cyclin D1 protein overexpression; in breast cancer, however, where the *D1* amplification frequency is only 13%, more than 50% appear to overexpress the protein. Aberrant overexpression of cyclin D1 is also seen in sarcomas, colorectal tumors, and melanomas, even though *D1* gene amplification frequencies are exceptionally low (*3*). That cyclin D1 can directly contribute to oncogenesis is supported by studies with transgenic mice, in which targeted overexpression of D1 in mammary epithelial cells leads to ductal hyperproliferation and eventual tumor formation (*39*). Conversely, mice nullizygous for *D1* show profound defects in mammary lobuloalveolar development during pregnancy, indicating that cyclin D1 plays a critical, uncompensated role in the maturation of this tissue (*40*). This special dependency of breast epithelial cells on cyclin D1, coupled with the ability of the same regulator to induce breast cancer, points toward a striking concordance between normal developmental controls and neoplastic processes. Yet, one must bear in mind that overexpression of cyclin D1 also occurs in many other tumor types, including those involving B cells that normally express only cyclins D2 and D3. Constitutive overexpression of the *D2* and *D3* genes has not been reported, possibly because they reside in chromosomal regions that do not readily undergo amplification.

Mutations that inactivate the CDK inhibitory function of the *INK4a* gene (also called *CDKN2* or *MTS1*, on chromosome 9p21) are associated with familial melanoma and occur at high frequencies in biliary tract (~50%) and esophageal (~30%) carcinomas (*3*, *7*). Reciprocally, a mutation in CDK4 that prevents its interaction with p16 has been found in melanoma (*41*). Homozygous deletions of the *INK4a* locus occur commonly in gliomas and mesotheliomas (~55% each), nasopharyngeal carcinomas (~40%), acute lymphocytic leukemias (~30%), sarcomas, and bladder and ovarian tumors. Pancreatic, head and neck, and non–small-cell lung carcinomas sustain both *INK4a* mutations and deletions (*3*). Although the *INK4b* gene (also called *p15* and *MTS2*) maps in tandem with *INK4a* and is usually included in the deletions, *INK4b* is not targeted by inactivating mutations. Nor have mutations or deletions of *INK4c* or *INK4d* been reported in tumors. The hypothesis that *INK4a* disruption is critical gains further credence from studies of *INK4a* nullizygous ($INK4a^{-/-}$) mice. These animals spontaneously develop a spectrum of different tumors by 6 months of age, with the rate of tumor formation accelerated in response to carcinogen treatment (*42*). Cultured $INK4a^{-/-}$ embryo fibroblasts do not senesce, and unlike their wild-type counterparts, they can be transformed by oncogenic *RAS* alone. Although the *INK4a* locus also encodes a second, potentially contributory protein ($p19^{ARF}$) from an alternative reading frame (*43*), the weight of current evidence favors the primary involvement of $p16^{INK4a}$ in tumorigenesis (*42*).

Inactivation of *RB* itself is the sine qua non of retinoblastoma (*44*), but overall the gene is targeted more often in adult cancers, particularly small-cell carcinomas of the lung (*3*). Similarly, inherited allelic loss of *INK4a* confers susceptibility to melanoma (*9*), but the gene is inactivated at a much higher frequency in sporadic tumors of different types. Presumably, $p16^{INK4a}$ loss might mimic cyclin D1 or CDK4 overexpression, each leading to RB hyperphosphorylation and physiologic inactivation (Fig. 2). Support for this functional interrelation stems from observations that inactivation of any one component of this pathway in a tumor greatly decreases the probability of identifiable damage to other components. For example, tumor cells that overexpress cyclin D1 or lose p16 tend to retain wild-type RB, but those with inactivating *RB* mutations generally express wild-type p16 and show no elevation in D1 levels (*7*).

If p16, cyclin D1, and RB function in the same pathway, why do alterations of their genes sometimes yield different tumor types? Mouse embryos nullizygous for *RB* survive beyond midgestation but die in utero with erythroid aplasia and neuronal degeneration, implying that only specific cell types depend crucially on RB during prenatal development (*45*). Mouse $Rb^{+/-}$ heterozygotes develop midlobe $Rb^{-/-}$ pituitary tumors (versus retinoblastoma in humans), so these cells are uniquely susceptible to losses of *Rb* later in life. In humans, inactivation of *RB* is most commonly observed in retinoblastomas, osteosarcomas, carcinoid tumors, and small-cell lung cancers, again suggesting that specific

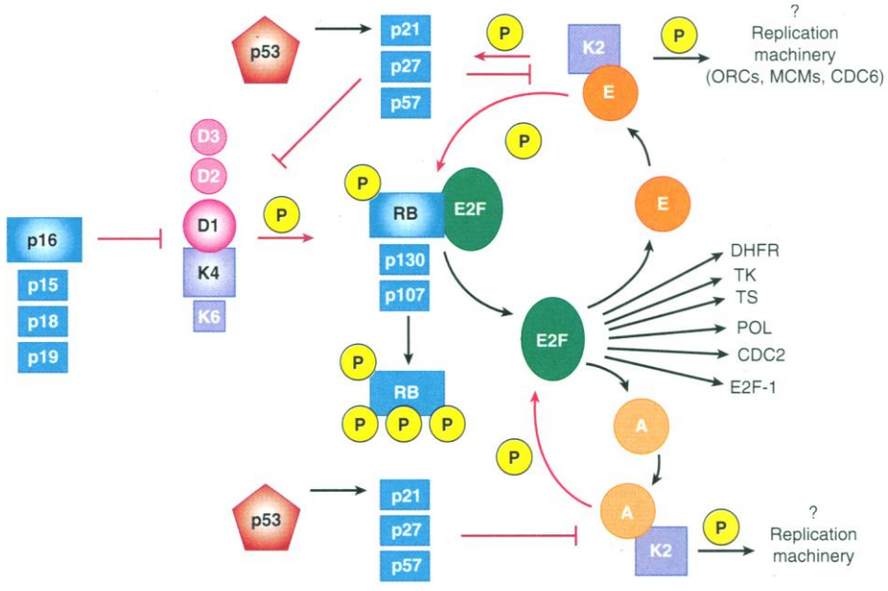

Fig. 2. Restriction point control. RB phosphorylation triggered by cyclin D–dependent kinases releases RB-bound E2F. Rather than illustrating the many E2F-DP heterodimers that are differentially regulated by various RB family members [see text and (*14*, *15*)], E2F "activity" is shown for simplicity. E2F triggers the expression of dihydrofolate reductase (DHFR), thymidine kinase (TK), thymidylate synthase (TS), DNA polymerase-α (POL), CDC2, cyclin E and possibly cyclin A, and E2F-1 itself. This establishes a positive feedback loop promoting RB phosphorylation by cyclin E–CDK2, contributing to the irreversibility of the restriction point transition and ultimately making it mitogen-independent. In parallel, cyclin E–CDK2 may oppose the inhibitory action of $p27^{KIP1}$ by phosphorylating it (*35*). This allows cyclin A–CDK2 and possibly cyclin E–CDK2 to start S phase. Possible CDK substrates include those of the origin-recognition complex (ORC), minichromosome maintenance proteins (MCMs), and CDC6, all of which assemble into preinitiation complexes (*26*). Once cells enter S phase, cyclin A–CDK2 phosphorylates DP-1 and inhibits E2F binding to DNA (*28*). Like p27, p53-inducible $p21^{CIP1}$ can induce G_1 arrest by inhibiting the cyclin D–, E–, and A–dependent kinases (*29*, *30*). In contrast, INK4 proteins antagonize only the cyclin D–dependent kinases (*9*). The proteins most frequently targeted in human cancers are highlighted. Arrows depicting inhibitory phosphorylations (P) or inactivating steps are shown in red, and those depicting activating steps are shown in black.

cell types are particularly sensitive to RB loss. But, in cells in which the loss of RB function is better compensated by expression of other RB family members, tumors would not arise. Because cyclin D1–CDK complexes can phosphorylate the other RB-related proteins as well (*12, 46*), overexpression of D1 may have farther reaching consequences than does RB loss. Similarly, inactivation of $p16^{INK4a}$ might upregulate the cyclin D2– and cyclin D3–dependent kinases in addition to cyclin D1–CDK complexes. The predicted frequency of involvement of these genes in cancers would then be *INK4a* > *D1* > *RB*, which matches what is observed. However, this model does not explain why the *INK4b*, *-c*, and *-d* genes seem not to be disrupted in tumors, or why loss-of-function mutations in p107 or p130 have not been found in cancer cells. Thus, although groups of INK4 proteins, D-type cyclins, and RB family members may differentially contribute to restriction point control in various cell lineages, some special role in oncogenesis seems to be played by $p16^{INK4a}$, cyclin D1, and RB. Perhaps $p16^{INK4a}$ selectively functions in a signaling pathway that detects certain oncogenic perturbations and brakes the cell cycle in response. Positive selection of cells deficient in this putative surveillance mechanism would be manifested by a recurrent disruption of the RB pathway in tumor cells (see below).

Other G_1-S Regulators in Cancer

Although the *E2F* genes are the apparent targets of the RB pathway, their overexpression, mutation, or inactivation has not as yet been reported in human cancers. In mice, the elimination of both wild-type *E2F1* alleles leads to developmental defects in some tissues and to tumors in others (*47*), but in humans, alterations in a single E2F complex might be adequately compensated.

Alterations in the cyclin E and cyclin A genes in human cancers also appear to be rare (*3*). Very few cases of cyclin E amplification have been reported in established tumor cell lines, and there is only one instance in which the cyclin A gene was found to be altered in a hepatoma (*48*). Nonetheless, sustained overexpression of cyclin E is tolerated under experimental conditions (*21*), and the protein is aberrantly overexpressed in carcinomas of the breast, stomach, colon, and endometrium, and in some adult acute lymphocytic leukemias (*49*). Overexpression of cyclin E could result from its failure to undergo ubiquitin-mediated degradation. Homozygous inactivation of the *KIP1* and *CIP1* genes has not been reported either, but reduction in $p27^{KIP1}$ levels in a subset of colon and breast cancers correlates with poor prognosis (*35, 50*). Identification of components of the protein synthetic and degradation machinery that determine cyclin E and $p27^{KIP1}$ turnover rates may provide the key to understanding their altered expression in tumor cells and whether it is a cause or consequence of cell transformation. Perhaps there is a class of oncoproteins and tumor suppressors awaiting discovery whose role is to regulate protein turnover.

The p53-Dependent G_1 Checkpoint

Although cell cycle transitions depend on the underlying CDK cycle, superimposed checkpoint controls help ensure that certain processes are completed before others begin. A critical conceptual distinction between cell cycle phase transitions and these surveillance operations is that components of checkpoint control need not be essential to the workings of the cycle. Instead, their role is to brake the cycle in the face of stress or damage. By allowing repair to take place, checkpoint controls become crucial in maintaining genomic stability (*51*).

The *p53* gene is the most frequently mutated gene in human cancer (*52*) and is an archetypal checkpoint regulator. Although it is not essential for normal mouse development (*53*), one of its roles is to ensure that, in response to genotoxic damage, cells arrest in G_1 and attempt to repair their DNA before it is replicated (*54*). Although p53 is ordinarily a very short-lived protein, it is stabilized and accumulates in cells undergoing DNA damage or in those responding to certain forms of stress (*54–56*). The precise signal transduction pathway that senses DNA damage and recruits p53 has not been elucidated, but is likely to include genes like *ATM* [mutated in ataxia telangiectasia (AT)] (*57*). The p53 protein functions as a transcription factor, and cancer-related mutations cluster in its DNA binding domain (*55*). MDM-2, a p53-inducible and amplifiable proto-oncogene product, neutralizes p53 action by binding to and inhibiting its transactivating domain (*58*). The gene encoding the CDK inhibitor $p21^{CIP1}$ is another target of p53-mediated regulation (*29*) and is at least partially responsible for p53-mediated G_1 arrest (*59*). When treated with DNA-damaging drugs, cells lacking $p21^{CIP1}$ appear to undergo repeated S phases, possibly reflecting aberrations in controls linking the completion of S phase with mitosis (*60*).

Ionizing radiation not only triggers arrest at the G_1-S checkpoint but it also slows S phase and blocks progression in G_2, allowing additional time for the repair of chromosome breaks before entry into mitosis (*51*). The loss of p53 predisposes cells to drug-induced gene amplification and decreases the fidelity of mitotic chromosome transmission (*61*). Duplication of the centrosome normally begins at the G_1-S boundary, but in the absence of p53, multiple centrosomes appear to be generated in a single cell cycle, ultimately resulting in aberrant chromosomal segregation during mitosis (*62*). Barring changes so severe as to precipitate mitotic catastrophe, the resulting genetic instability leads to changes in chromosome number and ploidy, further increasing the probability that such cells will more rapidly evolve toward malignancy by escaping immune surveillance, tolerating hypoxia, and becoming angiogenic, invasive, metastatic, and, ultimately, drug resistant in the face of chemotherapy.

In some cell types, p53 induces apoptosis when overexpressed (*63*) and is required for apoptosis in response to severe DNA damage, chemotherapeutic drugs, or MYC or E1A overexpression (*64*). Launching this apoptotic program does not depend on p21 (*59*), and p53 may directly activate death genes, such as *BAX*, or down-regulate survival genes, such as *BCL-2* (*65*). Hence, G_1 arrest and apoptosis appear to be alternative p53-induced outcomes. Cell suicide is arguably the most potent natural defense against cancer, because it eliminates premalignant cells that enter S phase inappropriately after genetic sabotage of restriction point controls (*64, 66*). Consistent with the idea that p53-induced $p21^{CIP1}$ can limit RB hyperphosphorylation (Fig. 2), loss of RB function can bypass p53-mediated G_1 arrest (*67*). However, overexpression of E2F-1 not only drives quiescent cells to synthesize DNA, but it induces p53-dependent apoptosis (*68*). Cooperation between the RB and p53 pathways likely determines whether p53 induces G_1 arrest or apoptosis in response to DNA damage, with the loss of RB tilting the balance toward the latter. In cells that have sustained lesions in the RB pathway, there could be a strong selection for the loss of normal p53 (*66*).

A Final Accounting

Of the more than 100 proto-oncogenes and tumor suppressor genes that have been identified, most function in signal transduction to mimic effects of persistent mitogenic stimulation, thereby uncoupling cells from environmental controls. Their signaling pathways converge on the machinery controlling passage through the G_1 phase, inducing G_1 cyclins, overriding CDK inhibitors, preventing cell cycle exit, and ultimately perturbing checkpoint controls. Some transcription factors such as MYC play important roles in cell cycle progression, directly reg-

ulating CDC25 phosphatases that control CDK activity (69) and, probably indirectly, cyclin expression as well (70). Other transcription factors, including many encoded by genes that are targeted by cancer-specific chromosomal translocations, instead seem to control lineage-specific differentiation and developmental decisions (71), including apoptosis (72).

Despite this plethora of oncogenes, an accounting indicates that pathways dominated by two tumor suppressor genes, *RB* and *p53*, are the most frequently disrupted in cancer cells. The functions of p53 are subverted by mutations in about half of human cancers, but other less direct mechanisms also contribute to p53 inactivation. For example, proteins like MDM2 or human papillomavirus E6 are likely to be oncogenic because they antagonize p53 function. How p53 senses DNA damage or induces apoptosis remains unclear, but we might guess that those tumors that retain wild-type p53 instead accumulate epistatic lesions that mirror a loss of p53 function. In short, most if not all cancer cells may have lesions in this pathway. Preventing p53-dependent apoptosis appears to be key to tumorigenesis. If so, loss of a death gene or overexpression of a survival gene might also mimic p53 inactivation.

What about the RB pathway? The discovery of *RB* in the context of familial retinoblastoma pointed toward its specialized role in the retina (44). Yet, RB's biochemical behavior connoted a more generalized function during the cell cycle, and so it seemed surprising that it was completely dispensable throughout much of mouse development (45). *RB* therefore appears to be unnecessary for the cell cycle per se, and even the eventual lethality imposed by its loss during gestation may not be due to cell autonomous mechanisms (73). Similarly, mice nullizygous for *D1* show only focal developmental anomalies (40). Although $p18^{INK4c}$ and $p19^{INK4d}$ are ubiquitously expressed during mouse gestation, $p16^{INK4a}$ is not (74), and *INK4a* nullizygotes develop normally (42). Even p16-null humans have now been identified (75). Clearly, restriction point control during development does not critically depend on *RB*, *D1*, or *INK4a*, although it may well be governed by families of redundant RB-like proteins, D cyclins, and other CDK inhibitors in a tissue-specific manner. There is some evidence for this. For example, mice nullizygous for either the *p107* or *p130* genes are normal, but animals lacking both *p107* and *p130* show severe anomalies in bone development; mouse embryos deficient in both *p107* and *Rb* die earlier than mice lacking *Rb* alone (76). But whether or not their inactivation is compensated by other family members, the loss of RB, D1, or p16 during much of development is tolerated and does not foreshadow their later importance in cancer.

In children who inherit a mutant *RB* allele, retinal tumors lacking both copies of the gene appear early in life with almost 100% penetrance, emphasizing the particular susceptibility of retinoblasts to *RB* loss (19, 44). The overall incidence of cancer in persons under 15 years of age is one-thirtieth that of the population as a whole, and even in children, familial and sporadic retinoblastomas are rare (together, 3% of all pediatric tumors) (77). Indeed, most pediatric cancers consist of leukemias, lymphomas, and sarcomas, or arise elsewhere in the nervous system. Thus, although retinoblastoma provided the historical basis for Knudson's now classic "two-hit hypothesis" for tumor suppression (44), the very short developmental history of these tumors in humans [and of pituitary tumors in $RB^{+/-}$ mice (45)] is atypical of cancer in general. The loss of *RB* or *INK4a* in childhood tumors need not stem from inherited defects because their inactivation is also observed in sporadic pediatric cancers, with disruption of RB function occurring in osteosarcomas and that of p16 in a high percentage of childhood T cell leukemias and glioblastomas (3, 7).

In contrast, more than 80% of adult cancers in the United States are carcinomas (tumors arising from basal epithelial cells of ectodermal or endodermal origin), and 8% are hematopoietic with a higher preponderance of myeloid leukemia than is observed in children (77). Carcinomas are rare in persons under age 30, rising exponentially in incidence thereafter, and their appearance with increasing age emphasizes the importance of cumulative exposure to environmental carcinogens in their induction. The cardinal property of the affected target tissues is that they undergo replacement throughout life. In this setting, stem cells must continuously enter the cell cycle to produce differentiated progeny, and over time, they are vulnerable to carcinogenic attack. Cyclin D1, p16, and RB figure most prominently here (78). In terms of overall cancer incidence per annum, RB inactivation is at least 50 times as prevalent in lung cancers than in retinoblastomas. It is striking that in lung or esophageal carcinomas, and possibly in other tumor types not yet analyzed, almost 100% of cases have detectable lesions in either *INK4a*, *D1*, or *RB* itself. To date, the incidence of p16 aberrations in human cancer appears to be second to that of p53.

The dynamics of cell cycle entry and exit in cell populations undergoing homeostatic renewal may differ considerably from those in cells exiting the cycle during development. Like p53, p16 may play a nonessential but otherwise important checkpoint function in self-renewing tissues, being selectively induced in response to certain types of damage, or to "inappropriate" mitogenic or constitutive oncogene-mediated signals. Alternatively, p16 may be a senescence gene whose expression is triggered by a generational alarm clock that records an allocated number of cell divisions before promoting cell cycle exit. The observation that p16 levels rise as cells age, although consistent with a role for p16 in cell senescence (79), is also compatible with an inducible surveillance function. RB-negative tumor cells, but not fibroblasts from $RB^{-/-}$ mice, express uncharacteristically high levels of p16 (7, 9, 13), so RB loss may occur in the face of elevated p16 expression, bypassing the putative p16 checkpoint. Cyclin D1 amplification would represent yet another way to override p16's braking effects on the cell cycle. If this is true, an inability of cells to exit the cycle is likely to be more important than their absolute proliferative rate in tumor formation, at least in the earliest stages of oncogenesis. Identification of the alarm or senescence signals to which p16 responds should be telling. Whatever the explanation, p16, D1, and RB must play a special role in somatic cell divisions after birth. Cancer cell cycles tell us this.

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78. A notable exception is colorectal cancer, in which loss of function of p16 and RB and amplification of cyclin D genes are rarely observed. A distinct spectrum of oncogenes and tumor suppressor genes are targets of genetic alteration in colonic epithelium. These include *APC* in the majority of cases, as well as involvement of a group of genes that regulate DNA mismatch repair in nonpolypoid cancers [B. Vogelstein and K. W. Kinzler, *Cold Spring Harbor Symp. Quant. Biol.* **59**, 517 (1994); R. Kolodner, *Genes Dev.* **10**, 1433 (1996)]. Colonic epithelium may be unusual because of its extremely high rate of self-renewal. Given that mutation of a gene can abrogate the selective pressure for inactivating other genes in the same pathway, a provocative possibility is that the *APC* gene product regulates p16. Alternatively, defects in the RB pathway may induce genomic instability, which, in colon cancer, would instead be a consequence of faulty mismatch repair.
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